6.
Effects on Plants

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PLANT DISPERSAL

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ABSTRACT

In the British landscape species exist in a mosaic of patches. Because many of the patches are small there will always be a probability of extinction of a species in a patch, and the species persistence through time in an area becomes as much a matter of its movement between patches as its performance within a patch. Dispersal between patches is imperfectly known and for a full assessment of the impact of pesticides should be better studied. From a review of what is known about dispersal, lines of further work are suggested. In particular, for wind dispersed species number of seeds produced and plant height are important, but for many species dispersed by animals the effect on animal ecology must also be assessed.

INTRODUCTION

This symposium is addressed to field methods for the study of environmental effects of pesticides. You may therefore be surprised to find a paper on Plant Dispersal, but it is my thesis, which I hope to substantiate in this paper, that plant dispersal is as important as plant performance in the context of this symposium.

That context I take to be, in current jargon, a discussion of methodological matters in Environmental Impact Assessment of pesticide usage. Now I am a botanist and a conservationist, and as such my objective is to ensure that plant species persist at places through time. That is not to be taken as requiring weeds to persist for ever in cornfields: I would happily define plants, places and time so that farmers may make a living! However, I would insist that all these three features, of plant, place and time, are emphasised. I do this to give weight to the dynamics of systems. I have no doubt that this symposium will very adequately discuss the methods for assessing the impact of pesticides on plants, at a place, at a time and chart the consequent changes at that place. It is, however, one thing to measure an impact in this way, and quite another to assess its significance. We may well have methods to measure this impact of, say, spray drift into a pond or hedgerow on the organisms in that pond or hedgerow. We may well be able to model and predict the changes consequent upon the changed competitive interactions between the organisms surviving the pesticide. Can we estimate the significance of these changes in the context of a farm with several ponds or many hedgerows? My view is that we cannot, unless dispersal is taken into account.

Population biology and island biogeography

I am sure these days that every schoolboy knows that the number of individuals at a site depends upon the rates of birth, death, immigration and emigration at that site. All too often we, as experienced ecologists, look at the first two only and ignore the second pair. We treat our pond or hedgerow, field or wood, as closed systems. They are not.

To be fair there is the general theory of island biogeography (MacArthur & Wilson, 1967), plus work on disturbance (e.g. see Mooney &

Godron, 1983) and patch dynamics (e.g. see Burgess & Sharpe, 1981) which do not treat sites as closed systems but open ones, and do consider immigration and emigration. These approaches focus upon the pattern of the landscape (Godron & Forman, 1983) and for the British rural landscape I would suggest that there are three basic semi-natural 'habitat' types, woodland, water, and grass, arranged in solid or linear patterns of spinney and hedgerow, lake or pond and river or stream, meadow and roadside verge. These habitats can be charted and classified, but to an extent these approaches can have only limited success because detailed measurements of one factor, plant dispersal, are not easily available for such habitats.

Plant dispersal mechanisms

That many plants have morphological or anatomical modifications to assist dispersal is very well known, and there are a number of descriptions in the literature (e.g. Chapter 5 of Salisbury, 1961. Chapter 19 of Good, 1964). There are also reviews (e.g. Howe & Smallwood, 1982) of the ecology of seed dispersal, but these commonly discuss the topic at an evolutionary or theoretical level. It is all very well to suggest that dispersal is an escape from a high probability of mortality of seedlings near the parent plant (Janzen, 1970) or to contrast this with a 'directed dispersal' hypothesis (Thompson & Willson, 1978) or a 'colonisation' hypothesis, that dispersal has its primary advantage in allowing occupation of safe sites which are unpredictable in time and space (Hubbell, 1979). We may accept all three as valid for specific cases and, as I do, accept the 'colonisation' hypothesis as more generally applicable but we actually need, for our environmental impact assessment, measurements of dispersal rates and distances.

Wind dispersal

Sufficient measurements exist to show that quite often with wind dispersal the largest proportion of propagules come to earth at some distance from the parent plant and, whether it be about 4 metres as with Verbascum thapsus (Salisbury, 1961) or 24 metres as with Eucalyptus regnans (Cremer, 1965), this maximum may be interpreted as evidence for the escape hypothesis. Nevertheless, it is the further tail of the distribution which is the important feature for persistence of a plant in the landscape of patches.

One model has been proposed by Green (1983) who relates the various distributions of seed numbers with distance from the parent plant to the variation in occurrence of safe sites with distance from the parent plant.

Consider a tree in a forest and imagine it surrounded by a series of concentric rings of equal width. The area of each ring will increase linearly with distance from the tree. Hence assuming the forest is more or less homogenous, and safe sites are therefore uniformly distributed, then the number of safe sites will also increase linearly with distance from the parent. If the tree is tolerant of a wide range of conditions then there will be a sufficient number of safe sites near to the tree and seed dispersal will be more efficient if it is limited in distance. If the safe sites for the tree are rare then there is more importance in the long tail of the dispersal curve. Green discusses this model for three genera (Acer, Fraxinus and Liriodendron) which have winged samaras and shows how the differences in dispersal curve could be related to the distribution of safe sites (canopy gaps) which differ for each genus. This is, however, a theoretical analysis only and all theory based on measurements of terminal velocity of a falling winged seed must be reassessed in the light of Rabinowitz and Rapps (1981) conclusions that "orderly differences in dispersal behaviour among species due to subtleties of morphology observed under laboratory

conditions are masked in nature", and "terminal velocity is a misleading descriptor of dispersal because dispores with similar terminal velocities have differing movement patterns".

Despite these caveats it does seem to me that Green's model has value. Where one might part company is in the assumed uniform distribution of safe sites. Jantzen's (1970) escape hypothesis provides one reason: predators may be more frequent nearer the parent. In rural Britain we should also remember the pattern of patches and linear habitats and consider whether extreme winds dispersing seeds over kilometres may not be more relevant than dispersal over tens of metres which may be sufficient for regeneration of gaps in a forest.

Animal dispersal

Consideration of our rural mosaic may prompt the query of whether or not dispersal would be more efficient in getting propagules to safe sites if animals were the medium for dispersal rather than wind. Many species appear to have seeds or fruits adapted to external adhesion by burrs, hooks and sticky substances. Some may merely adhere in mud to animals feet, and there is evidence that many of our ruderal weeds, such as Plantago, Urtica, Stellaria and Bellis are distributed by mud on our own boots (Clifford, 1956). Despite the proliferation of forms of burr or hook and the frequency of species with such adaptions in our flora (Galium aparine, Myosotis arvensis, Circaea lutetiana, Arctium etc.) there have been few experimental studies which demonstrate the distance of transport or numbers of seeds transported. Also given the passive mode of transport there can only be an assumption that an animal to which the seed adheres will continue to move in the same habitat type and therefore that the seed has a probability of reaching a safe site.

For seeds transported by ants there has been one demonstration, for Viola odorata and V. hirta (by Culver & Beattie, 1980), that this transportation considerably improved seedling emergence. Given the number of ant dispersed genera may reach a third of the total flora and includes Lamium, Primula, Ajuga as well as Viola it seems to me that there may be a complicated co-evolution and a story akin the Blue butterflies (Thomas) awaiting elucidation.

Dispersal by frugivory has been somewhat more generally studied, and it does appear that we have detailed data on such points as taste preferences of thrushes among the hedgerow berries (Sorensen, 1983) or the differences between titmice and thrushes (Sorensen, 1981) but although many details are known the distances seed commonly travels in a bird's gut are not. My own observations of colonisation of hedges by shrub species suggest that no hedge is isolated from seed sources by distance alone: it appears that birds will fly from a source in, say, a wood to any hedge within half a mile. Distribution of propagules within that hedge bottom, however, can be very uneven. It seems that birds prefer to perch on a high point of the hedge to regurgitate or defaecate and there is, as a consequence, a higher concentration of seeds beneath such high points. This does not persist, apparently because of preferential predation on such concentrations of seed and seedlings by small mammals. Nevertheless, the tail of the dispersal curve is very long.

The impact of pesticides

With such a scenario of species persistence in a mosaic landscape dependent upon moving between elements of that landscape, several types of impact may be predicted. Spray drift may make the pond and hedgerow less

safe sites. Using Green's model this has the effect of making safe sites rarer and putting even more emphasis on the tail of the seed distribution.

Pesticides may affect the tail more directly. For example, in any given wind dispersed species the size of the tail will depend upon the number of seeds produced and the height at which they are released. A pesticide which reduces the height of the plant or the number of seeds it produces will thereby reduce its dispersal. These parameters should be measured in, for example, work on spray drift if its full significance is to be assessed.

It is not only direct effects on plants which have to be considered, but upon animals too, as so many plant species are dispersed by animals.

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METHODS TO INVESTIGATE SUB-LETHAL EFFECTS OF HERBICIDES ON PLANT SPECIES

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ABSTRACT

Little evidence is available on the sub-lethal effects of pesticides on plants, especially species in natural habitats for which there is no perceived economic interest. At the level of response by individual plants, a variety of methods is available, and the usefulness of some of these is discussed. In many cases, the wider application of methods is limited by the lack of suitable conceptual frameworks to analyse data. Inter-plant variation is a serious practical problem, and it is suggested that one of the best ways to obtain dose response data is to construct a time course of non-destructive measurements. No evidence appears to exist for the effect of competition stress on the dose response to herbicide, even though such information is essential to understand the long-term consequences in a plant community. Methods to measure this should be sought.

INTRODUCTION

The increased use of pesticides by the agricultural industry, as well as the greater public concern for environmental matters, has brought about a need for a better understanding of the effects of pesticides, and in particular herbicides, on plants. Whereas the questions raised in the past for herbicides have largely concerned weed species and lethal doses, there is now an interest in a wider range of plant species and natural communities and the way they could be affected by low doses of herbicide, for example from drift. Problems such as these often present greater conceptual and experimental difficulties than those facing the agronomist.

It is not at present possible, given the lack of a suitable body of evidence, to review methods to study sub-lethal effects of pesticides on plants in the field without borrowing heavily from laboratory-based studies for ideas. It could be argued that laboratory conditions are a necessary starting-point for studies that have a large quantitative component, because methods that cannot be proven successful in relatively controlled conditions are doomed to failure in the field.

The two main questions considered here are: what are the sub-lethal effects of herbicides on individual plants, and are these effects similar when plants grow in commmunities? Only responses of whole plants are considered because studies at the organ or cellular level are rarely, at least with today's technology, applicable to the field. Nor is chemical analysis of herbicides considered, even though such methods could be used in the field when measuring responses by plants. The paucity of the literature means that many of the examples are drawn from work of the Weed Research Department, Long Ashton Research Station.

In practice, many of the studies of the environmental effects of herbicides on plant species concern spray or vapour drift, and the methods discussed in this review are applicable to either or both of these. There appears to be no recent review of this subject, although the chapter entitled

'Susceptibility of crop plants to very low doses of herbicides' in Elliott & Wilson (1983) collates much of the available data on minimum damaging doses. There are other examples, besides drift, of herbicide contamination which might come under the general topic of 'environmental effects', such as soil persistence and contamination of groundwater, both of which can lead to damage to plants, but these will not be specifically mentioned here.

METHODS TO STUDY THE RESPONSE OF THE INDIVIDUAL PLANT

Experimental design

It is likely that most experiments to study the response of the individual plant to herbicides will be quantitative, for example to obtain a dose response, and so will require statistical analysis. Much time can be saved, however, if some idea of inter-plant variation is known in advance, and it is unfortunate that this aspect of experimentation has received little attention. Few crops or wild species have been studied in detail (Breeze & Milbourn 1981), and it could be worthwhile to make some preliminary measurements before embarking on large-scale experiments with new species.

Another problem in designing experiments is that at high doses of herbicide, some plants may die and so reduce the number of replicates. Only in certain circumstances can the analysis include these. For example, in an analysis using relative growth rate, it is valid to score dead plants as zero. But using weight alone presents difficulties because it is often not possible to obtain a biologically meaningful weight of a dead plant, especially if it has been dead for some time before harvest. One solution to the problem is to take measurements before treatments are made, and to express results as a difference following treatment.

How many harvests?

This question frequently arises in both laboratory and field experiments for cases where plants can reach similar sizes or weights and the imposition of treatments only affects the time of the attainment of the final state. Measurements soon after the dose is applied indicate a treatment effect, whereas the effect decreases at later harvests. Eventually, the initial effect may disappear completely. Similar problems arise if the plants recover in other ways as, for example, in the case of leaf extension by oilseed rape (Breeze & Timms 1986). Here, leaf growth was greater in the fourth to seventh leaf grown following application of mecoprop at 10 g/plant than in controls. Thus the herbicide reduced growth of earlier leaves but the plants later recovered, and grew new leaves at a faster rate than the controls which already had a large amount of leaf. A similar effect could be caused by partial defoliation, and it is probably not strictly correct to regard it as a stimulation of growth by the herbicide. For practical purposes, an entirely different and erroneous conclusion might have been drawn if measurements had been made on one occasion, on leaf four. In fact, had leaf seven only been measured on day 35 following treatment, all of the leaves on the treated plants would have been longer than the controls, even at 100 ug/plant. Thus time course measurements are essential unless information about the development of symptoms is available. Also, if repeated non-destructive measurements can be made on the same plant, the problems of interplant variation can be reduced.

Measurement of response

It is not surprising that different characters of a plant show different responses to herbicide. For oilseed rape, the rate of leaf extension was reduced by 1 ug mecoprop/plant, and shoot dry weight (at 5 weeks from

treatment) was reduced by 10 ug/plant. However, if 6-week old plants were allowed to grow to maturity, seed yield was unaffected in plants receiving less than 100 ug/plant (Breeze & Timms 1986).

For reasons of recovery and interplant variation, it is better to make repeated non-destructive measurements, but weight measurements are often essential. In this case it is usually possible to obtain relative growth rates and moisture contents with little extra work. Relative growth rate is useful for example when there are dead plants in a treatment and in comparisons of response for species of different sizes. It is also a convenient way of handling time-course data, and its interpretation is well understood (Hunt, 1982). Moisture content, although rarely used, may be a sensitive indication of the effects of certain herbicides on plants (Breeze 1988a, Breeze 1988b).

Of all the non-destructive methods available, assessment of visible symptoms is simple and reliable but difficult to quantify. A great variety of schemes are available but all depend on subject observation. They can at best be used as an adjunct to other more rigorous methods. An interesting discussion of visible injury, although for damage by gaseous air pollutants, is given by Heath (1980).

Leaf extension rate has not been used in many studies, but potentially has advantages of sensitivity and simplicity. The main disadvantage is that the biological interpretation is not simple. Its usefulness could be improved, however, by measuring leaf area (as this is an important factor determining growth rate), although this would only be feasible for plants with regular leaf shape unless sophisticated methods of recording (such as photography) are used. It can also be used in the field (Dennett et al. 1978, Peacock 1975) on tagged plants. Further studies using a range of different species and herbicides would demonstrate whether the method has a wider application, but at present it appears to offer the best opportunity to measure effects of very low doses on plants in the field.

Although methods to measure the effects of environmental factors on whole-plant photosynthesis have been available for many years, they have rarely been used to determine quantitative responses to a range of doses of herbicide. These methods are complex but can show the effect of very low doses within a few hours. Furthermore, such responses are usually reflected in the dry matter production of the plants several weeks later (Breeze 1988a). Although the methods of carrying out such experiments are well documented (Merritt & Simmons 1985, Breeze & West 1987a), the interpretation of data is less well known. As with leaf extension, it is difficult to predict the long-term effect of small responses, and the change in rate of carbon dioxide assimilation may be due to epinastic leaf movement and not a direct effect on uptake or carbon dioxide reduction (Breeze 1988a). Even though there have been several attempts to relate the short-term carbon balance of plants or crops to dry matter production in the long term, this does not appear to have been attempted in the case of herbicide studies. For example, the 'synthesis and maintenance' model of the carbon balance (Ryle et al. 1976), in spite of the drawbacks discussed by Breeze & Elston (1983), could be useful in explaining the effect of a herbicide on the carbon balance. Synthesis respiration represents the fraction associated with the growth of new plant material, and is thought to be related to the previous amount of photosynthesis. Maintenance respiration, on the other hand, is due to processes such as protein turnover in existing structures, and is approximately related to the size of the plant. Thus a herbicide which

affected only photosynthesis would eventually cause a lowering of growth rate and respiration initially due to a reduced rate of synthesis; this has been observed in tomato plants following exposure to 2,4-D butyl vapour (Breeze 1988a). Obviously, much more experimentation and refinement is necessary before a model such as this becomes workable. However, if it ever became essential to understand the physiology of the processes of competition in a natural community between species susceptible to a herbicide and others that were not, then a model of the carbon balance would be necessary.

Of relevance to studies of the carbon balance is the movement of photosynthates within the plant. In one of the few studies of its kind, Whipps & Greaves (1986) observed the effect of a high dose of mecoprop on the distribution of 14-C within wheat plants. The herbicide permanently lowered the rate of 14-C fixation and caused the proportion of carbon dioxide translocated to the roots to decrease for 2 days and then increase (by 9 days after spraying). Later, there was no difference between treated plants and the controls. No estimate was given of respiratory efflux of carbon dioxide, although this would usually be related to the photosynthetic rate.

The lack of good methods to measure the sub-lethal effect of herbicides means that the investigator needs to be prepared to devise new techniques. One possibility is the measurement of ethylene production from dosed plants (Abeles 1968, Hall et al. 1985). Essentially, the ethylene is trapped and measured using standard methods following application of herbicide to a plant. Whether the response is sensitive enough to be of use, and whether the production can be quantitatively related to herbicide dose, are questions that must await further investigation. Even so, it is difficult to see how such a method could be used in the field, and its main application might, for example, be in studies involving screening a large number of species for sensitivity to different herbicides. Some encouragement for the development of this method is provided by studies of the role of ethylene in promoting epinasty during waterlogging, where quantitative responses have been observed (Jackson 1985).

Dosing techniques

Often, it is adequate simply to spray plants with standard equipment, for example using a range of concentrations. However, the precise dose received by the plant is often difficult to deduce, and this method cannot be regarded as offering precision. For example, it is inevitable that different sized plants will receive different doses, and species with erect leaves will receive less than those with prostrate leaves. Breeze & Timms (1986) dosed individual plants with 1.0 ul doses of mecoprop dissolved in acetone using a microsyringe; although this may not be the same as spray drift in the field, it is at least reproducible. In this case, the oilseed rape plants were dosed at the base of the petiole of the (approximately) sixth leaf. No data were provided on the amount of herbicide reaching the site of action in this or other studies.

Whereas it is relatively simple to dose plants with liquid droplets of herbicide, the vapour phase presents greater problems. Some workers have used a simple enclosure method, with a plant placed alongside a container of herbicide (Grabowski & Hopen 1985). It is doubtful whether this method can demonstrate anything except the general effect of vapour-phase phytotoxicity. It is limited to concentrations at or near the saturated vapour pressure, that are many times greater than normally found in the field. Often, it has been used to compare effects of two or more herbicides (Savory 1973), but it is questionable whether it is reliable for this because the amount of uptake

by the plant depends in part upon the rate of evaporation of the herbicide. To overcome some of these drawbacks, Breeze & West (1987a) have developed an air-flow system, in part based on concepts used for studying nutrient uptake at low concentrations (Clement et al. 1974) and the gas exchange of plants (Sestak et al. 1971), and similar to that proposed by Hartley & Graham-Bryce (1980). This uses high flow rates of air to minimise both boundary layer formation and vapour concentration depletion due to uptake. Vapour concentrations similar to those reported in the field can be generated and plants may be exposed for long or short periods.

With unlabelled herbicides, it is normally not possible to measure plant uptake from the vapour phase due to the extremely small amounts of herbicide involved. A maximum estimate of uptake can be made from the product of vapour concentration, flow rate and duration of exposure. For example, for a 6-hour exposure to 2,4-D butyl vapour at 0.12 - 0.82 ng/l, the maximum uptake by tomatoes was 1 - 8 ug/plant (Breeze 1988a). However, using labelled herbicide, uptake can be measured more precisely. In preliminary experiments at Long Ashton, the uptake of 2,4-D butyl was 11.5 ug/plant following a 2-hour exposure to 25.5 ng/l; this represented only 8% of the maximum herbicide uptake possible. Symptoms of phytotoxicity developed very rapidly and were much more severe in the second case, suggesting that the actual doses in the first experiment were much less than the maximum estimate. This may be the only time in which vapour uptake has been directly measured, but the technique is clearly a versatile one and will make possible a greater understanding of sub-lethal effects of herbicide vapour. It may even be possible to use a modified air-flow system in the field, perhaps to expose communities instead of individual plants. Also, an understanding of vapour uptake should give an indication of the relative toxicity of herbicide in liquid and vapour phase, a question of considerable importance when considering the contamination of the environment by herbicides.

Comparisons between species and different herbicides

Frequently, there is a need to compare the response of different species, or the effect of a variety of herbicides; at the level of sub-lethal doses, surprisingly little data are available. For example, Way (1964) studied the effect of TBA and some phenoxyalkanoic herbicides in the liquid phase on a number of vegetable crops. Breeze & West (1987b) observed the effect of 2,4-D butyl vapour in the range 3 - 50 ng/l on the growth of six crop species, and found that the ranking of species for sensitivity to vapour did not agree with the ranking for liquid doses. Nor was it straight-forward to rank species because different characters showed different sensitivities. Sunflower and field bean were the most sensitive on the basis of relative growth rate and mortality, with tomato, lettuce and cabbage less damaged and clover the least affected. Dead plants were included in the analysis using relative growth rate as zero. Otherwise, a small number of survivors greatly biased the comparison. Breeze & West (1987b) concluded that reports of the sensitivity were so widely different that it was difficult to compare separate studies. Standardisation of methods is essential for progress to be made, especially in the study of vapour effects. An unsatisfactory feature of species' comparisons is that it may be difficult, especially for wild plant species, to explain the response in terms other than empirical ones unless detailed physiological studies are made.

Plants as biological indicators of pesticide pollution

The concept of using a plant which has a particular sensitivity to a pollutant, to indicate the presence of that pollutant, is a very attractive

one. For example, the type of lichen community may be related to the average concentration of sulphur dioxide in the atmosphere (Gilbert 1970). A cultivar of tobacco (Nicotiana tabacum cv. Bel-W3) has been used to indicate the relative atmospheric concentration of ozone (Bell 1984). The method has the great advantage of simplicity because it avoids the need for sampling and analysis.

For pesticides, there do not appear to be suitable indicator species, and information about the distribution of pesticides in the environment has been obtained directly by analysis. In the case of phenoxyalkanoic herbicides, this has been reviewed by Que Hee & Sutherland (1981). Farwell et al. (1976) and Grover et al. (1976) monitored airborne 2,4-D using samplers and, at present, this appears to be the more reliable method. Although some species such as tomato respond to very low doses of herbicide (Breeze 1988a), much more work would be necessary before it could be used as an indicator species. The most immediate difficulty is to quantify the response of the plant to the herbicide. A more promising line might be to use the properties of certain plants, such as mosses, to retain atmospheric pollutants in their tissue (Brown & Beckett 1985). Until a simple method of indicating the amount of pesticide present in the environment is found, information on the subject of pesticide movement from spraying operations in crops is necessarily restricted. The need for such information becomes more urgent with the increasing use of pesticides of high activity per unit weight and the demonstration of effects such as the ability of atmospheric fogs to concentrate pesticides (Glotfelty et al. 1987).

Interactions with other pollutants

The possibility that combinations of pesticides and atmospheric pollutants could cause damage in the field is one that has received little attention, even though synergistic effects between gases such as sulphur dioxide and ozone are well established (Roberts et al. 1983). In a biochemical study, Hodgson et al. (1973) showed that the metabolism of the herbicide diphenamid by tomato was altered by fumigation with ozone. Another study by Carney et al. (1973) showed synergistic phytotoxicity between the herbicide pebulate and ozone, measured as dry weight, on tobacco plants (cv. White Gold) in two experiments, but not on the cultivar Dehli 34. The combination of ozone and the herbicide chloramben showed synergism on the cultivar Dehli 34 in only one of two experiments conducted. Trifluralin, chloramben and monolinuron on white beans, and diphenamid or trifluralin on tomatoes, in all cases in combination with ozone, showed only an additive response. Benefin and ozone showed an antagonistic response in tobacco. Barley leaves showed damage related to sulphur dioxide gas concentration following spraying with pesticides and a growth regulator (Baker & Fullwood 1986). In a study of the effects of simulated acid rain and the herbicide 2,4-DP on several crop species (Larsen 1985), only additive responses was observed.

Few studies have been made of herbicide vapour and gaseous pollutants, however. Preliminary experiments at Long Ashton (unpublished) suggest that tomato plants exposed to both sulphur dioxide (0.1 ul/l) and 2,4-D butyl vapour develop symptoms of phytotoxicity more rapidly than those exposed to the herbicide alone, but that effects on dry weight are only small. Possible mechanisms of interaction in the gas phase include sulphur dioxide induced stomatal opening, especially at low concentrations, which may facilitate uptake of herbicide vapour (Unsworth et al. 1972). Further work is needed, both to identify the sites of herbicide vapour uptake during exposure to a

second pollutant and to measure the rate of uptake. Until such information is available, a question must remain over interpretation of effects of very low doses of herbicides on plants growing in areas subjected to other forms of pollution.

RESPONSE OF PLANT COMMUNITIES

Whereas there have been many reports of herbicide damage to crops due to spray and vapour drift (Elliott & Wilson 1983), cases of damage to natural communities are less well documented. Biologically, the effect on communities is far less easy to predict and measure than on individual plants, due to the presence of neighbouring plants, both of the same or different species and perhaps damaged or unaffected by the herbicide. A monoculture, in which all plants are similarly damaged, presents the best opportunity for recovery by the individuals but, in a community, even a small amount of damage could lead to a decline in numbers of a particular species by the end of the growing season.

The response of plant communities is so difficult to investigate that it is not surprising that it is poorly understood. Not only do the responses of plants have to be considered in herbicide studies, but other components of the community may show unexpected changes. For example, paraquat is toxic to aphids (Smith et al. 1987). In the case of fungicides, the soil mycoflora could be damaged, so that nutrient uptake, and hence growth rate, by plant mycorrhizal associations is affected (Fitter 1986). It would not be difficult to draw up a list of responses sufficiently long to deter the potential investigator, or at the least, to provoke interest in alternative approaches.

In spite of the complexity, the problem can be addressed with one simple question; does the additional stress due to competition affect the dose response to a herbicide? This could come about in a number of ways, for example, if the effect of herbicide was related to the growth rate. Also, stress may lead to plant variation (Cooper 1959) and thus modify the response to herbicide. Defining what is meant by competition stress is not easy, but may be avoided by growing plants in artificially-arranged combinations following the method of de Wit (1960). In this way, it is possible to compare two types of plants at different degrees of competition stress, and in the simplest arrangement, dosed and undosed plants of the same species might be compared. Further experiments could use different species and thus better reflect a natural plant community, for example, by using a vigorous grass species and a broadleafed type which is susceptible to a herbicide. It, nevertheless, remains most important to study responses both in natural conditions and the long term. Perhaps the demographic studies of Ranunculus species, in which the progress of individual plants was followed over several years, carried out by Sarukhan & Harper (1973), or of Ophrys sphegodes by Hutchings (1987), serve as useful models. Even so, it would be a formidable task to follow the growth and reproduction of individual plants in a population, each with one of several different doses of herbicide, for a number of years. Such a study, especially if carried out in conjunction with dose-response data obtained from plants grown in spaced trials, would be a major contribution towards understanding effects of herbicide contamination in the environment, and be valuable in determining policy on the use of herbicides and different spraying systems as well as the management of conserved habitats.

CONCLUSION

The lack of data and conceptual frameworks for their interpretation is a serious problem in understanding the effects of sublethal doses of herbicides in the field. The widespread use of herbicides at rates of less than 10 g/ha, for example in the case of metsulfuron (Worthing 1987), which may cause phytotoxicity in susceptible plants at one-thousandth of this rate, means that studies should be undertaken, if only with the object of providing reassurance that damage to the environment is not being caused.

Apart from accidents, herbicides may damage the environment in many ways. Spray and vapour drift are probably among the most serious. Whereas spray drift damage is usually localised to a few tens of metres from the spraying operations in most conditions (Thompson & Ley 1982), vapour has been reported to travel many kilometres (Grover et al. 1976). It may also be concentrated by atmospheric mist (Glotfelty et al. 1987) or rain. Although many herbicides are non-volatile, there is little doubt that the ester formulations will be produced for the foreseeable future because they are generally more efficacious than salts and can be mixed in one spraying operation. It is this type of herbicide that presents the greatest potential threat to the environment and for which there is most need for further study. Work on volatile herbicides should not be confined merely to screening plant species for susceptibility. Factors determining the rate of evaporation from a sprayed crop, photodecomposition of the vapour, and the effect of environmental conditions (including other pollutants) on uptake are a necessary part of any investigation.

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ASSESSMENT OF HERBICIDE EFFECTS ON INTERACTIONS OF WEEDS, CROP PLANTS, PATHOGENS AND PESTS.

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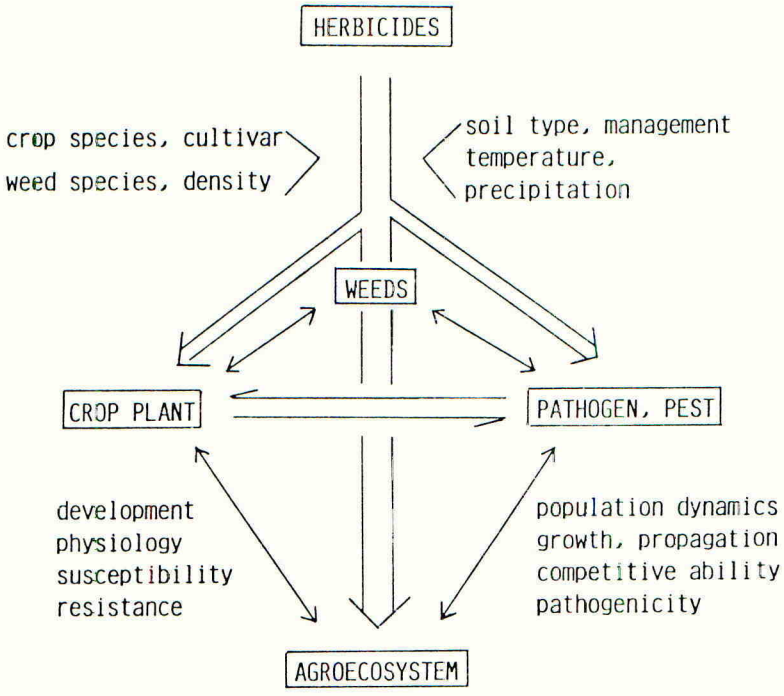
ABSTRACT

Herbicide application and chemical weed control may affect the occurence and intensity of diseases and pests in crops. Decrease or increase of pathogens and pests can be the result of a complex of interacting factors including direct and indirect effects of herbicides. With diseases in arable crops some examples are described, the results discussed and the consequences for planing of weed control measures indicated. Points which should be considered in field studies and supplementary analysis of such non-target effects of herbicide application are elaborated.

The main effect of herbicide application to remove weeds as competitors of crops can be reached today very effectively with a high number and considerable variation of different compounds. Non-target- or side-effects of herbicides have attracted interest ever since chemical weed control has developed from early attempts to great practical importance. Several reviews summarize the results obtained and most of them conclude, that more must be known about such side effects in order to optimize the results and minimize the risks of chemical weed control. This applies especially to the interaction of herbicides and weeds with plant diseases and pests (Altmann & Campbell 1977. Heathcote 1970. Heitefuss 1970. 1972. 1973. 1986. Katan & Eshel 1973. Moore & Thurston 1970. Way & Cammel 1981. Zweep, van der 1970). The purpose of this paper is to focus again on these interactions, whereby special emphasis will be placed on methods to observe such side effects in the field and to analyse the results by supplementary experimentation in the greenhouse or laboratory. Due to the limited space examples must be restricted mainly to herbicide - weed - crop - disease interactions.

Complex of possible interactions

An attempt to demonstrate the multitude of posssible direct and indirect interactions is made in figure 1. It includes among other assumptions the fact that weeds can exert negative effects on the crop by competition, harvest impedimentation, sequential weed population increase and as alternative host



Interactions of herbicides, crop plants, weeds, pathogens and pests

Fig. 1

plants for pathogens and pests. On the other hand, positive desired influences may be a reduction of mono-culture features, better soil cover and firmness, support of higher species diversity especially of arthropods and provision of alternative food for pests.

Weed control by herbicides depends in its success on crop species and cultivar, weed species and density; on the other hand on soil-type and -management and weather conditions. It will remove the yield reducing influences, but also the desired

influences of a limited, non competitive weed cover. Although herbicide effectivenes depends largely on the principles of selective action on weeds, also the crop plant may be affected by certain compounds leading to stress conditions. The results can be changes in development and physiology and thereby alterations in susceptibility or resistance of the plant to diseases and pests. Their attack furthermore may be facilitated or impeded by changes in microclimate within the crop as conditioned by removal of the weeds. Direct actions of herbicides on pathogens and pests are also possible mainly by fungicidal or insecticidal effects or repellent action on insects. This will lead to consequences in population dynamics; growth or propagation may be inhibited, competitive ability or pathogenicity affected. Finally the role of weeds respectively their removal must also be considered in the consequences for the agroecosystem within a crop. Members of the epigaeic and hypogaeic flora and fauna including microorganisms may be affected, antagonists and competitors, predators and parasites and also apparently indifferent organisms may decrease or increase. In consequence, if side effects of herbicides on diseases and pests occur they may be the result of different influences in a rather complicated system of interactions. The cause and effect analysis requires careful experimentation and multifactorial approaches.

Removal of weeds as alternative hosts of pathogens

Numerous investigations have shown, that weeds can be infested with pathogens or pests which also will attack the crop plant (cf. Thurston 1970. Heitefuss 1986). Sclerotinia sclerotiorum for example, a major pathogen of rape, has a host range of more than 360 plant species including many weeds (Purdy 1979). It was reported that in rape fields without weed control a higher number of crop plants with Sclerotinia were found, although quantitative data about this and the effect of weed removal by metazachlor are lacking (Saur & Löcher 1986). For the two most important foot and root-rot diseases of wheat and barley, Pseudocercosporella herpotrichoides and Gaeumannomyces graminis many weed grasses can serve as host plants (Cunningham 1965. Hartz 1969. Asher & Shipton 1981). Whereas the grasses Agropyrum repens, Alopecurus myosuroides and Apera spica venti are considered epidemiologically of no importance for P. herpotrichoides, this is apparently different for G. graminis (Hoffmann & Schmutterer 1983). Due to the low competitive saprophytic ability, carry over of G. graminis can be facilitated by alternative grass hosts or volunteer barley plants and thereby increase the level of take-all in wheat (Rovira & Venn 1983).

When Agropyron repens, quack grass was controlled by glyphosate, G. graminis disease incidence was higher compared to mechanical control. Unfortunately no exact comparisons to plots without any control of quack grass were included (Mielke 1983).

Consequences of weeds as alternative hosts for plant pathogens are not only relevant during one growing season but must be evaluated during the crop rotation or at least for the

following crop. Much more exact data about these effects are required in order to include such informations into the considerations about weed control according to economic thresholds.

Direct and indirect action of herbicides on pathogens

Herbicides may have direct stimulatory or inhibiting effects on pathogenic fungi, even at the concentrations applied or calculated for the upper few cm of soil surface (cf. Heitefuss 1973), although such influences are more the exception rather than the rule under practical conditions. Tests on agar medium containing different concentrations of herbicides do not represent natural conditions but will give first indications of fungicidal potential. Table 1 gives an example of such test, whereby the amount of 10 mg/l commercial product aproximates the concentration range which can be reached in the top soil. At 1 mg/l the inhibition of the three tested fungi does hardly exceed 10%, at 10 mg/l around 25 - 35% are reached. At 100 mg/l prometryne exhibits the highest fungistatic potential with 57% inhibition compared to the control. Other herbicides, for example dinoseb-acetat have a much higher fungicidal effect, at 10 mg/l G. graminis was inhibited by 85% in tests on agar media (Heitefuss 1973).

TABLE 1

Radial growth of cotton pathogens on agar medium containing various concentrations of herbicides (Youssef et al. 1985)

Herbicide	mg/l	Fusarium oxysporum f.sp.vasinfectum mm	Rhizoctonia solani mm	Sclerotium rolfsii mm
Control	-	40	40	40
Fluometuron	1	36**	38*	36**
	10	30**	31**	28**
	100	23**	20**	22**
Prometryne	1	36**	34**	37**
	10	30**	27**	26**
	100	24**	17**	22**
Trifluralin	1	36**	40	35**
	10	30**	36**	30**
	100	23**	25**	22**

Differences to control significant at *) $p \leq 5\%$; **) $p \leq 1\%$

Applying the herbicides at practical rates to soil infested with the pathogens and then observing the extend of disease on the host planted into such soil will give further information. Table 2 shows the result of such tests with cotton

and Rhizoctonia solani and Sclerotium rolfsii. It can easily be recognized, that the results do not parallel those of the previous test. Treatment with fluometuron leads to a decrease of damping off caused by R. solani, prometryne and trifluralin cause a significant reduction of disease by S. rolfsii. In contrast prometryne and trifluralin at the high concentration stimulate R. solani, fluometuron increased S. rolfsii (Youssef et al. 1985). The picture is further complicated by field studies, which however must be the ultimate and most important means to assess the practical importance of herbicide side effects on diseases. In order to differentiate between weed and herbicide influence, hand weeded plots were included as additional controls (table 3). Assessment at 45 days after sowing showed the most conspicuous difference with the prometryne treatment, leading to a decrease in seedbed failure, R. solani and a disease complex including several fungi. In contrast, trifluralin caused a significant increase in cotton seedbed failure and incidence of R. solani (Youssef et al unpublished). The latter confirms earlier studies of Neubauer et al. (1973) and Chandler & Satelman (1968), that trifluralin increased R. solani incidence on cotton but fluometuron did not. However, depending on the inoculum density and herbicide concentration, also a decrease or increase of resistance of cotton against R. solani after aplication of trifluralin has been observed (Grinstein et al. 1976. 1984). From our different experiments to explain the observed effects, only the analysis of root exuda-

TABLE 2

Influence of herbicides on incidence of cotton seed and seedling diseases in soil inoculated with Rhizoctonia solani and Sclerotium rolfsii respectively. Greenhouse tests, cultivar Giza 75 (Youssef et al. 1985)

Herbicide	kg/ha	% of seed preemergence and seedling post emergence damping off Rhizoctonia solani	Sclerotium rolfsii
Control	-	73	64
Fluometuron	1	71	70**
	3	60**	74**
	5	47**	89**
Prometryne	1	70	53**
	3	81**	41**
	5	86**	34**
Trifluralin	1	56**	58**
	3	67**	42**
	5	87**	37**

Differences to control significant at **) $p \leq 1\%$

TABLE 3

Influence of herbicides on incidence of cotton soilborn diseases. Field experiments El-Goumeza, Egypt (Youssef et al. unpublished)

Herbicide	kg/ha	% disease incidence [1] seedbed failure [2]	Rhizoctonia solani	disease complex
Handweeding	-	17	30.1	18.2
Control	-	19	26.6	16.4
Fluometuron	3.75	19	24.6	16.8
	5.00	21	19.5*	12.5
Prometryne	2.50	12**	17.4**	11.5
	3.75	14*	14.6**	8.8**
Trifluralin	2.50	24*	34.3**	20.5
	3.75	28**	38.4**	18.0

[1] disease incidence at 45 days after sowing
[2] % of seedbeds with ungerminated seeds
Differences to control significant at *) $p \leq 5\%$ **) $p \leq 1\%$

TABLE 4

Influence of herbicides on the amount of reducing sugars and amino acids in cotton seedling root exudates (Youssef & Heitefuss 1983)

Herbicide	mg/l	reducing sugars mg/g root dry weight	amino acids
Control	-	4.0	0.34
Fluometuron	0.5	5.9	0.51
	1.5	7.0*	0.55
	2.5	7.4**	0.59
Prometryne	0.5	9.4**	0.77**
	1.5	10.9**	0.88**
	2.5	12.9**	1.05**
Trifluralin	0.5	9.5**	0.93**
	1.5	11.1**	1.12**
	2.5	12.3**	1.28**

Differences to control significant at *) $p \leq 5\%$; **) $p \leq 1\%$

tes from herbicide treated cotton seedlings will be shown (Youssef & Heitefuss 1983). All three herbicides when applied at nonphytotoxic concentrations to cotton seedlings grown in liquid cultures stimulated the exudation of sugars and amino acids, whereby the effect of trifluralin was especially evident (table 4). Further microbiological studies revealed, that growth of Fusarium oxysporum was stimulated by root exudates obtained from herbicide treated plants, however that also a population increase in the rhizosphere-microflora of cotton plants grown in herbicide treated soil could be observed (Youssef & Heitefuss 1983). The final answer remains open, whether this contributes to the differences in disease incidence observed in the field.

TABLE 5

Influence of herbicides on mildew of spring wheat (E. graminis) under field conditions (Ibenthal & Heitefuss 1979)

leaf number	days after herbicide treatment	control	Methabenz-thiazuron 4.5 kg/ha	Terbutryn 3.0 kg/ha	Chlortoluron 4.5 kg/ha
		pustules / leaf			
4	13	18.8	3.1**	4.5**	4.8
5	33	40.1	37.0	46.5	73.9**
6	42	61.4	72.7*	89.6**	97.4**
7	60	32.3	48.1**	50.7**	44.5**

Differences to control significant at *) $p \leq 5\%$ **) $p \leq 1\%$

Another example of indirect herbicide non-target effects is the initial reduction and following stimulation of powdery mildew (Erysiphe graminis) on wheat and barley, which has been observed after application of triazines or substituted ureas in field and greenhouse studies (Brandes & Heitefuss 1971. Ibenthal & Heitefuss 1979a). Table 5 summarizes data from a field experiment, in which the number of mildew pustuls was counted on consecutive leaves and at different days after herbicide treatment. Less mildew incidence is evident on the fifth leaf at 13 days after herbicide application. A change to a higher pustule number becomes very conspicuous on the 6th and 7th leaves under the influences of all three herbicides tested. This observation has been confirmed many times under field conditions for example with isoproturon by Springer & Heitefuss (1986) and Kuhlmann (1988). The phenomenon occurs also under weed free conditions. The fungicidal effect of the tested herbicides is rather low. Decisive is apparently the stress situation in the host plant as induced immediately after application by the herbicides which interfere primarily with photosynthesis and related carbohydrate and amino acid metabolism. Later on the plant recovers from the "herbicide

shock", whereby the development is slightly retarded in the treated plants which reach the stage of adult plant resistance later compared to the control plants and thereby exhibit a higher degree of mildew incidence.

The physiological and biochemical changes associated with this delayed senescence and higher adult plant resistance can not be treated here in detail (cf. Ibenthal & Heitefuss 1979b). For field studies it is however important to note that such interactions between herbicides and mildew occur especially pronounced with susceptible cultivars at higher nitrogen supply to the crop. Under practical conditions the phenomenon is often unnoticed because of routine fungicide spraying. In oder to develop systems of integrated crop protection, these interactions however should be taken into account.

Consequences for field testing of herbicide-weed-disease interactions

The examples elaborated so far in view of the general scheme of possible interactions and the multitude of factors of influence demonstrate, that a simple approach will not be sufficient to observe, proof and analyze these effects and interactions. For field studies however several points must be considered:

1. Side effects of practical relevance should be evident after application of normal, practical rates of herbicides to the respective crop.

2. With airborne leaf diseases observations during the growing season of the crop are sufficient. With soil borne diseases the effects should be evaluated also in the consecutive, susceptible crops or the crop rotation, especially if they may be due to removal of weeds as alternative host of the respective pathogens.

3. Epidemiological consequences of weeds as alternative hosts of pathogens should be analysed in relation to weed density and weed control in the respective crop.

4. Effects of herbicides on disease incidence should be evaluated also under weed free conditions.

5. Field results in which non-target, side effects of herbicides have been shown should be supplemented by greenhouse and laboratory studies in order to distinguish between direct and indirect effects of herbicides and weed control.

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PROBLEMS IN THE INTERPRETATION OF EFFECTS OF HERBICIDES ON PLANT COMMUNITIES.

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ABSTRACT

Problems in the interpretation of survey data and the results of simple, uncontrolled experiments are discussed. The need to obtain a quantitative measure of the size of an effect and to establish a herbicide as the cause is highlighted; examples are drawn from published studies. It is concluded that most present approaches are more suited for generating hypotheses. They do not test those hypotheses and are not capable of giving the strong conclusions that are required. Detailed base-line studies, followed by carefully designed experiments with limited but precise objectives are required. Even then, the objectives may not be fully attainable.

INTRODUCTION

It is all too easy to embark on a weed monitoring programme with the minimum of planning. The methodology of assessment is long- established and simple. Standard designs of study, which 'worked' previously, can be copied again. It is also easy, however, to find that the data generated during the programme do not provide answers to the questions posed, either through poor experimental design or failure to appreciate the true nature of the question. To be able to say at the end of a 10 year, £300,000 study that "pesticide X appears to have had a slight effect on species 1 and we cannot be sure, but it doesn't appear to have affected species 2 to 35" in answer to the question "is X safe to use on this Site of Special Scientific Interest" is clearly poor value for money, if not completely useless for management purposes. Many environmental studies, however, are only capable of producing such statements, and we must put their usefulness in perspective.

To many, the objective of environmental monitoring is to ask whether or not a particular treatment, or event, has an adverse effect on the environment. As scientists, we often cannot determine what is adverse, except in extreme cases, since this is indefinable and subjective. We can determine whether the treatment had a measurable effect. For any study to be truly worthwhile, we need to be able to make a confident statement of whether there was any effect, what that effect was and what the cause was. For any failure to observe an effect we also need an estimate of the sensitivity of the study, so that we can state what size of effect may have escaped observation. Conclusions of this type, however, are rarely made. Yet, in order to substantiate arguments, there is a need for more than just repeats (often contradictory) of previously published work. Better designed, better planned work and conclusions that cannot be faulted or

explained in any other ways are essential. A claim that "in a small number of detailed experiments chemical X reduced yield (or species diversity) by an average of 0.3 t ha^{-1}" cannot be countered by the argument that "in our (simple) numerous experiments (with minimal replication, analysed separately and with an average LSD of 0.7 t ha^{-1}) we consistently found no (significant) effect". Public support for the use of chemicals in sensitive areas is unlikely to be forthcoming if studies are poorly designed, of limited depth and inconclusive. Later publication of the fact that the studies were statistically inadequate, poorly thought-out and the conclusions scientifically unwarranted, will benefit no argument and will reduce future confidence. Good design, leading to clear and precise statements, is therefore of paramount importance.

It may be that in many cases the problems are insoluble, but an appreciation of why this is so can be informative. Can we expect to achieve our objectives from present studies? In this paper we will examine the meaning of 'measurable effect', discuss what types of effect we might expect, how we can record this in the case of plant communities and question whether our aim of a strong conclusion can ever be attained. We will base our discussion on the assumption that we are concerned only with long-term studies. How long such a study should be will be dependent on the objectives and on the system under study; effects may show up in detailed, short-term experiments or only in long-term studies where complex or subtle interactions may need time to develop. This particular question, we feel, does not have a general answer and we will not address it here.

WHAT IS MEASURABLE?

Many ecological reports present measures such as species number or diversity, forgetting that they are only estimates of the true assemblage of species, and discuss differences without any attempt to show that they are due to other than sampling variation. Agrochemical use is an emotive subject and such data are insufficient evidence, whatever side of the fence you sit upon. In order to justify conclusions that differences are likely to be real, data must be accompanied by appropriate statistical analyses.

In biological studies it is usual to regard a 'measurable' effect as one which can be established as statistically significant. From statistical analyses it is possible to state that, within a certain degree of confidence, an apparent effect is highly unlikely to have occurred by chance. However, many studies attempt to do this on the basis of inappropriate statistical methods (see, for example, discussion by Perry 1986). Furthermore, one of the most common statistical errors is to conclude the converse for a non-significant result, that there is probably no effect (Cousens & Marshall 1987). For example, it has often been concluded from a non-significant result that a chemical did not affect crop yield, or that a chemical treatment could be omitted without affecting yield. Both conclusions are misleading. A non-significant result means only that an effect was not detected, i.e. that it was not measurable. Clearly, if it is only possible to detect very large effects, then even quite sizable real effects could be missed.

In arable crop studies, it is common to obtain a coefficient of variation of 5-10% from the analysis of variance of yield data. With replication of about 4, the detectable difference is likely to be in the region of 10% of the grand mean; any real effect smaller than this will

usually be designated as non-significant. What is the limiting value for detection likely to be for species abundance, for diversity, or for cover in a hedgerow ? If we do not know, then we cannot design surveys or experiments to meet a specific objective or to test an hypothesis. This highlights the need for a detailed background survey before the programme is begun. This will give estimates of the variability of measures of interest, so that suitably sized experiments can be planned. Initial information on, for example, species presence and absence can also be used as covariates in later analyses to allow for differing initial states of the plots. For most studies similar in size to yield experiments, it is unlikely that detectability will be as great as for yield effects. Therefore, we are unlikely to be able to detect (with confidence) quite large effects on the species composition of the flora, and certainly not subtle or sub-lethal effects of herbicides, all of which could be classed as adverse effects.

The ability of a significance test to detect differences depends on the number of treatments, replication, experimental design and inherent variability of the data collected. Hence, even a large number of small experiments on a very variable subject will 'consistently fail to observe any significant effect of a treatment' if analysed separately, because they simply do not have the capability to see most effects when they exist. There are methods available with which to calculate, for example, the replication or sampling frequency required to observe a given size of difference (Geng & Hills 1978). For one example, given by Cousens & Marshall (1987), it can be calculated that in order to detect, with a frequency of 95%, an economic threshold response due to a particular weed, 137 replicates would be needed. Only three replicates were actually used in the study. If a researcher is serious about his aims, then regardless of claims about limits of time, cost and manpower he must design an experiment capable of answering the stated objectives. Of course, there may be pressures not to detect too small a difference, in which case a weak design may be favoured. Alternatively, pressure to find a difference at all cost, or to be as sure as possible that a chemical is safe, must indicate highly detailed studies.

CAUSE AND EFFECT

It is not sufficient to be able to conclude that a flora has changed. If we are interested in the effects of chemicals, then we must be able to confirm that the cause was the herbicide treatment. We expect that over time any community will change, if not in the species present, at least in their relative abundances. How can we determine whether those changes are caused by herbicide use?

Consider the case of a survey of species abundance in a single field over a long period of time. Chancellor (1985) describes an excellent study of this type. Over a period of 20 years, seedlings were counted in quadrats located on a grid in one field each year. Records were kept of all the farm husbandry, including chemicals used. Species number, total weed density and abundance of each species were examined over time, either visually or by regression. It was concluded that the distributions of some species reflected earlier periods of cultivation, that perennial species from former grassland steadily declined and some arable weeds increased or decreased as a result of spring or winter crops. No conclusions were reached concerning the effects of herbicide, and it was noted that this was difficult to do.

Though it was noted that they were 'effective' in controlling weeds within any one year, since seedling numbers increased with time, control was not perfect.

Chancellor's study was not intended to look at herbicide effects, but rather to be a general description of long-term changes. Could any information be extracted regarding herbicide effects on the flora? Some sort of multiple regression analysis could be used, with species abundance as the dependent variable and husbandry practices as independent variables. The analysis would then indicate those variables having significant contributions to the regression. The relative size of the coefficients would indicate the magnitude of the effect. However, over the 20 years there were 6 crops grown, drilled in 6 different months, sprayed with 8 different herbicide sequences, assessed in one of three months, along with numerous differences in tillage, straw disposal, pesticide use and fertiliser applications. Many of these other variables are either partially or wholly correlated, making it difficult to separate individual effects. With so many variables, it might be that a small number of them would be significant even where they had little effect, simply due to chance. In short, too much changed over the study period. Herbicide effects would have to be very large to stand out, and, if confounded with other effects, almost impossible to pick up. Such surveys would be of little use in our aim of making conclusive statements.

If such surveys are not sufficient, then experiments must be designed which will determine the effects of herbicides. A major experiment of this type has been conducted at Boxworth E.H.F. over the last 5 years. The intention was to examine the effects of pesticide management on the fauna and flora, both in the fields and in the hedgerows. Fields were divided into three blocks, each block receiving a different programme of pesticide inputs. Broad-leaved weeds were surveyed on a grid system in the spring, and grass weeds in the summer. Results of the first three years for one field in each block were presented by Marshall (1985). Over this period there was 'some indication' that the number of broad-leaved species was increasing in the 'integrated' (reduced input) treatment area but that of grass weeds was decreasing in the 'full insurance' (high input) treatment. Attention was drawn to the higher diversities in the reduced input area by the third year. These apparent trends in the fields, along with similar indications from the hedgerows, were clearly encouraging for aspects related to conservation.

Marshall's report allows more insight into herbicide use than was possible from Chancellor's example. However, since there was no replication involved (only one field in each area was included), the conclusions remain no more than speculation. Also, although pesticide input differed between areas, so did other factors such as cropping and tillage. From the Boxworth study, there are data for replicates, which allow confidence limits to be calculated for the various community measures. Even so, it is still not possible to draw conclusions regarding the causes of changes and the effects of herbicides with any certainty. For various reasons, the experiment was laid out such that the fields (replicates) of a given treatment were not randomised and each set of fields was spatially separated from those receiving other treatments. The problem is therefore analagous to that of growth cabinets, where although conditions are as near as possible equal (apart from the treatment) between cabinets, it cannot be certain that there are no differences and any treatment effects are inevitably confounded with positional effects. Without randomisation, statistical tests are invalid;

however, if there are large differences, then they will show up and will probably reflect cause and effect.

With growth cabinets, care is taken that the experimental material is the same at the start of the experiment. With Boxworth this was not the case. There were clear initial differences between the areas in species present. In the first year, 27 species were recorded, of which 9 were found in only one of the field blocks, 14 were in all blocks, and only two species were recorded in all fields. There was an average of 12 species per field in the high input treatment, whereas there were 13.8 and 16.3 species per field in the two reduced input treatments. Hence, even at the start of the experiment the number of species was least in the area to be given the treatment most likely to reduce diversity. The question to be asked of the data, therefore, is not 'do they differ at the end of the trial period?' but 'has the change in each area from year 1 to year 5 been different?'. Given that the floras were initially not the same, how can we assess whether a change has been similar? We may be comparing very different things. Although we may use measures such as diversity indices (Fig.1), is a change in such an index from 0.2 to 0.5 the same as a change from 1.2 to 1.5? Is diversity (or whatever the measure is) on a linear scale? The problem is just as acute if we consider the abundance of a single species. If two populations increase at different absolute rates, is this simply what would be expected from their initial abundances and density-dependence, i.e. a similar response to treatment given that they were not at the same level to begin with? It is difficult to envisage any situation where the degree of change in two initially different values can be compared with confidence.

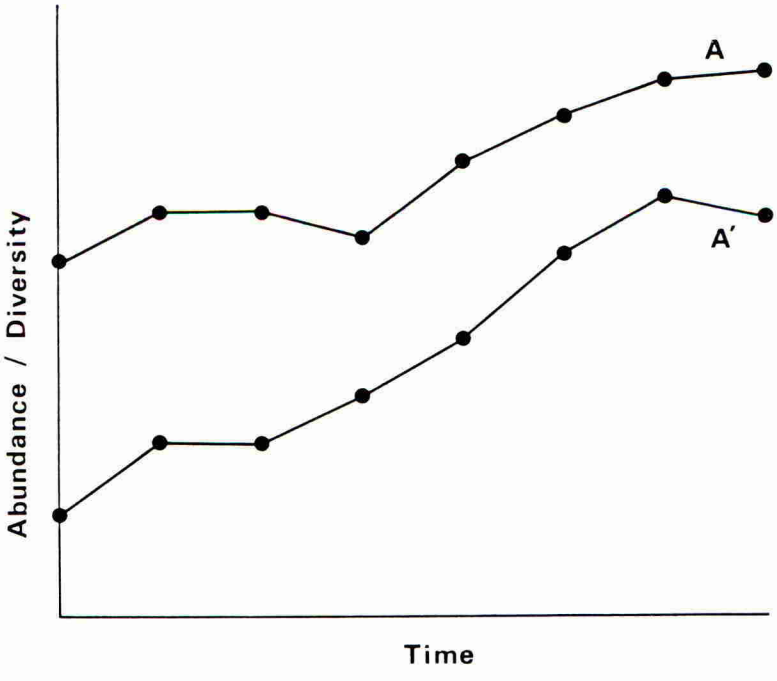

Fig. 1. Hypothetical effect of two identical treatments, A and A', on populations starting from different initial states.

There is perhaps a tendency in ecology to regard the aim of a study as to produce output from multivariate (or other) analyses in which can be found evidence in accord with expectation. Such evidence, when 'enough' has been accumulated, may then be presented as confirmation of a theory, for example that the use of herbicides reduces the species diversity of hedgerows. Although the theory may be true, the collection of supporting evidence does not, in itself, prove a belief, since it may also not refute other possibilities (weak inference - Platt 1964). In addition, there is also the problem of what constitutes 'enough' evidence. It is necessary, also, to produce evidence to eliminate alternatives. The best way to do this is through carefully designed experiments, not through survey work.

Hume (1987) reported the results of a study of the weed flora in an experiment on long-term application of 2,4-D. Over a period of 36 years the only differences in treatments between plots were the use of this herbicide. There were six replicates, randomised in complete blocks, of two rates of herbicide and a control. Cropping was rotated on a three year cycle, but so that in any year two plots of each treatment were at the same stage of the cycle. Although other factors undoubtedly changed with time, the husbandry of the three treatments was the same in a given year. At the end of the study the abundances of the species were recorded. It was found that although the relative abundances had changed, all species present at the start were still there, even those susceptible to the herbicide. Hume noted that other long-term studies had also found that species number was little affected. Such a clear, interesting conclusion was only possible through the use of a carefully designed experiment. If we are to look at effects of herbicides on species diversity, the number of species may not be a sufficiently sensitive index.

MEASURING CHANGE

One of the basic requirements of environmental studies is to be able to detect and to quantify change. For a single species, its abundance has changed if it increases or decreases in number. The increase may be expressed in absolute terms, or relative to the initial value, such as a percentage increase. If regressions are used to relate abundance to time, change may be expressed as a rate or some other parameter of a fitted curve. However, change may be more difficult to assess for a community. If the abundances of all species change equally, has the flora changed? Presumably, a community has changed if the abundance of even one species changes differentially to the rest. In other words, in order to assess change we need to examine the relative abundances of all species and to express this quantitatively.

We could, of course, go through each species one-by-one, looking for those in which their relative abundance has changed significantly (needing the application of valid statistical tests). As pointed out earlier, if we examine enough species we might expect some changes to be significant simply by chance, giving spurious relationships. Alternatively, we must examine the species-abundance assemblage as a whole. A great many ways of expressing diversity or even-ness of communities have been devised (Pielou 1975). Some of these are sensitive to the rarer (or under-sampled) species, others are most sensitive to the dominant species. All are, however, difficult to interpret and have, perhaps, become less common. It has also been popular to compare parameters of fitted species-abundance curves. Although such curves, along with diversity indices, indicate the balance in

numbers between species, the particular species are ignored. Hence, a change resulting in the replacement of one common species by a rare one may not affect the measure of diversity, but there has clearly been a change in the community which would probably be regarded as beneficial. The measures discussed above can all be given confidence intervals and can be compared using significance tests. If the floras were similar to start with, then diversities can be compared between end-points for each treatment.

If we are to allow for the fact that a species may change its position in the abundance hierarchy, we may decide to use non-parametric rank tests, such as for concordance. By far the most common methods, however, are those under the general heading of multivariate techniques. Marshall (1985), for example, mentions that these will be used on the Boxworth data, and cluster analysis has already been used. Most multivariate methods produce groups of sample units according to the species found in them (and their abundances), either in the form of a table or as a graph on some transformed axes representing the maximum variation between units for that number of dimensions. The most similar units will be grouped together. Programs commonly used at present include TWINSPAN and DECORANA (e.g. Post 1986); statistical packages, such as GENSTAT, have a considerable number of classical multivariate techniques available.

If there is a difference resulting from the treatments (assuming similar initial floras), then it might be expected that a separation of groups may increase with time. Often, however, such analyses are hard to interpret, requiring much subjectivity. Slight effects may not be obvious because of considerable overlap in the scatter of units. The methods of analysis most often used are not capable of giving a quantitative assessment of degree of effect, only a qualitative indication of which units may have become different. The significance of separations of groups of units (given by some external factor, such as herbicide treatment) may be tested using canonical variates analysis (e.g. Chatfield & Collins, 1986, p.153). Here, the linear combinations giving the new transformed axes are selected so that the between-group variation is maximised relative to their within-group variability. Confidence circles may be constructed for the group means in the transformed space.

CONCLUSIONS

The intention of this paper is to highlight problems in the study of environmental effects of herbicide use. The discussion has emphasised that current monitoring programmes, experimental designs and methods of analysis are generally suitable for hypothesis-generation, not for hypothesis-testing. Many of the methods cannot give a quantitative measure of the size of an effect, only a qualitative statement of whether an effect is likely. We began by stating that because of the emotive nature of the subject, unsubstantiated hypotheses are insufficient. Precise statements are needed, and current approaches are usually incapable of these. If such an aim is indeed required, the only solution is to design experiments capable of answering our questions, with replication, randomisation and control of all variables other than those of interest. We cannot state here what they should be, since each will depend on the precise objective. We can, however, argue for far more care and detail. Small numbers of replicates and infrequent sampling will only be able to detect large effects during the course of the study. Small effects, which may not be classed as adverse over that time-scale but which may become serious, will not be

detected.

A great deal of time and money is invested in long-term studies. The setting up of the programmes is critical. It is surely inexcusable to gloss over critical considerations at the start, only to find inadequacies at the end. Detailed base-line surveys of the flora (in far more detail than expected to be used for the duration of the experiment) will allow the selection of an adequate sampling frequency and will help to calculate sizes of effects likely to be detected (e.g. Marshall, in press). Statisticians are trained in such considerations and for the good of the study every attempt should be made fully to involve one at the start of such a project. If the capabilities do then not meet the requirements, a decision can be made at the outset, not after it is too late.

This paper has been somewhat negative and critical, outlining problems without giving solutions and without saying exactly how we would prefer to see things done. In the absence (within the rigid guidelines of herbicides and weed communities) of perfectly designed experiments it is difficult to be other than critical. The examples we have used were not, perhaps, intended to be other than hypothesis-generating by their designers; in this, they have proved excellent value and must be applauded. Boxworth is valuable as an experience in management, such as using thresholds, and has generated hypotheses, but it is not capable of giving conclusive statements on the effects of herbicide use. We now leave the question open as to how to test the hypotheses. Ecological experiments designed to look at effects on an entire ecosystem must inevitably make compromises in their design. Too many compromises and too many objectives in an experiment will inevitably lead to situations in which most of the aims cannot be achieved. Our final warning, therefore, is to beware of such experiments; do not expect too much!

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INTERPRETING THE RESULTS OF TERRESTRIAL ENVIRONMENTAL IMPACT STUDIES WITH REFERENCE TO MODELLING AN ECOLOGICALLY SIGNIFICANT EFFECT.

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ABSTRACT

Before deciding on what methods to use in environmental impact assessment, it is important to consider three things. Which species or ecosystems should be chosen as indicators, what constitutes an ecologically significant effect on them and is this effect acceptable? When predictions of the level of effect (hazard) are made from observations on indicator species the differences in size, activity pattern and life-style between the indicator and other species must be considered in relation to the availability of the chemical in the environment. The long-term effects were examined theoretically. Whether the species survived regular pesticide induced mortality depended on the size of the mortality and the net rate of increase of the species, as did the time to achieve a new equilibrium and the magnitude of this new equilibrium. The nature of the intra-specific competition was only important in determining the level of the new equilibrium. In certain cirumstances the acceptability of such effects can be valued in a risk/benefit calculation but for a majority of cases further work is necessary to determine the true nature of the risks.

INTRODUCTION

Recently, public and regulatory interest in the environmental impact of pesticides has increased resulting in demands for more research. Much of this new research is concerned with perfecting the methods of study, adapting a wide variety of existing techniques to measuring the effects of agrochemicals on non-targets. The variety of these methods is due to the diversity of habitats studied and the personal preferences of the methodologists. Given that there are no "absolute" techniques in biology (or in any other science, according to quantum theory), it is accepted that all methods are to a degree imperfect and so it is often personal preference which dictates their ranking.

Though method development is of the utmost importance, it must not precede the setting of aims or the method itself becomes the objective, regardless of its ultimate utility. This paper proposes that before the merits of competing methods are judged, there are three things that must be considered:-

1) How should the species and ecosystems to be studied be selected?
2) What constitutes an ecologically significant effect on these species or ecosystems?
3) Is this effect on the non-targets acceptable?

Having answered these questions, the research objectives will have been focused to guide the development of the necessary techniques.

SELECTION OF SPECIES AND ECOSYSTEMS

In environmental toxicology the impact of a pesticide is evaluated in a stepwise manner, begining with laboratory studies and progressing through small plots to field experiments where necessary (eg Urban and Cook 1986). During this process, increased realism is gained at the expense of decreased control of variables. It is never possible to test all the species or ecosystems which will be exposed to the chemical and so some selection of 'indicator' species or ecosystems has to be made.

Species

The potential environmental hazard of a chemical to a species is a function of the chemical's intrinsic toxicity and the exposure to it in the field (Johnson 1982). Such hazard can be represented as a percentage effect (mortality or other effect, depending on circumstances) caused by the chemical in use. The quantitative information needed to predict this would be:-

i) the acute, and where necessary chronic, effects of the chemical on the species in the laboratory;
ii) the environmental concentration (residue level and availability) of the chemical to which the species is exposed in the field.

To be of use as a predictor of hazard to other species, the indicator must be of similar sensitivity and route of exposure.

The similarity of the intrinsic sensitivity of an indicator species to a chemical, to the sensitivity of other species is generally determined by taxonomic relatedness.

For many non-targets, regulatory authorities accept data concerning two species from each taxonomic class (eg Mammalia, Aves etc) that might be exposed (eg Bunyan and Stanley 1979). In some cases, such as with the Insecta, much more information will probably be considered, at least to the level of taxonomic order (eg Coleoptera, Lepidoptera etc). Numerous detailed studies show that substantial differences in susceptibility can be found at least between genera within a single family and frequently between sexes of the same species. However, unless these differences exceed an order of magnitude, they are unlikely to produce different measurable effects in the field.

Two aspects of exposure of a species must be considered in relation to its utility as an indicator of direct (Bunyan and Stanley 1983) effects in the field. These are size, activity pattern and life-style.

Size

Even if the intrinsic toxicity of a chemical to a range of species is not affected by the size of the organisms alone, there may be practical problems concerning the mechanisms of entry into and loss from the body of the exposed species which make smaller ones more susceptible. For example, Balcomb *et al* (1984) showed that, in practice almost any exposure of small bird species to carbofuran was fatal, whereas larger species may survive because of both the increased quantity of carbofuran needed to cause mortality and the reversible nature of carbamate poisoning.

Activity pattern and Life-style.

The time during which the chemical is available for the non-target species to be exposed in the field is crucial to the resulting hazard. After application the chemical may rapidly become biologically unavailable to non-targets either through absorbtion into plant material, adsorption onto soil, hydrosoil or organic matter or through degradation soon after application. In such cases laboratory toxicity data alone will overestimate the effects on species not active at the time application, or species which are protected from the chemical by being under cover, in the soil or in the field margin.

Conversely, chemicals which are chronically available in the field, such as those formulated in baits, will be available to a wider range of species and sometimes at locally high concentrations. Under these circumstances, appropriate laboratory toxicity data on suitable laboratory species may be of use in predicting effects to a wider range of species than in the case of the biologically unavailable chemical.

Non-target species which avoid contact with the chemical during application or its subsequent persistence on foliage, the soil etc. can still be exposed at a later date if the chemical has been absorbed by, but not eliminated from, their food. This phenomenon is known as secondary exposure and is relatively uncommon (Hardy et al 1986). Where secondary exposure does occur, it is best not to rely on general indicator species but to carefully examine the diet and behaviour of the species concerned and, where necessary, do experiments involving species at risk as indicators of a smaller specialised group (eg Townsend et al 1981, 1983).

Ecosystems

If trials are to be done to establish the effects of a chemical in the field, it is important that the environment chosen is the one of most utility. This should be the one most representative of the expected use of the chemical. However, for regulatory purposes, it may be necessary to include applications in excess of the usual one to demonstrate a safety margin.

WHAT CONSTITUTES A SIGNIFICANT EFFECT : A SIMPLE MODEL

As proposed in the previous section, environmental hazard will be represented by a percentage effect, usually the additional mortality of the non-target species within a generation. Having determined the percentage mortality, it must be decided whether this is ecologically significant. To do this experimentally is fraught with difficulty and so as a first attempt, a modelling approach is adopted. This allows a range of species with differing population dynamic characteristics to be examined for their sensitivity to a regular pesticide-induced mortality over many generations. By using an analytical model it is not intended to make precise predictions for individual species but to examine the relative importance of basic population dynamic parameters in determining the outcome.

A useful general population model for this purpose is that developed by Hassell (1975). This is applicable to species with minimal overlap of adult generations, typical of many species of temperate non-target arthropods. This model (1) is a non-linear difference equation describing changes in population density by discounting the product of net rate of increase (λ) and population size by a density dependent function.

$$N_{t+1} = \lambda N_t (1 + aN_t)^{-b} \quad (1)$$

where N_t = population density (N) at generation t.
N_{t+1} = population density at generation t + 1
λ = net rate of increase per generation
a = a constant defining the threshold of density dependence
b = a constant describing the intensity of density dependence

This model was orginally introduced to study the effects of "scramble" and "contest" intra-specific competition (Nicholson 1954). When b = 1, the model is representative of extreme contest competition where each successful animal gets all it requires, whilst the unsuccessful ones get insufficient for survival or reproduction (Varley et al 1973), this is typical of territorial behaviour. As b tends to infinity, the model is representative of scramble competition where resources are divided more equally between individuals.

When a pesticide is applied to each generation, a proportion of the non target species (p) are killed. This is analogous to the percentage mortality derived experimentally. The proportion surviving (s) is then:

$$s = 1-p \quad (2)$$

The population at N_{t+1} then depends on whether the pesticide is applied before density dependence acts, in which case it will be:

$$N_{t+1} = \lambda s N_t (1 + asN_t)^{-b} \quad (3)$$

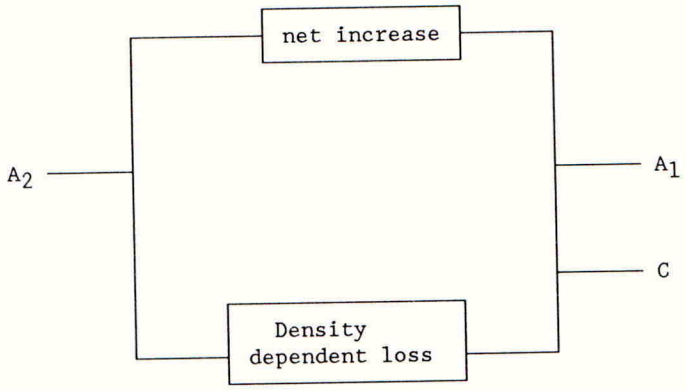
or after it acts, in which case it will be:

$$N_{t+1} = \lambda s N_t (1 + aN_t)^{-b} \quad (4)$$

Classical evidence for the importance of considering the timing of application of a control measure in relation to the onset of density dependence is the lack of control of wood pigeons achieved by autumn shooting (Murton et al 1974). However, time of censusing the population also effects the form of the equation. At point C in Fig 1, the resulting model becomes (4) regardless of the time of pesticide application and so these are the conditions used.

FIGURE 1

The lifecycle of the hypothetical species in the model



A_1 and A_2 are the points at which the chemical could be applied, point C is the censusing time.

The stability conditions for the model can then be found to be:

The species persists if:

$$\lambda s > 1 \qquad (5)$$

The equilibrium population is:

$$\overset{*}{N} = [(\lambda s)^{1/b} - 1] / a \qquad (6)$$

The return time (T_R the number of generations to reach stability) is:

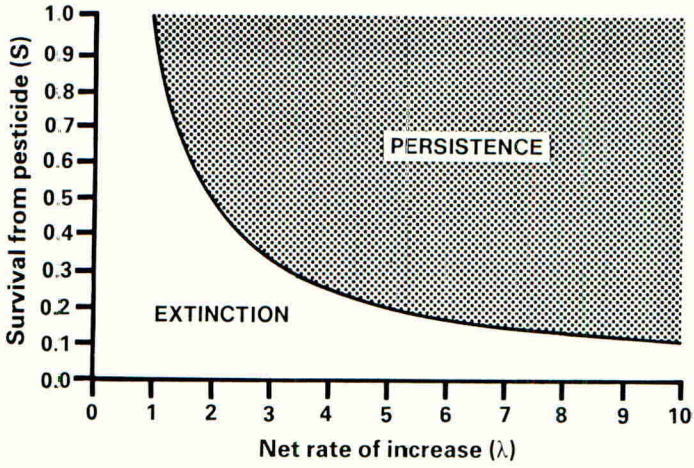
$$T_R = [b(1-(\lambda s)^{-1/b})]^{-1} \qquad (7)$$

Results (5) and (7) are independent of censusing time.

Whether or not the species persists at all following a pesticide-induced mortality in every generation is dependent principally on its net rate of increase and the extent of the mortality (Fig 2) and not on the nature of the intra-specific competition. Similarly, net rate of increase and extent of the mortality affect the time taken for the population to reach its new equilibrium level following the start of regular pesticide induced mortality to each generation (Fig 3) and the level of the new equilibrium. The nature of the intra-specific competition (b) has a trivial effect on the persistence of the species and the return time but is important in the level of the equilibrium population achieved (Fig 4). For any given net rate of increase and survival value, the more territorial the species (lower b) the higher equilibrium population it can maintain.

FIGURE 2

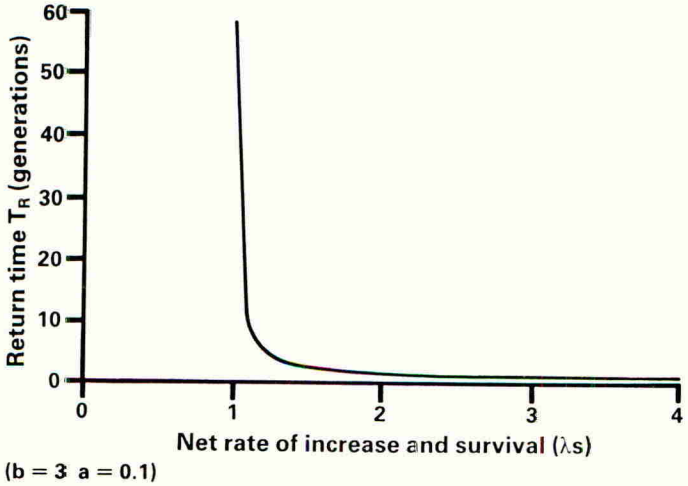
Extinction or persistence of species following pesticide-induced mortality in each generation



A species will survive pesticide-induced mortality in each generation if $\lambda s > 1$

FIGURE 3

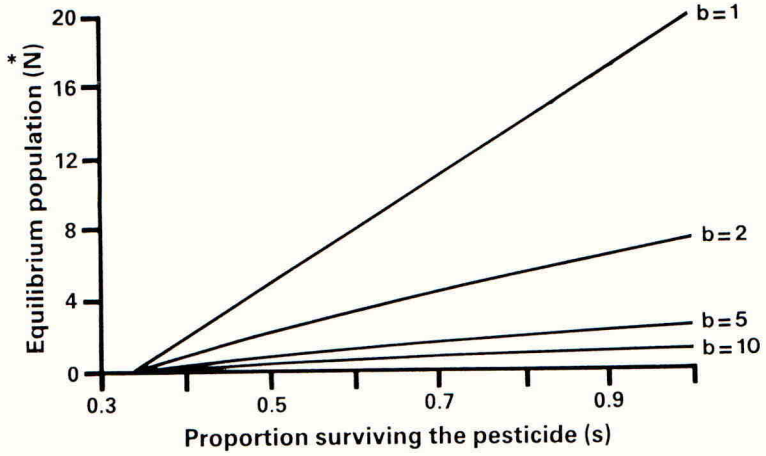
The relationship between return time to equilibrium and the product of net rate of increase and survival



As λs increases, the species reach their equilibrium populations more quickly.

FIGURE 4

How the form of competition affects the relationship between the population at equilibrium and survival of the pesticide at a fixed net rate of increase.



Highly territorial species achieve higher equilibrium populations for a given survival value and net rate of increase.

This model only serves to show how the significance of pesticide-induced mortality to a range of species with differing population dynamic characteristics can be examined. To make firm predictions using this approach, the model would have to be developed to include the effects of overlapping generations and properly parameterised.

In addition to numerical effects on species and communities, it is also valuable to look at effects on the functioning of communities. For example effects on the carbon and nitrogen cycle (Greaves et al 1980) or on predation (eg. Brown et al 1988a).

ARE THESE EFFECTS ACCEPTABLE

One way to judge if the predictions of observed affects on a species or group of species is acceptable is to examine the risk/benefit ratio. If this ratio is high (high risks, low benefits) the effects are unacceptable, if it is low (low risks, high benefits) then the effects probably are acceptable (Brown et al 1988).

Certainly, for the effects of a chemical to be acceptable in terms of environmental impact, the risk/benefit ratio must be less than unity (risks=benefits).

There are two cases in which this approach must be considered.

1) The benefits are in terms of pest, disease or weed control and the risks are in terms of effects on important natural enemies of a pest only. For example, insecticides applied to pome fruit colonised by spider mites and Typhlodromus pyri.
2) The benefits are in terms of pest, disease or weed control and the risks are in terms of effects on a range of natural enemies and non-target species. For example, fungicide use in cereals.

In the first case, it is possible to quantify the ratio as both quotient and divisor can be cast in terms of yield. However, in the second case, though it may be possible to quantify the benefits, the risks can only be partly assessed. Here the ratio becomes a value judgement and further progress cannot be made until the risks to a range of non-targets are better understood. Until then the risk/benefit ratio is a political rather than scientific reltaionship.

In conclusion, as it appears that there are some answers to what indicator species should be used but few as to what constitutes an ecologically significant effect or how this should be valued, it would seem opportune to consider these in more detail as work on methodology progresses.

ACKNOWLEDGEMENTS

The authors acknowledge the advice of Mick Crawley and Charles Godfray.

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MATHEMATICAL APPENDIX

The conditions for a species to persist if $\overset{*}{N} > 0$

$$\overset{*}{N} > 0 \iff [(\lambda s)^{1/b} - 1]/a > 0$$

$$\iff (\lambda s^{1/b}) > 1 \qquad \text{(since } a > 0\text{)}$$

$$\iff \lambda s > 1 \qquad \text{(since } b > 1\text{)}$$

The solution for equilibrium population and return time are based on May (1983).

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DISCUSSION

B. Davis, ITE, Monks Wood Experimental Station: Can we start with a short consideration of what constitutes a significant effect and how we might measure it in agricultural land. We have talked this afternoon of vapour drift of herbicide; should we be thinking about neighbouring crops or about adjacent wildlife? What is a significant effect? I talked earlier about reduction of seed production of non-target plants and their dispersal and earlier to-day we heard about the reduction of spiders in a cereal crop. The title of the symposium as a whole is "Fields methods for the study of environmental effects of pesticides" so, perhaps, we could just think for a minute about what constitutes a significant effect.

P. Jepson: The model you described, Dr Brown, is a great contribution and is extremely positive. However, one thing it does is to emphasise mortality as an effect. You showed very lucidly yesterday, and so did Dr Burn in his work at Boxworth, that the indirect effects that are equally responsible, or perhaps more important, for removal of organisms from an area. Obviously, the analytical model is giving you an excellent indication of whether extinction will occur. However, other factors may result in the removal of organisms from the population. How might you try and integrate those with this approach?

R. Brown: I would consider them to be important but the objective of this exercise was really to elucidate how much mortality can be tolerated in populations of some species. I certainly do not deny that sub-lethal effects and also the indirect effects (which are probably mortality effects on some other species) are important. That is outside the scope of this particular study. I would like to understand what happens in terms of mortality before I try and consider those.

P. Mineau: There are a number of things here that we have problems with. One of them is the assumption that density-dependent loss is important. I am not sure to what extent that is valid.

R. Brown: There is a great debate as to whether you have density dependence. Many are convinced that density dependence is a very important feature of all naturally-occurring events. One issue, and this returns to one of the points made about statistics, is that almost all the studies which looked at density-dependence and which have not found it, have been set up in a limited way. They have not looked at the extremes of what happens. I believe that density dependence is very important but, in terms of birds, I could not claim that this model is realistic because it is strictly in discrete time. It is a first order, non-linear difference equation. Therefore, the numbers that you get at one time are affected only by the numbers that you had the time before and there is no survival through to the next time.

P. Mineau: I am not denying that density dependence processes can, theoretically, be important. However, we have heard speaker after speaker say that with full pesticide regimes in the type of agriculture that you have here, whether in an integrated system or a 'softer' system, you are probably not at carrying capacity, and then density dependence becomes a tricky concept.

R. Brown: The main point to be borne in mind is the non-linearity of density dependence, although I do not necessarily believe in the carrying capacity of the environment being a set ceiling and that things go up to

it. Across the range of density, the intensity of this particular density-dependent effect will vary. One of the points that did not come out clearly enough was that the overriding effect was not actually the density dependent one. The thing that affected most outputs was, quite straightforwardly, the net rate of increase. Density dependence only became important in one of the three things that I looked at, and then it was equally as important as the other factors.