INTEGRATED CONTROL OF APPLE PESTS

G.H.L. Dicker
East Malling Research Station, Maidstone, Kent

Summary

The possibility of integrating biological control of the fruit tree red spider mite (*Panonychus ulmi*) with chemical control of other apple pests is discussed. An effective predator complex is known to exist but its establishment in commercial orchards would depend on the availability of adequate food supplies, which is unlikely to be attained without some increase in the population of pest species. At present a very restricted range of selective insecticides is available, none of which is considered adequate for control of leaf- and fruit-feeding caterpillars.

It is concluded that such an integrated programme would be accompanied by some reduction in quality or yield of the crop but little, if any, reduction in the cost of pest control.

INTRODUCTION

Professor Smith has indicated the broader principles associated with the concept of integrated control. I shall discuss this topic in the more restricted context whereby biological and chemical control are both utilised to maintain pest populations at a level below the threshold of economic importance (Pickett *et al.*, 1946). Furthermore, my comments on this subject will be confined to the pests of apple, since this is the most important fruit crop grown in England. One disadvantage of this choice is that apple possesses the most complicated pest and disease complex of all the tree fruits, which increases the difficulty of developing an integrated control programme. On the other hand, any measures which proved successful for apple should, with slight modifications, also be applicable to other kinds of tree fruits, since the major pests of these crops are very closely related.

Before considering. in detail the possibilities of integrated control it is first necessary to appreciate the factors which influence current opinion on control of fruit pests. The biologist has acquired an extensive knowledge of the life cycles of individual species but still knows relatively little about the factors which control their abundance, and so is unable to predict population trends. The fruit grower has invested considerable capital in a perennial crop and is aware that, apart from yield, the main factor influencing his income will be the quality of the harvested fruit. His attitude towards pest and disease control is also influenced by memories of the magnitude of damage which occurred on occasions when control measures failed. This has led to a situation where sprays are applied annually to protect the crop against the pests which occur most regularly. There is little doubt that many of these are unnecessary, but in view of our inability to predict the occasions when pest populations would be of economic importance this basic spray programme is treated as an insurance policy.

THE PEST AND DISEASE COMPLEX OF APPLE

The pests of apple against which sprays are regularly applied are listed in Table 1. These are broadly grouped to indicate the growth stage of the host plant when populations of individual species are developing and when control measures are usually applied.

Table 1.

The major apple pests in relation to time of appearance

Pre-blossom (April)	Aphids (<u>Rhopalosiphum insertum</u>, <u>Sappaphis</u> (<u>mali</u>))
	Apple sucker (<u>Psylla mali</u>)
	Winter moth (<u>Operophtera brumata</u>)
Petal fall (mid-May)	Apple sawfly (<u>Hoplocampa testudinea</u>)
Post-blossom (June, July)	Fruit tree red spider mite (<u>Panonychus ulmi</u>)
	Codling moth (<u>Cydia pomonella</u>)
	Fruit tree tortrix (<u>Archips podana</u>)

It will obviously be impossible to introduce alternatives to chemical control simultaneously for all pests. Hence the success of an alternative method, using parasites or predators, will depend both on the intrinsic efficiency of the biological agents and on the absence of adverse effects from pesticides needed to control other pests.

Normally, even if biological control is successful a delicate equilibrium exists between the pest and its natural enemies which could be upset by factors having only a slight adverse effect if repeated frequently. Thus, the effect of fungicides, however mildly toxic, which are applied on many occasions each year to control apple scab and mildew, must also be considered.

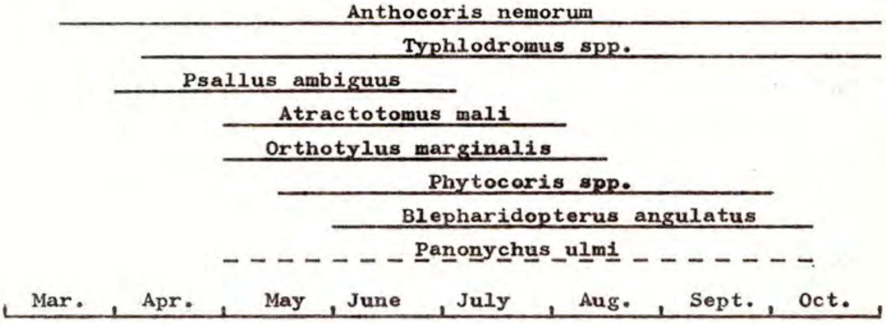
THE EFFICIENCY OF NATURAL ENEMIES

With the exception of the fruit tree red spider mite, all the species listed in Table 1 figured prominently in the older literature (see Theobald, 1909) as being destructive to the apple before regular control measures were practised. On this evidence it would seem that indigenous natural enemies were incapable of providing the consistent and adequate control required to maintain populations of these species below the economic threshold. In contrast, the fruit tree red spider mite became a pest only after a regular spray programme had been employed for some years, and it is the only example in this country of a fruit pest that has developed due to the use of pesticides.

Collyer (1953) showed that, on unsprayed apple trees the mite was a source of prey for many species of insects, mainly members of the families Miridae and Anthocoridae, and also mites of the genus _Typhlodromus_. The overall picture (Table 2) is one in which a succession of species prey on this phytophagous mite throughout its period of active life. The contributions made by individual species have not been assessed but there is considerable evidence (Muir, 1965b) that the black-kneed capsid (_Blepharidopterus angulatus_) is one of the more important mirids and, due to their relatively low food requirements (Chant, 1959) the typhlodromid mites are likely to play a vital role when prey populations are low.

Table 2.

The periods of activity of predators



Under these ideal conditions the fruit tree red spider mite provides an illustration of the classical example whereby populations can be maintained at a consistently low level. It results from the combined action of several species and is therefore less liable to disruption due to fluctuations in numbers of individual species of predator. Furthermore, none of these predators is solely dependent on the fruit tree red spider mite as a source of food; _Anthocoris nemorum_ is a general predator (Collyer, 1967), the typhlodromid mites can utilise pollen as a source of food in addition to preying on eriophyid mites (Chant, 1959), _Psallus ambiguus_ is probably primarily dependent on apple sucker (Morris, 1965), and _Blepheridopterus angulatus_ has been shown to feed on leaf hoppers (Muir, 1965a)

Potentially, therefore, a situation already exists whereby the mite can be effectively.controlled by predators if these can be re-established in commercial orchards. The full complex is unlikely to occur in all areas but it would be desirable to encourage the establishment of as many species as possible, as this would contribute to the stability of the controlling influence and also lead to a reduction in populations of the pest species which are utilised as alternative sources of prey.

FACTORS GOVERNING THE ESTABLISHMENT OF PREDATORS

Due to the regular use of non-selective insecticides the fauna of the average commercial apple orchard is composed of relatively few specimens of a very restricted range of species, most of which are pests. The rate of colonisation by beneficial species will largely depend on:-

(1) the presence in the neighbourhood of a reservoir from which dispersal can take place,

(2) the presence of adequate food to attract and maintain a resident breeding population,

(3) the absence of toxic materials from the immediate environment which could cause excessive mortality.

There is experimental evidence (Muir, 1966) that, on plots of ½ acre in size, B. angulatus can both recolonise and suppress a heavy infestation of fruit tree red spider mite within a period of three years, but this was an extreme case in which mite populations were permitted to develop naturally, irrespective of the injury to foliage, and the availability of an abundant food supply probably contributed to the rapidity of colonisation. In commercial orchards, serious injury to the host would be unacceptable, and regulation of prey populations, providing maximum attraction to predators with minimal damage to the host tree, may not only present serious problems but also extend the period required to obtain an effective population of predators. The mechanism by which typhlodromid mites disperse is unknown, yet these are considered to be an essential component of the predator complex if populations of the fruit tree red spider mite are to be maintained at a low level.

THE AVAILABILITY OF SUITABLE INSECTICIDES

Earlier in this paper the theme has been developed that the establishment of a predator complex in apple orchards would obviate the need for chemical control of the fruit tree red spider mite. The application of this principle to commercial fruit growing is dependent on the satisfactory control of other pests and diseases without disturbing the predator-mite equilibrium.

To what extent do the existing pesticides meet these requirements?

Let us first consider the fungicides as these present few difficulties. Captan, for control of apple scab, has been tested many times without detectable toxicity to either insect or mite predators. Dinocap, for apple mildew, also appears to be acceptable; as any slight toxicity to typhlodromid mites is counterbalanced by its acaricidal action against the fruit tree red spider mite (Muir, 1965c).

The choice of insecticides is not so easy. Those possessing contact action and persistence appear to be unsuitable for foliar application, but possible materials are listed in Table 3. As a starting point, from which improvements can be introduced as knowledge increases, either menazon or nicotine could be used against aphids and lead arsenate for control of winter moth. Paradoxically, the application of systemics via the bark or roots, to avoid the risk of contact action, may so enhance their efficiency that aphids and apple sucker would be eliminated and this would militate against their use, since the absence of these essential sources of food would prevent establishment of early predators such as Psallus ambiguus and Anthocoris nemorum. A similar effect may arise from the use of mevinphos, together with some mortality of typhlodromid mites, the majority of which have resumed activity by the green cluster stage

Table 3.

Some insecticides for an integrated control programme

Aphids	menazon	) nicotine, systemic	)
Apple sucker	γ-BHC	) via bark or roots	) mevinphos
Winter moth	lead arsenate		)
Apple sawfly	nicotine, γ-BHC		
Codling moth	lead arsenate, ryania		
Fruit tree tortrix	?		

(Niemczyk, 1965), when it is anticipated that such a spray would be applied.

For control of apple sawfly the choice would lie between nicotine and -BHC. Muir (1965c) could detect no deleterious effect from either of these insecticides in an experiment where the predators consisted only of *Typhlodromus pyri* and *B. angulatus*.

The most difficult problem will be to obtain satisfactory control of codling moth. This occurs at a time when the population of predators is approaching its maximum and mortality from insecticides must be avoided. The most suitable chemicals, lead arsenate and ryania, are of similar but only moderate efficiency, and the results of field trials indicate that infestations are unlikely to be reduced by more than 70% (Chiswell, 1962). Since biological efficiency and selectivity are of the same order the choice will be governed largely by price, at 15/- and 60/- per acre/application respectively for lead arsenate and ryania.

The absence of an effective insecticide for control of codling moth can be offset to some extent by introducing supplementary methods. Geier (1964) has emphasised the importance of available sites where mature larvae can spin cocoons. On old trees these occur in abundance under flakes of bark but on younger trees are restricted to cankers, cracks in stakes, and the tree ties. Attention to this aspect could possibly contribute materially to a reduction in populations of codling moth.

The attraction of artificial habitats to mature larvae has been exploited in the past by attaching bands of corrugated paper or sacking to the trunks of trees. There is some evidence that the former is preferable as the larvae are more accessible to birds, mainly tits, which utilise this source of food during the autumn and winter, and thus eliminate the need for human labour to remove the bands.

For control of fruit tree tortrix no insecticide is known which combines selectivity and biological efficiency.

THE ECONOMICS OF INTEGRATED CONTROL

The dependence of predators on an adequate and continuous supply of prey implies that the fauna of orchards under integrated control will be greater, both in the number of species and of individuals, than occurs where a non-selective spray programme is used. Certain components of this fauna will be represented by pest species, which will inevitably cause some reduction in either quality or yield, but the magnitude of these effects cannot at present be estimated.

In contrast, the costs of pesticides for the respective programmes can be accurately calculated. Similar fungicides can be used in each programme, and as all insecticides can be applied in combination with fungicides the costs of application will also be similar. At current prices the annual cost/acre for insecticides is approximately £6/10/- and £9/-/- respectively, for the integrated and normal programmes. To the former must be added the cost of any additional measures (e.g. tree bands) which may be required to reduce the damage caused by codling moth.

In general, it may be concluded that, with the insecticides available at the present time, the annual cost of pest control will be of the same order for each programme.

CONCLUSIONS

With the present emphasis on fruit quality, and as non-selective insecticides can provide satisfactory control of all the major apple pests, the fruit grower is unlikely to accept alternatives unless these are equally reliable or confer additional benefits. Integrated control, as outlined in this paper, does not yet meet these requirements.

A natural predator complex, capable of suppressing populations of the fruit tree red spider mite, is known to exist, but the mechanism by which these species may become established in commercial orchards without unduly increasing losses due to pests needs further study. Certain other advantages would accrue from the establishment of a resident population of general predators, as these would also prey on pest species other than the mite, and thus supplement the control obtained by selective insecticides, some of which may not be characterised by high biological activity. In addition parasites may increase, especially those of codling and fruit tree tortrix moths.

The most obvious shortcoming is a lack of suitable selective insecticides, especially for the control of leaf-feeding and fruit-infesting caterpillars. In its proposed form, integrated control is unlikely to result in reduced costs for the spray programme, and the possible hazards to vertebrates and invertebrates from the increased use of lead arsenate may attract the attention of toxicologists and conservationists, although residues on the harvested crop remain below the tolerance levels.

Major changes in a spray programme may, unwittingly, result in the removal of factors which previously had limited the population increase of a minor species. On apple, this could possibly occur with the common green capsid (Lygus pabulinus) which has been reported as a local and sporadic pest. In addition, three species which were listed as important pests in earlier years have so far been omitted from this discussion. Two of these, apple blossom weevil (Anthonomus pomorum) and apple capsid (Plesiocoris rugicollis) were eliminated from commercial orchards by DDT nearly twenty years ago and reinfestation has not occurred. Both species depended mainly on apple for their food supply and populations are now at such low levels that rapid reinfestation is unlikely. Even if control measures became necessary the evidence suggests that these would be required at very infrequent intervals and hence would not invalidate the development of integrated control. Populations of the third species, woolly aphid (*Eriosoma lanigerum*), have remained at a low level in all experiments carried out at East Malling in connection with integrated control, from which it could be inferred that natural enemies can effectively limit numbers of this species on the apple varieties in commercial use at the present time.

Finally, two factors could have a decisive influence on the future of integrated control. The continued and extended use of carbaryl for fruit thinning would introduce such a highly toxic element that many predators would be eliminated. On the other hand, the frequency with which resistance develops in populations of the fruit tree red spider mite may lead to a situation where effective acaricides were no longer available and another method of control became essential.

References

CHANT, D.A. (1959) Can. Ent. Suppl. 12, 5.
CHISWELL, J.R. (1962) J. hort. Sci. 37, 313.
COLLYER, E. (1953) J. hort. Sci. 28, 246.
COLLYER, E. (1967) Proc. R. ent. Soc. Lond. (A) 42, 107.
GEIER, P.W. (1964) Aust. J. Zool. 12, 381.
MORRIS, M.G. (1965) Entomologist 98, 14.
MUIR, R.C. (1965a) J. appl. Ecol. 2, 31.
MUIR, R.C. (1965b) J. appl. Ecol. 2, 43.
MUIR, R.C. (1965c) Rep. E. Malling Res. Stn for 1964, 167.
MUIR, R.C. (1966) J. appl. Ecol. 3, 269.
NIEMCZYK, E. (1965) Rep. E. Malling Res. Stn for 1964, 180.
PICKETT, A.D., PATTERSON, N.A., STULTZ, H.T. and LORD, F.T. (1946) Sci. Agric. 26, 590.
THEOBALD, F.V. (1909) Insect Pests of Fruit.

THE SCOPE FOR INTEGRATED CONTROL IN ENGLAND AND WALES: REPORT ON A N.A.A.S. STUDY

by D. W. Empson
National Agricultural Advisory Service, Cambridge.

In April 1967, entomologists of the National Agricultural Advisory Service of England and Wales held a two-day conference on "Integrated Pest Control". The aim was to consider to what extent their work, during the next ten years or so, should be influenced by the concept of multiple control methods. They were assisted by the Ministry of Agriculture's Plant Pathology Laboratory, invited research workers, and by members of the other Advisory Services of the United Kingdom.

After an introductory talk by Prof. J. S. Kennedy, F.R.S., President of the Royal Entomological Society of London, the subject was discussed in five syndicates, each dealing with a group of agriculturally related crops. The reports of these syndicates cover the scope for integrated control and its advisory implications, and make suggestions for further investigational work.

The full reports will be published in the spring of 1968 in the Journal of Applied Ecology. Some of the main conclusions and recommendations are discussed below.

THE NEED FOR INTEGRATED CONTROL

There were two main reasons for a general feeling that the development of integrated control was essential. The first concerned the question of wild life and the dangers of contaminating the environment with pesticides. This was stressed particularly for grass and cereal crops, which together occupy 90% of the cultivated land. Secondly, multiple control permits a reduction of the selection pressure exerted by any one method, with a consequent reduction in the probable rate of the development of resistance. The importance of a change to less persistent insecticides was seen as much from the point of view of reduced selection pressure as from that of reduced hazards to wild life.

The syndicate that discussed cereal pests had a special reason for wishing to limit the use of pesticides. It feared the uncontrolled increase of some organism at present relatively insignificant - a "red spider of agriculture". For this reason it recommended that chemical treatment of "minor" pests (aphids, gall midges, leaf miners etc.) should be discouraged unless and until it is clear that a species constitutes a real and continuing threat to yield or quality.

PEST TOLERANCE

Integrated control implies the presence on the crop of a population of the pest, albeit at a low level. It was pointed out that tolerance is exceedingly low for those pests that damage the quality of the final product. The vegetable syndicate stated that "little or no outlet exists for produce showing even small amounts of damage or pest infestation". The syndicates dealing with top fruit and glasshouse crops both stressed the demand for unblemished produce.

Low pest tolerance was seen as the most serious barrier to the general acceptance of the "integrated" approach.

AVAILABLE CONTROL METHODS

Each syndicate discussed at length the pest control techniques now available.

Chemicals. The advantages of limited persistence and high selectivity were stressed. The economic difficulties of developing and marketing highly selective pesticides were recognised, and there was a greater interest in using fairly broad-spectrum chemicals in a selective way. The vegetable syndicate, for example, pointed out the advantages of seed dressings, and the need for machinery capable of the very accurate placement of insecticides.

Because of the damage to beneficial insects and mites, the top fruit syndicate suggested that the use of insecticidal winter washes, and of carbaryl as a fruit thinning agent, could not be included in any integrated control programme.

The importance of fungicides was mentioned, and collaboration with plant pathologists was considered essential. For example, there is little prospect of using biological agents against red spider mite on roses under glass while the use of sulphur continues to be necessary.

Cultural Control. The reports contained little about such subjects as crop rotation, manuring, cultivations and the time of sowing. Much of this is "taken as read" by N.A.A.S. entomologists who have always, so far as possible, integrated chemical and cultural controls. It also reflects the realisation that cultural methods, for example adopting a rotation that foregoes an acreage of a profitable crop, are not necessarily less expensive than other pest control techniques.

Plant Breeding. All syndicates saw the production and use of resistant or tolerant plants as one of the most hopeful lines of attack. Resistant varieties, however, must be in no way inferior to the varieties they are to replace.

Biological Control. Only the glasshouse syndicate thought mainly in terms of introduced species of parasites and predators. They stressed the need for considering the pest complex as a whole, and not trying to deal with a single species in isolation. They also pointed out the need for much entomological guidance if biological control is to be attempted, either alone or integrated with chemical control, and the need for more facilities if the work is to develop.

The other syndicates were concerned with the protection and encouragement of native predators and parasites. This generally meant the avoidance of persistent chemicals, the use of the minimum effective dose, and the achievement of selectivity by careful timing or placement. Natural enemies could be harmed by certain fungicides, and by the over-enthusiastic use of herbicides. Herbicides, indeed, were viewed with some suspicion from more than one point of view. Apart from removing flowering weeds, which provide nectar and alternative prey for many beneficial insects, herbicides may be associated with increased populations of nematodes.

Opinions were divided on the question of shelter belts and hedgerows. They would undoubtedly provide more parasites, predators and pollinators, but they might also provide more pests.

Sterilization. It was considered that sterilization, by physical or chemical means, held considerable promise for the control of a number of pests, particularly wheat bulb fly, cabbage root fly and carrot fly. Before possible methods could be considered, however, there was a need for much more information about many aspects of the biology and ecology of these species.

Insurance. Quite small amounts of wireworm damage to potato tubers can lower the saleable value of the crop. The vegetable syndicate thought that there was a greater use of insecticides to prevent this than was justified by the true saving involved. They asked that serious consideration should be given to the possibility of a scheme for financial insurance against wireworm damage to ware potatoes, so that the use of insecticides against low wireworm populations could cease.

RESEARCH NEEDS

The syndicates made many suggestions for investigational work. These ranged from fundamental studies of population dynamics to the search for plants with genes conferring resistance to pest attack, and included such complex studies as the inter-relationship of various pest and disease organisms. Because the need for integrated control is urgent, and little progress can be made without more knowledge, the need for research is also urgent.

Particular importance was attached to assessing the damage caused by many species of pests - especially where there is some tolerance because they do not directly affect the quality of the final product. These include the "minor" pests of cereals, together with aphids and red spider mites on a number of crops. The successful forecasting of pest attacks could also lead to a more logical and economical use of pesticides.

THE SCOPE FOR INTEGRATED CONTROL

The urgent need for multiple control methods was accepted by each syndicate. The main difficulty was seen to lie in the many instances where pest tolerance is very low. Because the pest complex of the crop must be considered as a whole, a single pest to which tolerance is low may limit the development of integrated control of all pests on that crop. Except perhaps in the case of wireworm damage to potatoes, there was no suggestion for the reduction of the present high quality standards of the final product.

Within this limitation, however, much can be done to avoid, or at least reduce, the employment of persistent chemicals, and to use pesticides as selectively as possible. At the same time, and with the co-operation of plant pathologists and herbicide specialists, the use of other pesticides might be made less harmful to beneficial insects and mites.

The greatest need is for more information, particularly biological information. There are exciting prospects of new methods of pest control by the use of attractants and sterilants, and by subtle manipulation of pest populations. But all these need to be based on fundamental biological and ecological knowledge, which at the moment is sadly lacking.

It is under glass that most progress has been made towards introducing biological control, and integrating it with chemical control. Syndicates had been asked to list their recommendations under the headings "Advisory Work", "N.A.A.S. Investigations" and "Research Projects". The glasshouse syndicate found this tidy arrangement almost impossible, because of the close inter-relationship of all three aspects. Successful integrated pest control will require not only the combination of different control methods, but also the integration of research, development and advice.

N.A.A.S. entomologists see no immediate prospect of a revolution in pest control methods. Indeed they see little that is really new in this concept of integrated control, for they are used to viewing the pest in the context of the crop, and the crop in the context of the farm as a whole. Being citizens and biologists, they also see the farm in the context of the countryside. But above all, they see pest control as a problem of long-term economics. If they stress the biological dangers of reliance on pesticides alone, it is because they fear the ultimate consequences on the productivity of our farms. As Prof. Kennedy reminded us in his opening address, we in the N.A.A.S. have an axe to grind. It is that of agricultural production - now and in the future.

THE COMMERCIAL AND INDUSTRIAL IMPLICATIONS OF INTEGRATED CONTROL

D. Price Jones
I.C.I. Ltd., Jealott's Hill Research Station, Bracknell, Berkshire.

Summary The development of integrated control systems in the field of crop protection carries many implications for the pesticide industry. Given time, the need for more selective chemicals can be met, though not to the point of extreme selectivity. Restrictions on dosage rates, discouragement of calendar schedule spraying and prophylactic treatment where the context permits, should tend to diminish the growth rate of pesticides and ultimately lead to some kind of equilibrium. The pesticide industry as a whole is sufficiently flexible to adapt itself to changing requirements, but the production side of the industry will become even more dependent on world markets if the adoption of integrated control methods is accelerated. The development of integrated control implies integration with the economic fabric of our civilisation; it cannot be considered in isolation.

INTRODUCTION

The concept of integrated control, although essentially an old one, has not been actively promulgated in its present form for longer than about 10 years. Even this period is long enough for some effects on the pesticide industry to become apparent. It is the purpose of this paper to examine the possible implications of integrated control in the context of the pesticide industry, including its ramifications, and, if possible, to attempt to define the attitude of the industry.

The commercial and industrial implications of integrated control must in principle be very much the same in all industrial countries - certainly in those with a developed agriculture and a sophisticated attitude towards health and outdoor recreation. Nevertheless, to give substance and definition to my subject I must confine my remarks mainly to the United Kingdom.

THE REQUIREMENTS FOR AN INTEGRATED CONTROL PROGRAMME

In the establishment and maintenance of an integrated programme, involving the use of chemicals, certain practices are recommended, though it is recognised that their complete adoption is rarely feasible.

1. The use of selective chemicals
2. Minimal use of the chemicals concerned. This implies:-
   minimal dosage rates
   minimum numbers of applications
   avoidance of calendar schedule spraying
   avoidance of prophylactic treatment
   spraying only when necessary.
3. Accumulation of data relating pest populations to damage levels and particularly the establishment of maximum tolerable damage levels.
4. The acceptance of lower grade standards in marketed produce to facilitate the maintenance of reservoirs of parasites and predators.
5. The use of trained staff or independent consultants to assess pest situations and advise what and when action should be taken.

It is of some philosophical interest that most discussions about integrated control have, in relation to man, been outward looking. That is to say they have in essence been more concerned with man's environment than with man himself. I would like to redress the balance here by insisting that any description of the tenets of integrated control should include the two following principles:-

6. That any control system devised should as far as possible eliminate toxic

hazards to man.

7. That such control system should give due cognisance to the economic structure of our society.

THE STRUCTURE OF THE PESTICIDE INDUSTRY

Before going on to discuss in detail the implications of integrated control systems to the pesticide industry, it is necessary to give a brief description of the structure and economic significance of the industry.

The Ministry of Agriculture, Fisheries and Food (1967) lists more than 700 approved products in commercial use in the United Kingdom (Table 1). While many of these, of course, are only brand names or formulations of a more limited number of active ingredients, there still remains a nucleus of more than 130 different biologically active compounds in use for crop protection purposes in this country. Of these, 47 are classifiable as insecticides, 26 as fungicides and 42 as herbicides (the actual numbers are slightly higher, but not precisely definable).

Table 1

The U.K. pesticide industry: some statistics

Annual sales (e.g. 1966) £26·5 million

Officially approved products (1967):		
Branded products, total:		over 700
Biologically active ingredients,	total:	130
" " "	insecticides:	47
" " "	fungicides	26
" " "	herbicides	42
Firms with approved products:		69
Firms engaged in wholesale distribution:		about 900

In 1966, sales of all pesticide products in the U.K. exceeded £26,000,000, with insecticides at £7,000,000 and herbicides at about £15,000,000 (Board of Trade, 1967, Table 2). Until a few years ago the insecticide sales exceeded herbicides, but the general picture in the U.K., as in many parts of the world, is of herbicides taking the lead, and in fact far outstripping other classes of pesticide.

Table 2

Sales of pesticides in U.K., 1961-66

	Sales by larger manufacturers (£ million)			
	Total, all products	Insecticides	Fungicides	Herbicides
1961	14·6	5·8	3·2	4·7
1962	15·3	5·4	3·0	6·1
1963	18·4	6·0	3·6	7·8
1964	20·4	6·4	3·7	9·3
1965	22·6	6·1	3·6	12·0
1966	26·5	7·0	3·7	14·8

In this sense, despite the admittedly higher biological significance of insecticides, the vastly greater usage of herbicides may yet prove to be a dominating factor in the development of integrated control systems.

It is extremely difficult to define pesticide companies precisely, but again it is convenient to refer to Approval Scheme statistics, according to which the 700 odd products are produced and/or formulated by 69 different chemical firms. Many of these have major chemical interests, but some of the smaller firms are largely or entirely dependent on pesticides. Purely for discussion purposes one can assume that no more than 6 or 8 large firms enjoy 90% of the trade and a much larger number of small firms share the remainder. Changing the attitude of the industry as a whole would therefore resolve itself largely to a reorientation of the attitude of those relatively few larger firms.

In considering the structure of the pesticide industry in the U.K., it is essential to recognise that the outlet for crop protection chemicals in this country is too small to warrant the heavy costs involved in research and development, to which the industry is heavily committed. A world market is an essential prerequisite for such activities.

On the distribution side, the industry is much more complex. Pesticides are sold mainly through the agricultural merchants and the size of this sector may be gauged from the fact that the National Association of Corn and Agricultural Merchants have 2,200 member firms in England and Wales and that of these, 900 handle crop protection products. In Scotland and Northern Ireland the trade channels are a little different and potato merchants play a much more important role. Many of these agricultural merchant firms handling pesticides have technical staff with some training in the use of pesticides. They are often involved in discussing control measures with farmers and frequently in determining spray control programmes. Many of these crop protection officers are given training courses during the winter by some of the larger chemical firms. They are also in almost day-to-day contact with the representatives of those chemical firms. Finally, the Institute of Corn and Agricultural Merchants is responsible for an extensive educational programme (Putnam, 1966). There is, therefore, a very considerable technical service organisation diffused throughout the pesticide distribution sector.

SOME COMMERCIAL AND INDUSTRIAL IMPLICATIONS

Referring to the basic requirements for an effective integrated control programme, it is legitimate for the purposes of this discussion to omit those items concerned with produce grade standards and economic damage levels, as they are only obliquely relevant. The other items, however, are of direct and overriding importance.

Selectivity

Selectivity in the context of integrated control implies either "inherent" selectivity (a convenient but scientifically indefensible term) or the use of a non-selective chemical in a selective manner - for example by exploiting systemic properties. As the problem commonly arises in connection with insecticides, it is convenient to examine it in this context. There are said to be more than 600,000 species of insects, distributed over a wide range of taxonomic groups and habitats, and that some 2,000 species are economically important pests. In the U.K., more than 100 species or groups of species are listed as economically important and recommendations for their control are given (Martin, 1963). The ultimate in selectivity would of course be the use of more than 100 different chemicals, each one strictly specific in action. This would be absurd.

The next stage is to recognize the existence of a relatively homogeneous group, such as the aphids. The vast majority are sufficiently akin in physiology and habits to render them susceptible to the same mode of attack. Their economic status makes them a worthwhile commercial target. Phytophagous mites are almost in the same category. In both cases, beneficial (predatory) insects are included in nearly-related groups, thus complicating the position slightly. Nevertheless, materials with a good measure of selectivity are available for aphids and for mites.

One of the major problems facing the development of the selective approach to insect control is the existence of pest complexes on many important crops. In particular, the dominant role of caterpillars and beetles in many pest complexes implies the urgent need for specific materials for these particular pests. A few materials with some of the desired properties are beginning to penetrate commercial practice.

So much for the general concept of selectivity in insecticides. What are the implications to industry?

These are much more complex than would appear at first glance. In the first place, it follows that increasing selectivity would imply a diminished demand for any one product and perhaps an unsatisfactory return on the research and development costs incurred. This could well be so if the early onset of resistance were to terminate the commercial life of the product. There is, however, the hope - by no means a certainty - that resistance would develop only slowly, or not at all, to an effectively selective material. This hope is diminished by the threat of cross-resistance. On the whole, therefore, the reaction of the pesticide industry is likely to be to restrict the search for selective compounds either to groups of insects such as aphids, phytophagous mites or caterpillars, or to specific pests of outstanding importance, e.g. codling moth, cotton leaf worm (Prodenia litura), cotton boll weevil, and to adopt a very cautious attitude towards the development of any selective materials discovered.

Minimal dosage rates

Current recommended dosage rates for the control of specific pests are usually well above the minimum rates which can be applied under ideal conditions. Such increased rates are necessary to take care of the varying conditions encountered in practice, including of course some slight deviation from the recommended dose. In practice the recommended dose is such as to ensure that the failure rate is small enough to be tolerated financially and to be amenable to servicing through the normal complaints channels. It follows, therefore, that a widespread reduction in current recommended dosage rates without a corresponding tightening of the control of application could well render the sale of pesticides impracticable.

The other implication is perhaps the more obvious one. Reduction of the usage of a material must necessarily increase the cost per unit of the smaller quantity actually employed. This cannot but have repercussions not only on the pesticide firms, but also on the commercial user.

Avoidance of calendar schedule spraying and prophylactic treatment

Calendar schedule spraying and prophylactic treatment have had a two-fold origin. They are a direct response, on the one hand, to the requirements of management, i.e. a simple schedule of operations that can be easily followed, and on the other to the need for a steady off-take of pesticides from the chemical firms. It follows that the avoidance of calendar schedule spraying and of prophylactic treatments, such as seed treatment, may well have serious repercussions on both the pesticide industry and the commercial user. In this context it is useful to bear in mind that it takes a minimum of a month to get a product formulated, checked, packed and distributed to the grower. This month is considerably longer than the time available for the operation of many warning schemes. In practice, of course, commercial producers may need far longer than one month's notice.

Spraying only when necessary

With the provisos already made for calendar schedule and prophylactic treatments, I can only agree that chemical treatments should be applied only when necessary. Adherence to this policy should have no harmful effect on a healthy pesticide industry.

The use of trained staff or independent consultants

I have already indicated that within the pesticide industry there are representatives of both the producing firms and the distributing organizations qualified to advise on pest control problems. It may be argued that as they have products to sell their advice may be somewhat biased. While this may occasionally be true, in my own experience this advice has been largely impartial. Continued good relations demands impartial advice.

It is interesting to record that in the herbicide field in recent years sales techniques have divided into two distinct channels. In the one, products are sold together with the technical service required by the grower; this service includes an evaluation of the weed problems and advice on which crops to spray. In the other, similar materials are sold, generally at a lower price, but without any technical backing. It is significant that the former technique has gained greatly in favour. This seems to indicate that the grower really appreciates good technical advice.

In integrated control discussions, recommendations have been made that technical service on crop protection matters should be given by independent consultants, of whom there are perhaps a dozen or so in England and Wales. There is, of course, a great deal to be said in favour of this system. It should be borne in mind, however, than an independent consultant must at times be faced with very difficult decisions - often with inadequate data - and may be obliged to decide in favour of treatment, not only in the grower's interest, but also in his own professional interest.

Reduction of human toxicity hazards
Integration with human economy

I deliberately include these requirements among the principles of integrated control for two reasons. One, as already stated, concerns the need to ensure that man himself is fully considered in assessing a particular pest control situation.

The second is that they enable me to indicate how the pesticide industry has already reacted to some of the implications of integrated control. In this respect reference to the vast system developed for the control of toxic residues in food material can prove illuminating. Given the initial stimulus (new information on potential hazards), industry reacted by providing highly sensitive techniques for residue determination, residue data for pesticides and their metabolites under a wide range of conditions, withdrawal of products from uses where residues constituted a potential hazard and finally the provision of new materials with toxicologically insignificant residues. This is a clear demonstration of the ability of the pesticide industry to respond to new and exacting requirements.

The interdependence of pesticides and our economy is indicated by the gradual withdrawal of certain organochlorines from commercial use in the U.K. and elsewhere. The conduct of such operations has been determined by the need to find substitute materials. Similarly, parathion, despite its highly toxic nature and its application hazards, is still used on an immense scale because of its cheapness. Undoubtedly, it will be replaced in due course, but probably only when alternative, safer materials approach it in cheapness.

INTEGRATED CONTROL IN ITS BROADEST APPLICATIONS

So far I have dealt solely with the integration of chemical into pest control practice. I now want to say something about the broader concept of integrated control - the integration of all appropriate control measures - and to comment on its special significance to the chemical industry. Here I must confine myself to insecticides, where the issues are already alive and where, according to one's point of view, the position is either promising or threatening.

Control measures, excluding those involving conventional insecticides, may still be divided into chemical and non-chemical. Among the chemical measures, microbial insecticides are not far removed from conventional insecticides and any major developments in this field would have relatively little effect on the pesticide industry as a whole, although the effect on individual firms could vary greatly, mainly in proportion to their pharmaceutical interest. Other chemical approaches include insect growth disorganisers, behaviour determinants and chemosterilants. For many years to come these are going to be small volume chemicals, probably highly priced in comparison with conventional insecticides and with a sales and usage structure quite unlike that of current products. Here the implications relate to sales by tender to official or co-operative organisations, or, in some cases, to "package deals" in which production is linked with application backed by a comprehensive technical service.

The non-chemical control measures carry implications of a different character. The establishment of crop varieties resistant to particular pests could, for example, erode the field available to conventional pesticides or, in some cases, as envisaged by Stakman (1959) in discussing stem rust of wheat, chemical measures may still be necessary to prolong the life of a resistant variety. Developments in biological control appear currently to be at least partly complementary rather than wholly antagonistic to conventional insecticides. There are still further possibilities in cultural control and also in the application of physical or mechanical techniques, but many of these are still on the horizon; some may never get any nearer.

To me it would seem that the biggest single implication to the chemical industry lies in the gradual transference of spheres of influence to official, officially-sponsored, and perhaps even international, organisations (Price Jones, 1967). To exemplify this trend, I need only cite the growing and legitimate interest of the U.S.A. Food and Drug Administration in an avowedly free-enterprise country, and of similar bodies in many other countries. W.H.O. and F.A.O. are playing a leading role on an international scale. As we have just heard, F.A.O. have recently established a series of working parties to co-ordinate various research and administrative aspects of integrated control. Without detracting from the potential contributions of these working parties, I suggest that their immediate significance lies in the way they signal the establishment of a truly international intervention in crop protection.

CONCLUDING REMARKS

In discussing the commercial implications of integrated control, I find that I cannot isolate any insect control system from the economic environment in which it operates. This is only a slight extension of the more usual ecological concept, but it is an extension that is all too frequently overlooked. Man's innate interest in wildlife may have origins in his distant past and may currently be mediated by the complex behavioural demands of civilisation, whether urban or rural; it is certainly a strongly motivating factor. But in the last analysis his wildlife interest must take its place as one of a number of competing factors. Judgments, decisions may be crude and must inevitably carry political inflexions, but they are in all essentials economic decisions.

The role of pesticides therefore in the development of integrated control methods is determined largely by economic considerations. If a particular chemical is required by the prevailing economic system it will be retained; if a certain non-chemical method is economically more desirable it will be adopted. Against this background, the pesticide industry with its elastic structure is well able to adjust to the changing demands of crop protection. Let us also remember that the time scale on which developments in integrated control should be measured is long in relation to the intervals normally involved in industrial investment planning.

References

BOARD OF TRADE (1967) Business Monitor, Production Series: Pesticides and Allied Products, October - December 1966. H.M. Stationery Office, 1967.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (1967). Agricultural Chemicals Approval Scheme: List of Approved Products 1967 for Farmers and Growers. H.M. Stationery Office, 1967.

JONES, D. PRICE (1967). Chem. & Ind., 13 May 1967 (19), 770.

MARTIN, H. (Ed.) (1963). Insecticide & Fungicide Handbook. Blackwell, Oxford.

PUTNAM, M. E. (1967). Proc. 8th Br. Weed Control Conf., 3, 742.

STAKMAN, E. C. (1959). Proc. 4th Int. Cong. Crop Prot., Hamburg, 1957, 1, 1.

A STUDY ON THE EFFECTS OF DDT APPLICATIONS AGAINST *PIERIS RAPAE* ON THE CROP FAUNA

J. P. Dempster
Monks Wood Experimental Station, Abbots Ripton, Huntingdon.

The effects of spraying a crop with a non-selective and persistent insecticide, such as DDT, are complex and are often long lasting. In this paper I shall attempt to show the range of effects that can result from the use of DDT, taking my examples from a four year study on the control of *Pieris rapae* on brussels sprouts.

Broadly speaking the effects of an insecticide on a species may be divided into two. Firstly, there are the direct lethal and sublethal effects of the poison on the species itself; and secondly, there are the indirect effects of the chemical on some other organism which is affecting the numbers of the species in question (eg. a natural enemy, a competitor or a food species.). It is often difficult to separate direct and indirect effects of the insecticide without a considerable knowledge of the ecology of the species. Nevertheless, the examples that I shall give will, I think, show some of the factors determining the overall effect of the pesticide.

DDT is chemically one of the most stable insecticides used in agriculture, but its effective persistence differs in different parts of the crop habitat. The plants within the crop grow rapidly during the summer and produce new leaves which are free from the insecticide. This means that many plant living species can build up in numbers after spraying, since they will not come into contact with the chemical. Thus, *Pieris* caterpillars which feed mainly on the newly expanded leaves and within the heart of the plant, can build up in numbers again on the crop within 4 - 5 weeks of spraying.

Much of the DDT put on as a foliage spray finishes up in the surface layers of the soil and here it is so persistent that successive applications can accumulate. Thus 6 applications of DDT to a sprout crop, over a period of 3 years, amounting to a total of 477g pp'DDT has resulted in 159g in the top 6 inches of a heavy clay soil at Monks Wood Experimental Station. Species living in the soil, or on the soil surface, are then at greater risk from the insecticide than are many of the plant living species, and generally it is within the soil that the biggest changes take place.

Different species differ considerably in their sensitivity to DDT. As will be seen in Fig. 1, the accumulation of DDT in the soil caused the progressive elimination of millipedes from the sprayed area. Some other groups of animals such as the spiders and phalangids are also very sensitive to the spray, but owing to their greater mobility they are able to recolonise the sprayed area each year after cultivation when the surface concentration of DDT is diluted. It will be noticed, however, that fewer tend to return each year, as the chemical builds up in the soil. The data in Fig. 1 refer to a small plot, of ¼ acre, at Monks Wood, where there is a large area of unsprayed land from which many species can recolonise the sprayed crop. In a more typical agricultural area, with smaller areas of unsprayed land, this recolonisation is less marked.

Even within one taxonomic group there can be considerable differences in the effect of the insecticide. Thus, *Bembidion* spp. have been reduced each year by the spray but have recolonised the plot after cultivation. Another similar sized ground beetle, *Trechus quadristriatus*, has on the other hand, progressively built up in numbers on the sprayed plot. (Fig. 2)

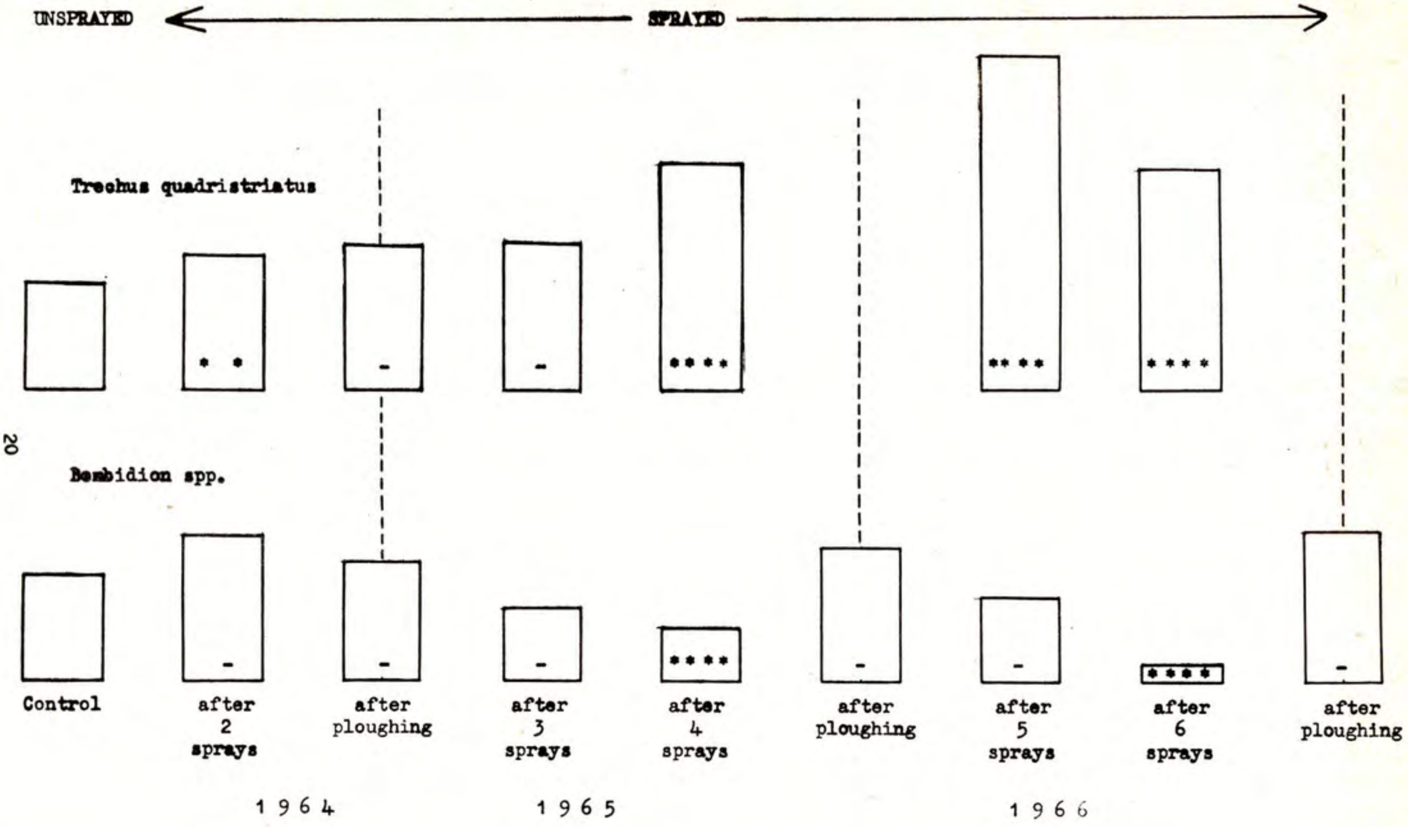


Fig. 2. Changes in the ratio of the numbers of <u>Trechus</u> and <u>Bembidion</u> on sprayed and unsprayed areas.
(- = not signif., * - p= 0.05, ** - p= 0.02, *** - p= 0.01, **** - p= 0.001)

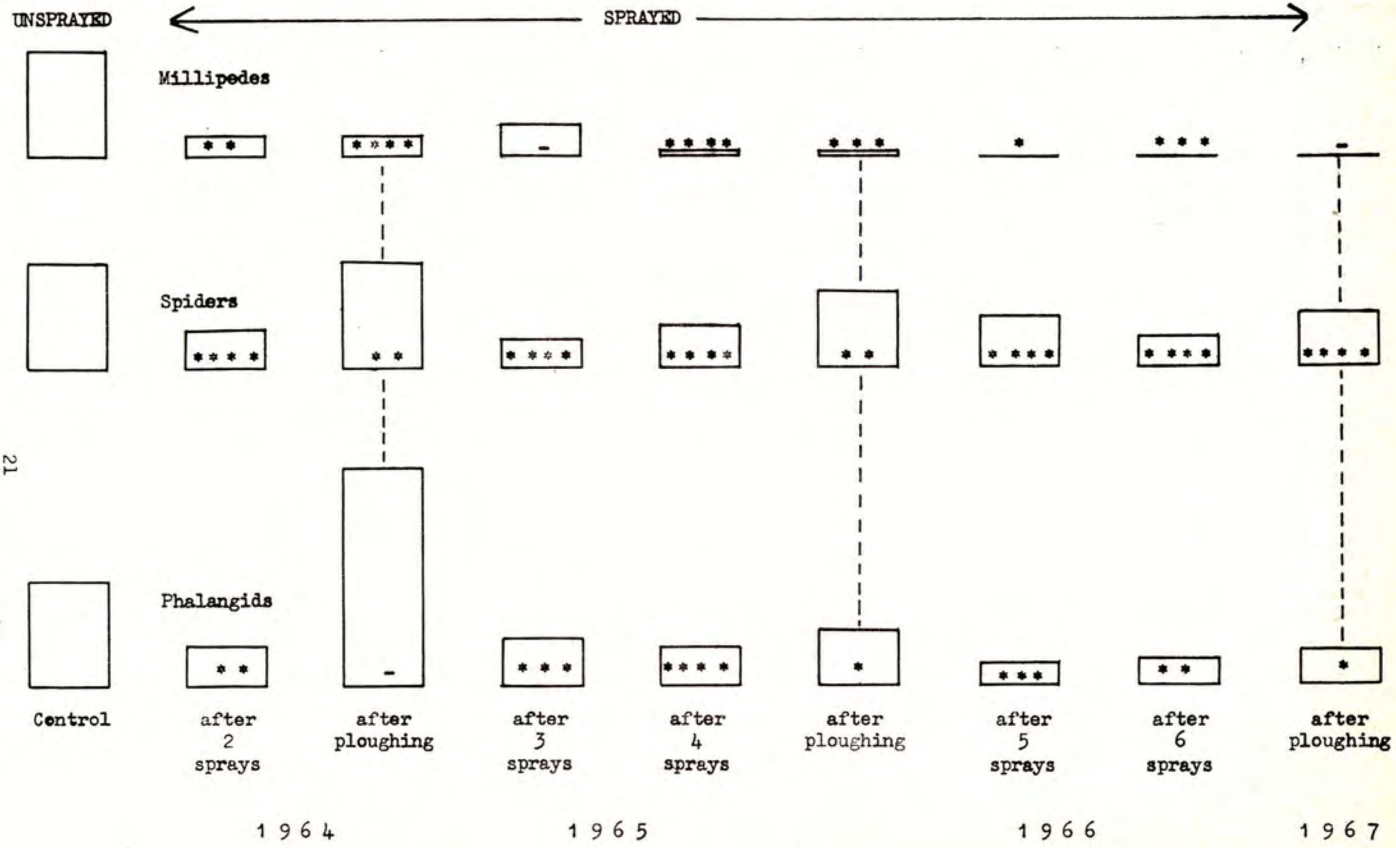


Fig. 1. Changes in the ratio of the numbers of Millipedes, Spiders and Phalangids on sprayed and unsprayed areas. (- = not signif., * - p= 0.05, ** - p= 0.02, *** - p= 0.01, **** - p= 0.001)

Different stages in the lifecycle of a species may differ in their susceptibility to the spray. For example, the adults of the ground beetle *Harpalus rufipes* are not markedly reduced in number by spraying with DDT, but its larvae are. Similarly, on the sprout plants the number of Syrphid larvae are reduced primarily because the adult flies lay fewer eggs. The larvae themselves are very resistant to the spray.

It will be seen then that the direct effect of DDT on the numbers of any one species depends upon the toxicity of the chemical to that species, the extent to which the species comes into contact with the chemical, and the powers of recolonisation of the species when the effect of the insecticide is reduced by either plant growth, cultivation or chemical breakdown.

Different elements of the fauna are interdependent so that the changing of the numbers of one species inevitably has repercussions on other species. This can be seen from some of the spectacular changes in the soil fauna following the use of DDT. A well known example is the upsurgence of Collembola, which at least in part is a result of the elimination of some of the predatory mesostigmatid mites (Sheals 1956). Little work has been done on the soil fauna in this study, but Collembola numbers have increased tremendously on the sprayed crop (Table 1). This has had an effect on the numbers of two ground beetles (*Trechus quadristriatus* and *Nebria brevicollis*). Both of these feed primarily on Collembola and are themselves relatively resistant to the DDT, so that their numbers have built up over the 3 years on the sprayed plot. This is shown for *Trechus* in Fig. 2. The numbers of both larvae and adults of *Nebria* have increased on the sprayed plot. Also the rate of growth of the larvae has been significantly higher on this plot, owing to the abundance of food (Table 2). Unlike *Nebria*, the larvae of *Trechus* are probably not feeding primarily on Collembola and their number has not increased on the sprayed plot. In this species the high adult numbers probably result from immigration. This is then an example of an indirect affect of DDT since it is acting on the food supply of these two beetles.

Table 1.

The number of Collembola on sprayed and unsprayed crop

	Mean no. per m^2 to depth 5 cm.		Signif. diff. of means
	Unsprayed	Sprayed	
Autumn 1965	448	11,115	$p = < 0.001$
Autumn 1966	2,735	8,557	$p = < 0.001$

Table 2.

The rates of growth by Nebria larvae 1965-6

	Mean length of body (mm.)		Signif. diff. of means
	Unsprayed	Sprayed	
December	4.31	6.52	$p = < 0.001$
January	6.68	7.52	$p = 0.01-0.02$
February	8.36	7.99	$p = > 0.10$

The interdependence of different species inhabiting the crop can be seen again from the effects of DDT on the pest itself (*Pieris rapae*). DDT gives a very good control of the caterpillars of *Pieris* which are present on the crop at the time of spraying. Control is, however, short lived since new leaves free from DDT are produced as the plants grow. The insecticide appears to have little effect on egglaying by the adult butterflies, so that the pests' numbers can build up again after a few weeks. In this experiment DDT was applied at 0.7 lb a.i./acre against the peak of each of the two generations of Pieris and it was found that the mortality of the young caterpillars of the second generation was markedly reduced in all three years, following an application of DDT against the first generation (Fig. 3). The bulk of the difference in survival between plots was due to the effect of the spray on the Arthropod predators of Pieris. These regularly account for 50-60% of the young caterpillars of this pest (Dempster 1967). Those predators living on the plants were all reduced in number by the spray. In all 3 years, the anthocorids and spiders were reduced by about half over the whole season, but syrphid larvae were more resistant. These plant-living species had little effect on Pieris, however, compared with some of the ground-living species, which climb the plants at night and feed on the caterpillars. Two species were of particular importance at Monks Wood, the harvestman Phalangium opilio and the ground beetle Harpalus rufipes. Phalangium spends the day in the litter under the plants and only becomes active at night. Harpalus adults also spend much of their time in the surface layers of the soil, while its larvae are confined to the soil. As might be expected both species have been affected by the accumulation of the insecticide in the soil. Phalangium has recolonised the area to some extent each year after cultivation, but with successive sprays the number doing so has been reduced. The adult numbers of Harpalus have not been affected significantly, but this is probably mainly due to immigration, since its larval numbers have been reduced on the sprayed plot. Laboratory studies have also shown that concentrations of DDT, far lower than is required to kill adult Harpalus, greatly reduce their rate of feeding (Dempster in press). In other words, although its numbers may not have been affected, the sublethal effect of the insecticide probably reduced its value as a predator.

Of the insect parasites of Pieris, Apanteles rubecula was eliminated each year from the sprayed crop. Apanteles glomeratus and two tachinid parasites were less affected. These are only of minor importance compared with the predators and during this study they have never accounted for more than 10% of the caterpillars. Added to this, they only kill their hosts when they have become large caterpillars or pupae; whereas the arthropod predators kill the young caterpillars before they have done any appreciable damage to the crop.

In all years, the incidence of granulosis virus disease was higher amongst the caterpillars on the sprayed than on the unsprayed crop. This suggests that sublethal doses of DDT may increase the susceptibility of Pieris to this disease.

Indirect effects of the sort that I have described are likely to occur to a greater or lesser extent with the use of any non-specific poison. DDT has, however, the additional disadvantage in being extremely persistent. This not only means that a longer period of time is required for other animals, including the pest's natural enemies, to recolonise the crop, it also results in the gradual accumulation of the pesticide in the surface layers of the soil. Soil and crop fauna are not independent of one another, as we have seen in the example of Pieris, important predators spend much of their time within the soil under the plants. In this case the persistence of the insecticide adds greatly to the chance of resurgence of the pest. In fact experiments carried out this year show that the mortality of Pieris caterpillars can be significantly reduced simply by the presence of DDT in the soil, that is with no foliage application of the insecticide at all.

Persistence of the insecticide is also the cause of many of the other changes in the crop fauna. Most species are sufficiently mobile to recolonise the crop after spraying, as can be seen from the data for spiders, phalangids and Bembidion (Figs. 1 and 2). Repeated applications of DDT can however, accumulate to such an extent that recolonisation is prevented.

Even the sublethal effect of DDT on Harpalus is dependent on the persistence of the insecticide. The rate of feeding by Harpalus adults immediately returned to normal when they were removed from contact with the DDT.

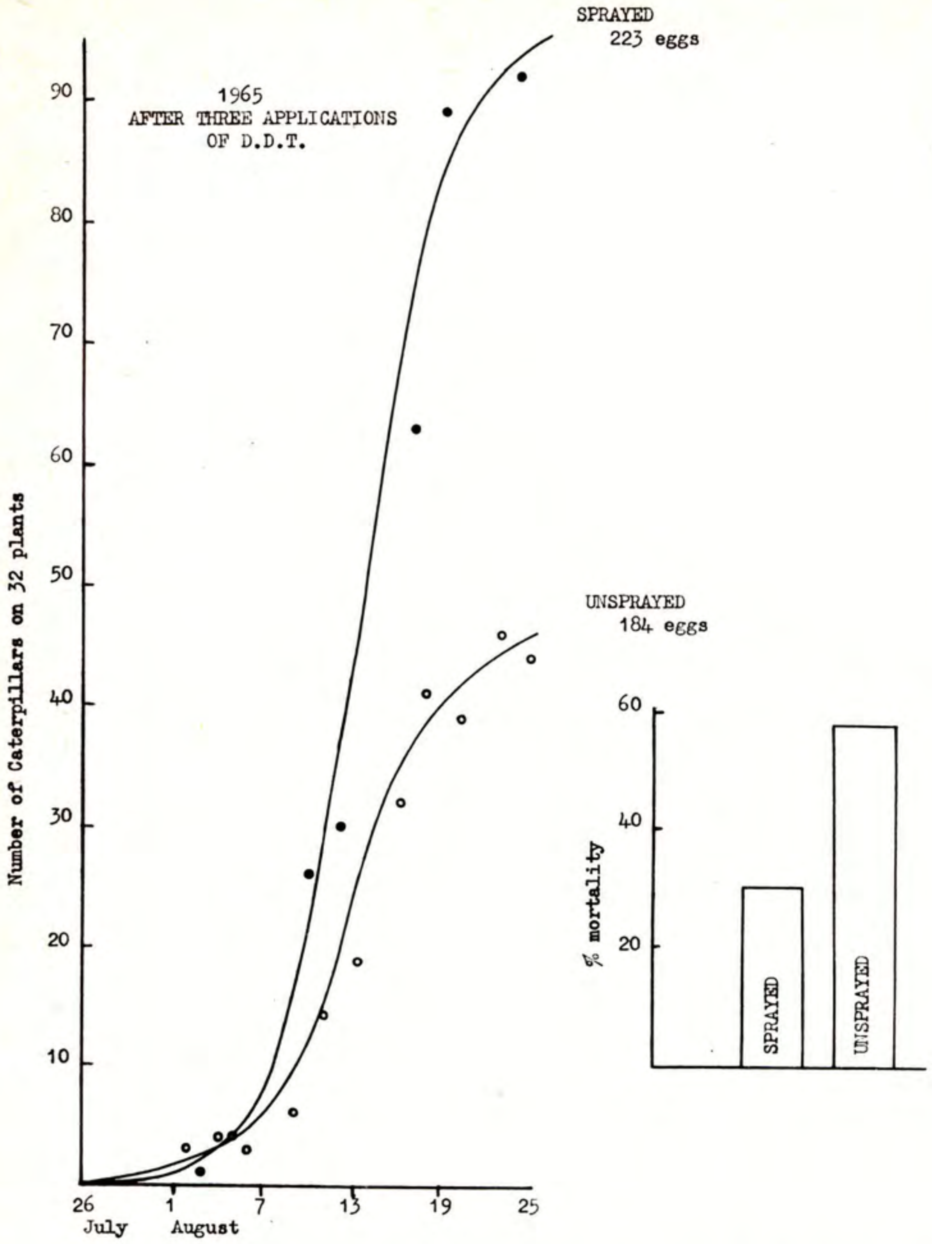


Fig. 3. **Pest population size and survival rate on sprayed and unsprayed areas.**

I have only been able to mention a few of the changes which have taken place in the crop fauna following the use of DDT. The examples that I have given show, however, that the considerable changes that occur are not due simply to the non-specificity of DDT, but also to its persistence. Clearly every effort should be made to produce more specific poisons for pest control, but many harmful effects would be avoided by the use of chemicals which breakdown rapidly.

References

DEMPSTER, J. P. (1967). J. Appl. Ecol. 4, 485.

DEMPSTER, J. P. (in Press). Entomologia exp. appl.

SHEALS, J. G. (1956). Bull. Ent. Res. 47, 803.

INVESTIGATIONS INTO POSSIBLE EFFECTS OF ORGANOCHLORINE INSECTICIDES ON WILD PREDATORY BIRDS

Ian Prestt
Monks Wood Experimental Station, Abbots Ripton, Huntingdon.

In 1964, following the voluntary ban on most uses of aldrin, dieldrin and heptachlor, work was started at Monks Wood Experimental Station on predatory birds. Its main purpose was to provide further data for the Advisory Committee on Pesticides and Other Toxic Chemicals, when they reconsidered the situation in 1967. Birds of prey and two (mainly) freshwater fish-feeding birds, the Heron *Ardea cinerea* and the Great Crested Grebe *Podiceps cristatus*, were selected for study because analytical data already available showed them to contain (on average) the highest residues present in wild birds in Britain (Moore & Walker 1964). Also several reports indicated recent declines in some species (Ash 1960, Ratcliffe 1963, Cramp 1963).

The research has been directed along four lines: (i) post-mortem examinations and chemical analyses, to establish the cause of death, incidence of disease and level of residues present; (ii) the collection and examination of existing historical information on numbers and distribution, to assess the reported recent declines in populations and evaluate possible causes; (iii) initiating, in cooperation with the British Trust for Ornithology, national surveys of the present distribution of common birds of prey, to provide a base-line for future studies of population changes; and (iv) studies of local populations and their breeding success, to provide detailed information on population changes, the means whereby residues are obtained and information on possible sub-lethal effects.

Post-Mortem Examinations

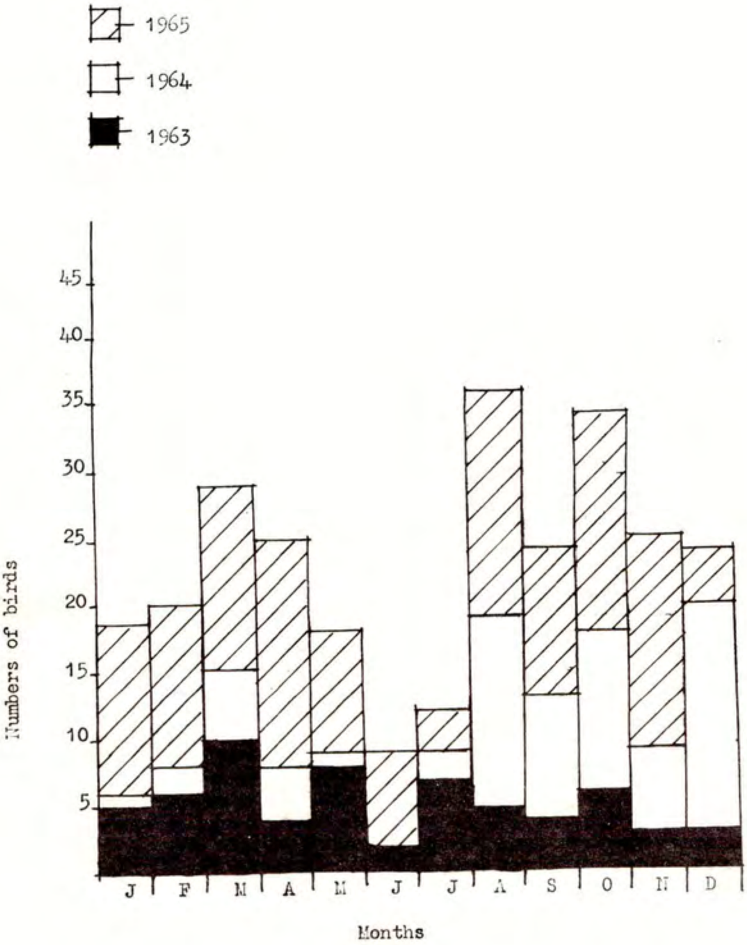
Specimens were obtained from all parts of Britain, mainly through members of the Royal Society for the Protection of Birds. A notice was published periodically in their quarterly journal 'Birds' requesting any predatory birds found dead or dying, together with information on the circumstances.

The Kestrel *Falco tinnunculus*, Barn-Owl *Tyto alba*, Tawny Owl *Strix aluco* and Sparrow-Hawk *Accipiter nisus*, in that order, accounted for most of the specimens received. Figure 1 shows the total numbers of these four species received each month during 1963-1965. In all years there was a peak in Spring and Autumn, with the months of March, August and October being outstanding. The Autumn peak is to be expected, reflecting mortality of juveniles; the March peak may represent the culmination of the effects of winter food shortage. The greater numbers received in the second half of 1964 and throughout 1965 were mostly of Kestrels and Barn-Owls, and reflect the cyclic fluctuations recorded for these species in certain parts of the country (Parslow 1967).

The examination of specimens was carried out at Monks Wood Experimental Station and also, on our behalf, at the Houghton Poultry Research Station (Agricultural Research Council) and the Lasswade Veterinary Laboratories of the Ministry of Agriculture, Fisheries and Food. Excluding possible deaths from insecticide poisoning, as confirmation of these is still a matter for doubt, the results from the 268 autopsies performed to date showed that the probable terminal cause of death was as a result of injury in 89 (33%), of disease in 50 (19%), of shooting in 21 (8%), and was not established in 108 (40%) of the cases.

The similarity of the pattern of the receipt of specimens in all three years, and the results of the autopsies accounting for the immediate cause of death as something other than insecticides in over half the specimens, suggest the sample is not particularly biased towards birds containing high insecticide residues. Indeed, as could be anticipated, a large number of the birds were found injured at the roadside.

Fig. 1. Showing the numbers of specimens of Sparrow-Hawks, Kestrels, Tawny Owls and Barn-Owls received each month from 1963 to 1965.



It can be assumed, therefore, that the residue data will provide a satisfactory indication of the range of residues present in predatory birds in Britain.

Chemical Analysis

Most of the specimens were analyzed, on behalf of the Nature Conservancy, by the Laboratory of the Government Chemist. Extraction and clean-up followed the method of de Faubert Maunder et al. (1964), followed by gas liquid chromatographic estimation of the pesticides using both silicone and 'Apiezon' columns. Confirmation of some results was possible by a paper or thin-layer chromatographic method.

The following number of specimens have been analysed to date :-

Falconiformes	Tissues	Eggs
Golden Eagle Aquila chrysaetos	6	54
Sparrow-Hawk Accipiter nisus	37	60
Peregrine Falcon Falco peregrinus	6	30
Kestrel Falcon Falco tinnunculus	119	26
Other species (total)	33	66
	201	236
Strigiformes		
Barn-Owl Tyto alba	75	3
Tawny Owl Strix aluco	58	5
Other Owl species	36	6
	169	14
Heron Ardea cinerea	91	100
Great Crested Grebe Podiceps cristatus	15	14
Grand Total	476	364

The tissue most commonly used in the analyses was the liver. The results from analyses of 459 livers and 364 eggs showed the following numbers to contain residues of the four most commonly occurring organochlorine insecticides or their degradation products:-

	dieldrin	pp'-DDT	pp'-DDE	pp'-TDE	isomers of BHC	hept. epoxide
No. of livers with residues > 0.01 ppm	445	100	454	125	109	307
% total examined	97%	22%	99%	27%	24%	67%
No. of eggs with residues > 0.01 ppm	354	127	362	102	125	257
% total examined	97%	35%	99%	28%	34%	71%

It is evident that in predatory birds residues of dieldrin and pp'-DDE are almost universally present and those of heptachlor epoxide are widespread. Most specimens, however, contain only relatively small residues of any particular compound. An indication of the levels of dieldrin, pp'-DDT and heptachlor epoxide found in the livers and eggs of six of the commoner raptorial birds in Britain is given in figures 2 and 3.

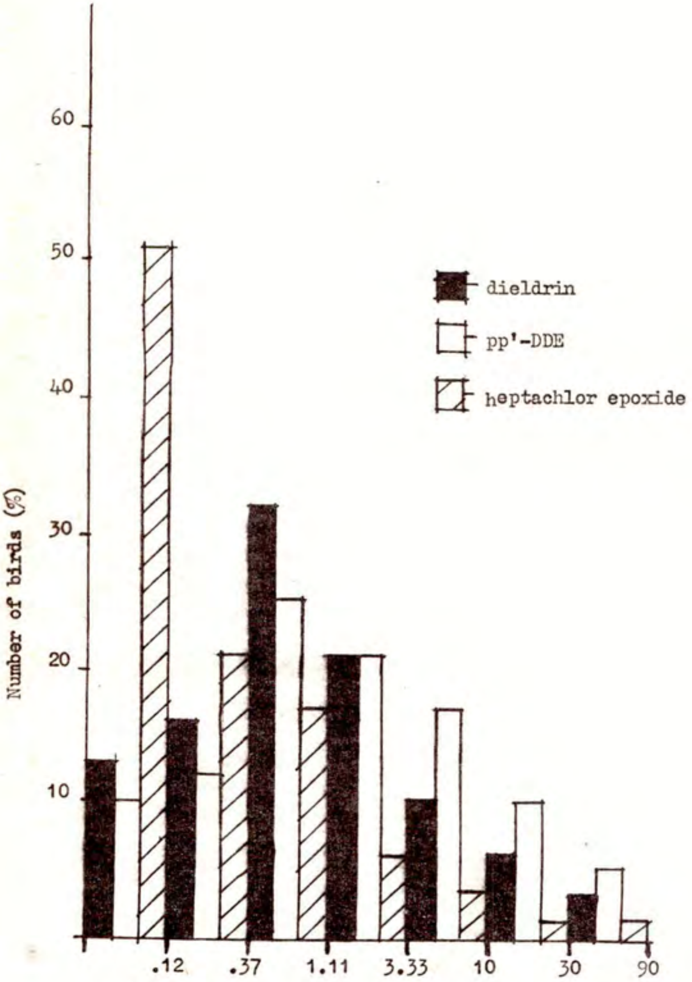
In both the eggs and livers the residues of heptachlor epoxide present are smaller than those of dieldrin and pp'-DDE, with almost three quarters of the specimens, of both livers and eggs, containing less than 0.37 ppm of heptachlor epoxide. In contrast the most commonly occurring liver residues of dieldrin and pp'-DDE lie between 0.38 to 3.33 ppm. The commonest dieldrin egg residues also lie between these same limits, but those of pp'-DDE are slightly larger. These larger pp'-DDE residues come from the eggs of the Sparrow-Hawk and Peregrine Falcon and are in marked contrast to the smaller residues of this compound present in the eggs of the other four species.

Robinson (1966) provided similar histograms for dieldrin and DDT-type residues obtained from analyses of a wide range of species of wild birds in Britain, in which only small numbers of birds of prey were included. Robinson's data showed 35% to have dieldrin residues of less than 0.12 ppm and 39% to have DDT-type residues of less than this figure. Figure 2 of this paper, however, shows only 13% of the raptorial birds to have residues less than 0.12 ppm dieldrin and only 10% with less than this of pp'-DDE, confirming that higher residues are usually present in birds of prey. Further confirmation of the higher residues present in birds of prey is provided by an examination of the total organochlorine residues present in a range of bird species analysed by the Nature Conservancy between 1963-1965 (Moore 1965). Out of 75 liver specimens of species other than predatory birds, only two contained a total higher than 4.0 ppm; whereas 54 of the 105 predators examined possessed residues greater than this.

Experimental data confirming lethal levels, or dangerous sub-lethal residues, of these compounds in wild avian predators is lacking. Moore (1965) suggested as a working basis that residues of 10 ppm of dieldrin and 30 ppm of pp'-DDT in the liver could be used as approximate indications of lethal concentrations. Robinson (1966) used those figures in his discussion of the effects of an organochlorine insecticide on wild birds. Jefferies and Prestt (1966), from post-mortem studies of a small number of wild Peregrine Falcons and some trained Lanner falcons *Falco biarmicus* concluded that a combined level of 5.2-9.3 ppm of dieldrin and heptachlor epoxide residue levels in the liver was probably indicative of death by poisoning by these compounds of larger falcons. More recently Jefferies (pers. comm.) has provided experimental confirmation that a Kestrel, killed by dieldrin, contained a residue of just over 12 ppm dieldrin in its liver.

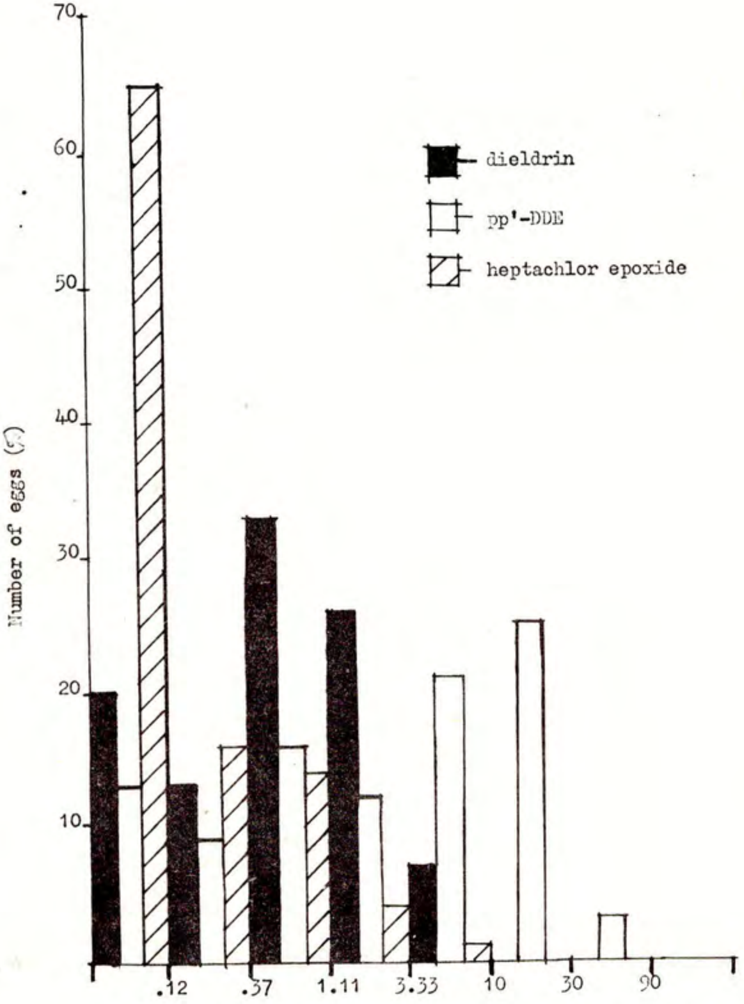
If residues of more than 10 ppm of dieldrin or heptachlor epoxide, and more than 30 ppm of pp'-DDE, are taken to represent high residues, the following numbers of specimens of each species are found within this category:-

Fig. 2. Showing the level of residues of dieldrin, pp'-DDE and heptachlor epoxide in the livers of Peregrine Falcons, Golden Eagles, Sparrow-Hawks, Kestrels, Barn-Owls and Tawny Owls.



Concentrations of 3 different organochlorine insecticide residues (ppm) in 6 species of raptorial birds

Fig. 3. Showing the level of residues of dieldrin, pp'-DDE and heptachlor epoxide in the eggs of Peregrine Falcons, Golden Eagles, Sparrow-Hawks, Kestrels, Barn-Owls and Tawny Owls.



Concentrations of 3 different organochlorine insecticide residues (ppm) in the eggs of 6 species of raptorial birds

	No. examined	dieldrin >10 ppm	pp'-DDE >30.0ppm	hept. epox. >10.0 ppm	Combinations of at least 2 of the other 3 categories	Total
Kestrel	116	12	3	1	2	18
% of total examined		10%	3%	1%	2%	16%
Barn-Owl	75	3	1	1	1	6
% of total examined		4%	1%	1%	1%	8%
Sparrow-Hawk	44	1	6	0	1	8
% of total examined		2%	14%		2%	18%
Tawny Owl	45	1	1	0	0	2
% of total examined		2%	2%			4%
Total	280	17	11	2	4	34
% of total examined		6%	4%	1%	1%	12%

If the birds in the next lowest category of the histogram (3.33-10.0 ppm dieldrin or heptachlor epoxide and 10.0-30.0 ppm pp'-DDE) are also included, as these levels are still relatively high when compared to other bird species and may therefore represent a potential hazard, there would be an additional 43 birds at risk giving a total of 77 (29%). It should be appreciated that all these analyses were made after the 1962 voluntary ban on dieldrin seed-dressings had come into force, and most were made after the more extensive bans in 1964 on aldrin, dieldrin and heptachlor.

Recent Changes in Populations

Ratcliffe (1963, 1965), after completing a survey of 600 (84%) of the known Peregrine Falcon nesting territories in Britain, confirmed that this species had undergone an unprecedented decline in numbers in the late 1950's. The decline started in southern Britain and eventually resulted in the virtual extermination of the Peregrine Falcon as breeding species in England and Wales.

Following an enquiry, conducted by means of a questionnaire sent to ornithologists throughout Britain, Prestt (1965) concluded: (i) the Sparrow-Hawk (like the Peregrine Falcon) had suffered a serious decline over most of England and parts of Scotland and Wales in the late 1950's; by 1960, from being a common widespread bird, it had almost disappeared as a breeding bird in the eastern half of England; (ii) about the same time the Kestrel had suffered a serious decrease in numbers in eastern England; and (iii) the Barn-Owl had decreased generally, but this had been occurring over a longer period. In contrast, during the same period, the Tawny Owl did not appear to have decreased and the Carrion Crow Corvus corone, Magpie Pica pica and Jay Garrulus glandarius were increasing. A fairly steady increase in numbers of Great Crested Grebes was also found to have taken place in Britain since the previous national census in 1931 (Prestt & Mills 1966); and the British Trust for Ornithology's national census of heronries (Stafford pers. comm.) showed no exceptional decrease in the British Heron population during the late 1950's.

The recent decreases recorded in the populations of Peregrine Falcon, Sparrow-Hawk and Kestrel contrast with the lack of change in numbers of the Tawny Owl and for the two fish-feeding predators, the Heron and the Great Crested Grebe. Surveys of the Sparrow-Hawk in study areas in different parts of Britain are being continued. These have shown a small but significant increase in 1966 and 1967 in the number of pairs in Cumberland, Anglesey and Surrey, but not in Yorkshire, Suffolk or mid-Anglia. A possible small recovery of the Peregrine in one area in England was recorded in 1967 (Ratcliffe pers. comm.). Objective data on the Kestrel are still being analysed, but a subjective assessment suggests a small recovery in 1967 in some parts of eastern England.

Changes in Breeding Success

Continuing field studies of the Sparrow-Hawk (Prestt in prep.) and Peregrine Falcon (Ratcliffe 1965 & in prep.) have revealed a serious decrease in breeding success, with up to half the pairs present in many areas failing to rear any young. In many cases the failure resulted from the parent birds destroying their own eggs. Lockie and Ratcliffe (1964) also found a similar decrease in breeding success, involving egg breakage, in the Golden Eagle; and current studies of the Heron (Prestt & Bell in prep. & Milstein et al. in prep.) have confirmed that in two Lincolnshire colonies some of the breeding Herons are destroying their own eggs. A detailed report by Ratcliffe (1967) on the egg breakage has shown that it appears to be a new phenomenon. It was first observed commonly in the late 1940's, at the same time as the eggshells of the Peregrine, Sparrow-Hawk and Golden Eagle underwent a decrease in weight (data on any possible change in eggshell weight in the Heron are not yet available). A remarkable aspect of these observations is that while prior to 1945 the eggshells of these species showed only a limited variation in size/weight ratio, after 1947 almost every egg obtained (apart from a few Peregrine Falcon eggs from the Scottish Highlands and Golden Eagle eggs from the eastern Highlands) was lighter than the previous 'normal' weight. Almost the entire British populations of these birds must, therefore, have been affected in a short period of time.

Discussion

The studies on predatory birds being carried out at the Monks Wood Experimental Station are primarily designed to find the cause, or causes, of the declines in numbers and breeding success that have occurred recently in certain species, and to establish whether or not certain uses of persistant organochlorine compounds could be a contributory factor; they will also provide information on the movement and persistence of these compounds in the terrestrial and aquatic environments. The main difficulty of the work arises from the fact that it is impossible to recreate in the laboratories the complex ecological circumstance existing in the field, particularly when the species deserving most attention are birds like the Peregrine Falcon and Golden Eagle. As a result laboratory experiments have largely been confined to other bird species such as domestic poultry, feral pigeons or Japanese Quail Coturnix coturnix japonica, selected solely because of their amenability to laboratory conditions. Therefore when, for example, Robinson et al. (1967) report laboratory experiments in which pigeon and Japanese Quail tolerated dieldrin residues considerably in excess of those found in dead wild birds thought to have died from dieldrin poisoning, it is difficult to know how pertinent these findings are to field conditions. Indeed, experiments in progress by Jefferies at Monks Wood have shown the activity and condition of a predatory bird at the time of the experiment can be crucial to the results, the innactive birds possessing considerably higher residues in their tissues than the active ones. It is also the case, however, that the necessarily limited data obtained from most field studies can often do no more than suggest a cause and effect relationship or provide a correlation with known events.

The main conclusions from the work to date are: a) that residues of several compounds are present in most predatory birds and their eggs in Britain, and generally these are significantly higher than those found in other bird species; and b) some raptores have recently undergone apparently exceptional decreases in numbers and are suffering widespread breeding failure, often associated with egg breakage by the parents and reduction in shell weight. It is also known that in the late 1950's and early 1960's thousands of seed-eating birds died from eating dieldrin dressed seed-corn.

There is a good correlation in both space and time between the decreases in numbers in the late 1950's and the use of dieldrin dressed seed-corn (Ratcliffe 1965, Prestt 1965), and then their recent recovery following the ban on its use. Jefferies and Prestt (1966) pointed out that only a small number of birds, containing high residues of dieldrin after feeding on dressed corn would be required to pass on a lethal residue dose to a hawk or falcon. A calculation (Young in press) of the effects, on the total numbers of the Peregrine Falcon population, of a widespread, longterm decrease in breeding success, has shown that only the death of a large number of the adult birds could account for sudden decreases of the type that have taken place. On the other hand any effect on breeding is going to affect the rate a bird population regains its former numbers. While it has not yet been established what, if any, particular compound is the cause of recent breeding failures, it is known that both pp'-DDT (Jefferies 1966) and dieldrin (de Witt 1956) can effect breeding success. Also, Jefferies (1967) has reported experimental evidence that pp'-DDT can effect the endocrine system of breeding birds, which could of course explain some of the behavioural and physiological aberrations reported from the field.

While the difference between the Tawny Owl and Great Crested Grebe and the other raptores can be explained, the contrast between the Heron and the others is more difficult to understand. Further investigation of the Tawny Owl and Great Crested Grebe have shown these species, in general, to possess smaller residues than the other predatory birds. In the Tawny Owl this undoubtedly largely arises from its hunting being restricted to small territories within woods. The presence of smaller residues is not the case with the Heron however, which on average contains higher residues than most other species (the geometric mean of a random sample of 93 eggs from a Lincolnshire colony for example being 3.47 ppm dieldrin with a range of 0.79-14.5 ppm). It is now known that the residues in the fish eaten by Herons are usually small, the intake of dieldrin is thus at a much lower level than that of Sparrow-Hawks and Peregrine Falcons eating graminivorous birds. This difference could account for the different effect, although it would also appear the Heron must be more resistent to dieldrin.

Other causes have been suggested for the changes in raptor populations, most of these however fail to take into account the sudden, exceptional nature of the decreases or the extent of their duration. Thus there is no reason to suppose the effect of game-keepers and game-preservation or egg collectors could suddenly have produced an exceptional effect in the late 1950's. Change in habitat could well produce a gradual change in the long-term, but again could not explain a sudden change. In the Peregrine Falcon, which has a history of known traditional nesting sites, there is evidence available to confirm there has been no outstanding decrease in numbers of breeding sites available; and the extensive increase in conifer plantations in all parts of Britain must have very considerably increased the amount of suitable habitat available for the Sparrow-Hawk. Natural fluctuations of wild populations have also been advanced as explanations for population decreases. There is no evidence to suggest the Peregrine Falcon or Sparrow-Hawk have in the recent past undergone natural fluctuations in Britain and, while the Kestrel in upland areas may fluctuate with vole numbers, there are insufficient data to show similar fluctuations have also occurred recently in south-eastern England (Snow in press); nor would this explain a decrease in numbers extending over seven or eight years when vole fluctuations occur approximately every four years. There is no evidence to suggest a lack of food for any of these predatory birds.

The results now available appear to confirm that the recent decreases in numbers, and the other effects noted, in several predatory birds, are real and unprecedented. That several species, occupying different ecological niches, have been affected almost simultaneously lends considerable strength to this view.

It is proving difficult to ascertain the possible cause or causes. In the pesticide field alone, not only could the changes recorded have been caused by several separate effects of known chemicals, but several other compounds have yet to be investigated. The recent effect of mercury on wild predatory birds in Sweden is one example; and the recent discovery (Holmes, Simmons & Tatton in press) of poly chlorobiphenyl residues in British predators is another.

References

ASH, J. S. (1960) *Brit. Birds*, *53*, 285.
CRAMP, S. (1963) *Brit. Birds*, *56*, 124.
DE WITT, J. B. (1956) *J. Agric. & Food Chem.*, *4*, 863.
de FAUBERT MAUNDER, M. J., EGAN, H., GODLY, E. W., HAMMOND, E. W., ROBURN, J., & THOMPSON, J. (1964) *Analyst*, *89*, 168.
JEFFERIES, D. J., & PRESTT, I. (1966) *Brit. Birds*, *59*, 49.
JEFFERIES, D. J. (1966) *Monks Wood Experimental Station Report, London: The Nature Conservancy 1960-1965*, 61.
JEFFERIES, D. J. (1967) *Ibis*, *109*, 266.
HOLMES, D. C., SIMMONS, J. H., & TATTON, J.O'G. (1967) *Nature* (in press).
LOCKIE, J. D., & RATCLIFFE, D. A. (1964) *Brit. Birds*, *57*, 89.
MOORE, N. W., & WALKER, C. H. (1964) *Nature*, *201*, 1072.
MOORE, N. W. (1965) *Bird Study*, *12*, 222.
PARSLOW, J. L. F. (1967) *Brit. Birds*, *60*, 2.
PRESTT, I. (1965) *Bird Study*, *12*, 196.
PRESTT, I., & MILLS, D. H. (1966) *Bird Study*, *13*, 163.
RATCLIFFE, D. A. (1963) *Bird Study*, *10*, 56.
RATCLIFFE, D.A. (1965) *Bird Study*, *12*, 66.
RATCLIFFE, D. A. (1967) *Nature*, *215*, 208.
ROBINSON, J. (1966) *New Scientist*, *29*, 159.
ROBINSON, J., BROWN, V. K. H., RICHARDSON, A., & ROBERTS, M. (1967) *Life Sciences*, *6*, 1207.
YOUNG, H. (in press) *The Proceedings of the Wisconsin Peregrine Conference, 1965*.

THE PROBLEM OF THE CHEMICAL ANALYSIS OF WILDLIFE SPECIMENS

J. Robinson

"Shell" Research Limited, Tunstall Laboratory, Sittingbourne, Kent, England.

An essential part of the study of the ecological aspects of insecticides is the analysis of wildlife specimens for the presence of these compounds. The analytical chemist is primarily concerned with the accuracy and reproducibility of his results: the accuracy of a determination is denoted by the closeness between the true value and the experimental value; lack of reproducibility (precision) indicates a lack of control of his experimental conditions.

Standard statistical techniques may be used to evaluate the precision of the results, but the determination of the systematic error of the results is less straightforward. Assuming that the addition of known amounts of the compound to samples of the animal tissue is representative of the biological incorporation of a residue into the tissue then the relationship between the amounts found and the amounts added may be determined by calculating the regression line for the two variables by standard statistical methods (Bennett and Franklin, 1954).

These concepts apply to all types of chemical analysis and are not confined to pesticide analyses, and they form a useful basis for the discussion of the difficulties encountered in the determination of residues of insecticides and their metabolites in animal tissues. Detailed reviews of the various analytical techniques used in residue analysis have been published by Elgar and Beynon (1966), but as this particular session of the 4th British Insecticide and Fungicide Congress is concerned with the ecological aspects of insecticides and as the organochlorine insecticides have aroused considerable interest and concern in this respect it seems appropriate to discuss the general principles of residue analysis in relation to these compounds. Most of analytical methods consist of three major steps:

(i) extraction of the compound(s) of interest from the sample,

(ii) removal or reduction in the relative amounts of co-extractives by appropriate 'clean-up' procedures,

(iii) detection of the compound(s) under investigation.

The analytical method used in Tunstall Laboratory, which is similar to that described by de Faubert Maunder et al (1964) and is essentially a subtractive method, is shown in Figure 1. The biological specimen may be regarded as a matrix of naturally occurring compounds and in this matrix residues of the organochlorine insecticides and their metabolites may be present. The concentrations of the latter may range from less than 0.001 ppm to 100 ppm, but the residues of most organochlorine insecticides are usually less than 1 ppm. A wide range of different types of compounds form the biological matrix and some of the more important classes of compounds are listed in Table 1. The more common of the organochlorine insecticides, or their metabolites, that may occur in the normal chemical environment of animals are shown in Table 2. The possibility of the presence of synthetic compounds other than the organochlorine insecticides should not be overlooked. In this connection the reported occurrence of unidentified halogenated compounds in wildlife specimens is important (Harrison, 1966; Robinson et al, 1967; Roburn, 1965; Simmons and Tatton, 1967), particularly as they do not appear to be of insecticidal origin and may be polychlorinated biphenyls or related compounds (Widmark, 1967). The occurrence of residues in wildlife of the more polar, hydrophilic metabolites, such as pp'-DDA, does not appear to have been studied.

The precision of the results obtained for a particular compound by the method mentioned above depends upon the reproducibility with which the various stages of the analysis are carried out.

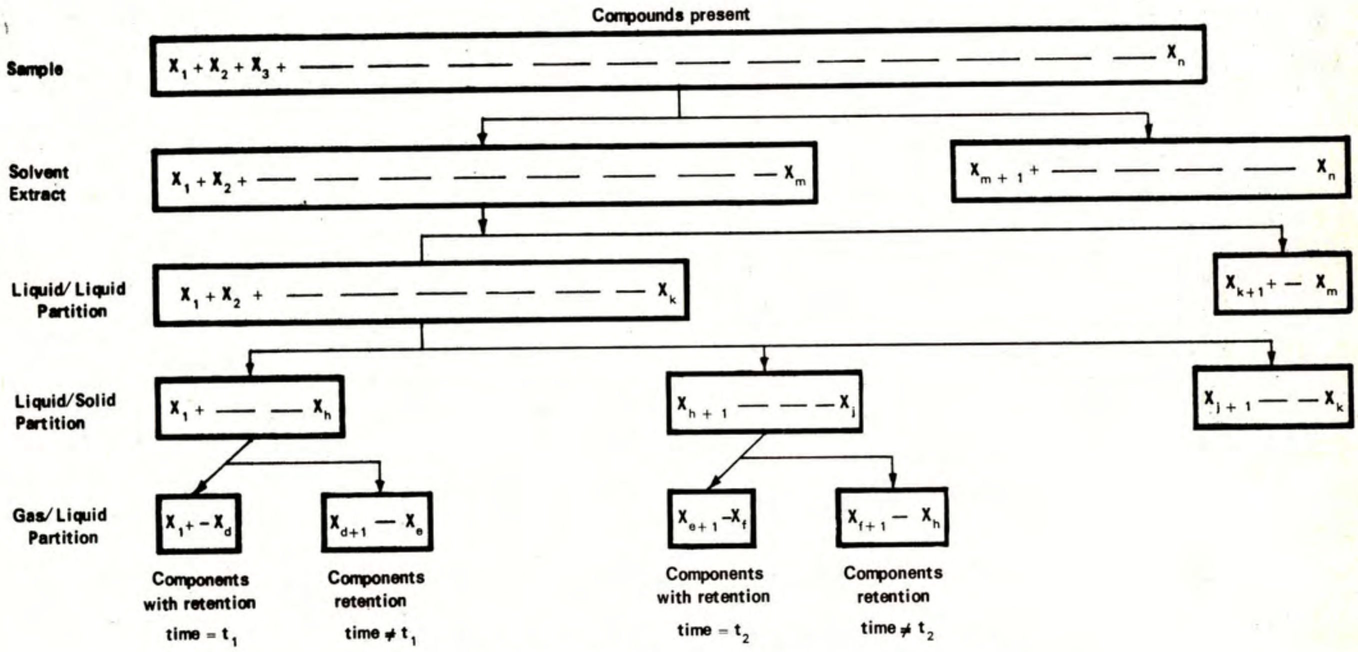


FIG 1 SCHEMATIC OUTLINE OF ANALYTICAL METHOD FOR THE DETERMINATION OF ORGANOCHLORINE INSECTICIDES IN ANIMAL TISSUES

Table 1.

Normal Constituents of Animal Tissues

Carbohydrates
(monosaccharides
(disaccharides
(polysaccharides

Lipids
(glycerides of fatty acids
(phospholipids
(lipoproteins
(steroids

Proteins, amino acids, and nucleoproteins

Porphyrins

Vitamins

Table 2.

Organochlorine Insecticides and their metabolites

pp'-DDT
pp'-TDE
pp'-DDE
DDMU[a]
DDMS[a]
DDNU[a]
pp'-DDA

α-BHC
β-BHC
γ-BHC
γ-Chlordane
Endosulphan
Heptachlor[b]
Heptachlor epoxide

Aldrin[b]
Dieldrin
Endrin
Dihydroaldrin-diol
Mono-hydroxydieldrin
Hexachloro-pentacyclo-dodecanone

[a] abbreviation used by Peterson & Robison, (1964) for metabolites of DDT formed sequentially by dehydrochlorination and reduction.

[b] residues of these compounds are rarely found as they are converted to the corresponding epoxides.

The accuracy of the method depends upon two factors: firstly, the efficiency with which the compound is transferred from the sample through the extraction and clean-up steps to the solution used for the final detection step; and secondly, the specificity of the method. If the transfer of material from the sample to the detection step is incomplete then the results will have a negative systematic error. On the other hand, if the combination of clean-up and detection procedures is not completely specific the systematic error will be positive.

The possibility that addition of a compound to a tissue in the analytical laboratory may not simulate the biological incorporation of the compound into the tissues of animals was referred to above. That this is a real problem is illustrated by some results obtained in the determination of dieldrin in the livers of experimentally poisoned birds. Recoveries of dieldrin from samples of control livers, to which dieldrin had been added in the laboratory, averaged 82% using an alkaline hydrolysis technique, a mean recovery which appeared satisfactory. However, the concentrations of dieldrin in the livers of experimentally poisoned birds, analysed by this hydrolytic procedure, were only about 50% of the concentrations found by a solvent extraction procedure. Subsequent work (Richardson, 1967) has shown that the alkaline hydrolysis of the specimen of liver degrades some of the dieldrin present to give two isomeric pentachloro derivatives. As a consequence this method gives significantly lower values than the solvent extraction technique although this could not have been foreseen on the basis of the results obtained with livers to which dieldrin had been added in vitro.

The results of this investigation of an extraction procedure involving alkaline hydrolysis is noteworthy for three reasons. Firstly, whereas dieldrin itself is stable to alkalies it is unstable to alkalies in the presence of liver tissue; secondly, in vitro addition of dieldrin to samples of liver gave recoveries which were apparently satisfactory; thirdly, in vitro addition of dieldrin has been found to be unrepresentative of in vivo incorporation of dieldrin into tissues.

Another example of the care required in checking the efficiency of extraction procedures is provided by the comparison of results obtained in the determination of dieldrin in blood using two different extraction procedures. One method involves a hexane extraction step (Radomski and Fiserova-Bergerova, 1965), and in the other acetone is used as the extraction solvent (Richardson, et al, 1967). It has been shown that the hexane extraction procedure gives results which are only 65% of those obtained by the acetone extraction method (Robinson, Richardson and Davies, 1967).

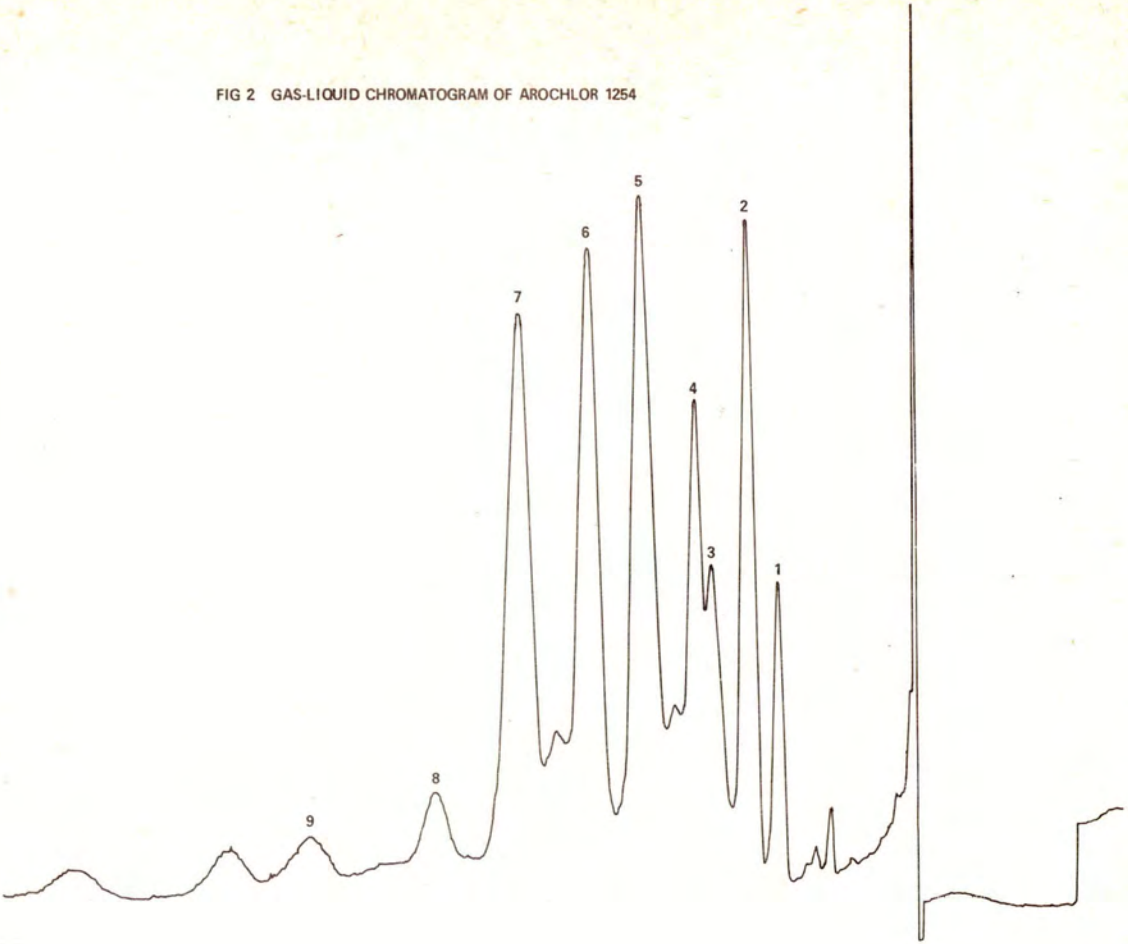
Experimental errors may also arise in the liquid/liquid partition step of the clean-up procedure. Emulsification tends to occur and this affects both the accuracy and the precision of the results. The other clean-up step, liquid/solid partition, also requires considerable attention to ensure that the various compounds are eluted completely in the appropriate eluates.

Most of the analyses of wildlife samples reported in recent years have been obtained using the electron capture detector. This detector has a high sensitivity for halogenated compounds and a significant response can be obtained with 1-10 picograms (10^{-12} - 10^{-11} g) of dieldrin for example. However, the response of this detector is not specific for either chlorinated or halogenated compounds (Lovelock, 1961) and many other types of compounds also have high electron affinities. In these circumstances the accuracy of the results obtained with an electron capture detector is dependent to a large extent upon the efficiency with which co-extracted materials are separated from the particular organochlorine insecticide that is being estimated. In particular, the accuracy of the results depends upon the efficiency of the separations achieved by the gas-liquid chromatographic procedure.

The difficulties that may arise in the separation of mixtures of organochlorine insecticides are illustrated in Tables 3 and 4; Table 3 is derived from the retention time data published by Burke and Holswade (1964, 1966) for two liquid phases; Table 4 gives some examples, using three liquid phases, of binary mixtures that are separated with difficulty. It is highly desirable that reports on residues of organochlorine insecticides should give detailed information on the separatory efficiency of the gas-liquid chromatographic column(s) used in the analyses. The separation factors for particular pairs of compounds, etc. are easily calculated from the retention times of the components and their respective peak widths at half-height. The number of theoretical plates required for a separation corresponding to a particular impurity fraction can be calculated from published graphical relationships or tables (Littlewood, 1962).

The separatory efficiency of the gas-liquid chromatographic columns is of great importance in all analyses using this procedure, particularly in the case of analyses of DDT-type compounds. Before the advent of gas-liquid chromatography the Schechter-Haller colorimetric procedure was used and residues of pp'-DDT and pp'-DDE only were reported. In recent years the results obtained with gas-liquid chromatography indicate that the mixture of compounds that may arise following exposure to pp'-DDT is more complex, and in the case of technical DDT, which contains some of the op'-isomer, the residue pattern may become very complex. The mixture of products arises from dehydrochlorination and reduction reactions. The analytical problem is intensified if members of another class of compounds, the polychlorinated biphenyls, are present. The gas-liquid chromatogram of one type of Arochlor is given in Figure 2; the column specifications were as follows: length, 3 ft; stationary phase, SE 30 (2%) + Epikote 1001 (0.2%) on 80-100 mesh celite; flow rate, 90 ml per min; and temperature, 170^0C. The chromatogram, using the same conditions, for the extract of a heron's egg is given in Figure 3. The numbered-arrows indicate the retention times of the various components in the Arochlor, the lettered-arrows indicate the retention times of a number

FIG 2 GAS-LIQUID CHROMATOGRAM OF AROCHLOR 1254



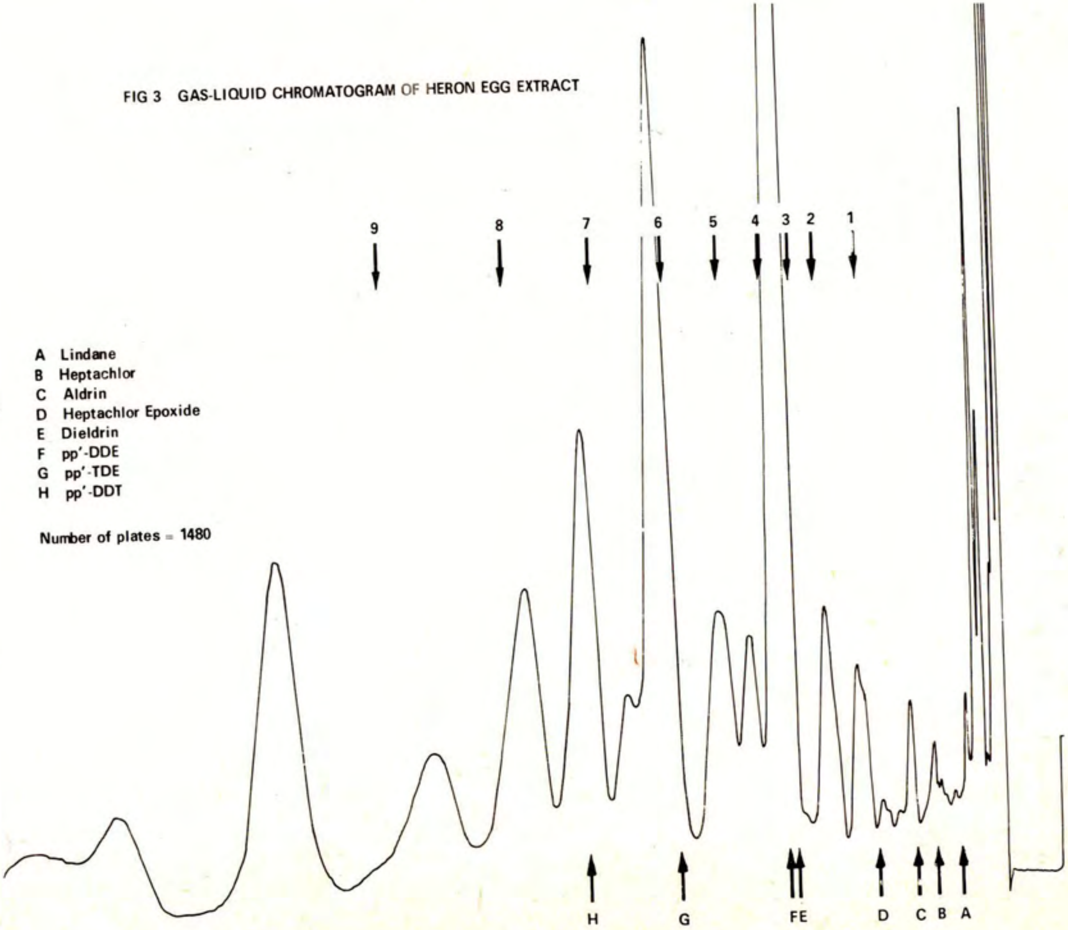


FIG 3 GAS-LIQUID CHROMATOGRAM OF HERON EGG EXTRACT

Table 3.

Groups of compounds with similar retention times

Column Specifications	Numbers of Compounds in Group	Number of cases in which the extreme members of the group have the following relative retention times		
		1.01	1.05	1.10
10% DC 200 on 80/90 Anakrom AB1, 6 ft x 4.5 mm id.	2	12	17	5
	3	5	7	3
	4	-	5	10
	5	-	3	6
	6	-	1	5
	7	-	-	2
	8	-	-	-
	9	-	-	1
15% QF1 + 10% DC 200 on Gas Chrom. Q, 6 ft x 4 mm id.	2	4	16	6
	3	3	7	13
	4	-	5	8
	5	-	1	1
	6	-	-	2
	7	-	-	2
	8	-	-	-

Table 4.

Relative Retention Times (HEOD = 1) of Chlorinated Insecticides

Compound	Stationary Phase		
	SE 30	Oronite Polybutene	GE, XE 60
Heptachlor Epoxide	0.66	0.58	0.65] 1.05*
pp'-DDMU	0.79] 1.05*	1.05* [0.95] 1.06*	0.68
op'-DDE	0.83	0.90	
HEOD	1.00] 1.04*	1.00	1.00
pp'-DDE	1.10* [1.04	1.27] 1.07*	0.81
Endrin	1.14	1.36	1.17] 1.02*
op'-DDT	1.44	1.74	1.15

* the ratios of the retention times of compounds which are difficult to separate. The numbers of theoretical plates required to give a separation corresponding to a 5% impurity fraction are:

	Ratio of Retention Times				
	1.02	1.04	1.05	1.07	1.10
Number of plates required	26,000	7,000	4,500	2,500	1,200

of organochlorine insecticides. Using eluates of differing polarity, as outlined in Figure 1, the polychlorinated biphenyls are easily separated from dieldrin, endrin and heptachlor epoxide, and some, or all, of the polychlorinated biphenyls may be at least partially separated from the DDT-type compounds by appropriate adjustments of the conditions used in the liquid/solid partition step of the clean-up procedure since the biphenyl derivatives are eluted from Florisil columns before pp'-TDE and pp'-DDT. The propensity for pp'-DDT to be degraded thermally, which has been reviewed by Ott and Gunther (1965), is another factor that must be considered in the determination of DDT residues by gas chromatography. Careful control of the column conditions is also required in the determination of Endrin to avoid thermal isomerisation to an aldehyde and a ketone (Phillips et al, 1962).

It is appropriate at this point to discuss the problem of confirmation of the chemical identity of the component estimated as insecticide X or Y, etc. From Tables 3 and 4 it is obvious that the retention time, using one stationary phase only, is quite inadequate as evidence of the identity of a component in a complex mixture. A number of techniques have therefore been used to confirm the chemical identity of a component as a particular insecticide. These include the use of multiple column and detector systems, paper chromatography, thin-layer chromatography, chemical reactions, infrared spectrophotometry, and mass spectrometry. The facilities for applying all these techniques to the investigation of the identity of residues in wildlife are available to few laboratories. The techniques which are most widely used are a combination of retention times on two or more stationary phases, R_f values in thin-layer and/or paper chromatography, and chemical reactions. The conditions under which valid conclusions may be drawn from the combination of these techniques require more precise study than is generally given to them (Robinson et al, 1966).

Finally, the concept of the lower limit of detection of an analytical method must be mentioned. The definition of the lower limits of detection of analytical procedures is becoming increasingly important in view of the increasing number of reports of minute residues of pp'-DDE, and other organochlorine compounds in rainwater, antarctic fauna, etc. It is desirable that the lower limits of detection of the analytical procedures should be precisely defined, e.g., on the bases of the concept of a statistically significant deviation from the background noise (Young, 1961).

However, the definition of the lower limit of detection of a particular analytical method is not merely a statistical one, as is apparent from the chromatograms obtained during the analysis of specimens of soil and animal fat that were collected and stored before the introduction of organochlorine insecticides (Bowman et al, 1965; Coon et al, 1966).

Outstanding advances in analytical methodology have occurred in recent years in the field of residue analysis; in some cases the analyst has outstripped the toxicologist or biologist by reporting residues of such small magnitude that their toxicological or ecological significance is not known. Similarly, the sensitivity of some of the techniques has raised questions of the analytical significance of the reported residues that, as yet, cannot be fully answered. The problems discussed above, whether they be concerned with the accuracy and precision of quantitative determinations, the qualitative confirmation of identity, or the definition of lower limits of detection, indicate the desirability of collaborative work by different analytical laboratories in order to standardise both the techniques used and the definition of terms such as column efficiency, separation factors, lower limits of detection and so on. A major problem in any such collaborative programme would be to obtain sufficient material to allow valid comparisons of analytical results to be made in statistically designed trials.

References

BENNETT, C.A. and FRANKLIN, N.L. (1954) Statistical Analysis in Chemistry and the Chemical Industry, John Wiley and Sons, New York.

BURKE, J. and HOLSWADE, W.(1964) J. Assoc. off. agric. Chem. 47, 845.

BURKE, J. and HOLSWADE, W (1966) J. Assoc. off. anal. Chem. 49, 374.

BOWMAN, M.C., YOUNG, H.C. and BARTHEL, W.F. (1965) J. econ. Ent. 58, 896.

COON, F.B., CHRISTENSEN, R. and DERSE, P.H. (1966) Paper presented at the 152nd Meeting of the American Chemical Society, New York, 12th - 16th September.

ELGAR, K.E. and BEYNON, K.I. (1966) Analyst, 91, 143.

de FAUBERT MAUNDER, M.J., EGAN, H., GODLY, E.W., HAMMOND, E.W., ROBURN, J. and THOMSON, J. (1964) Analyst, 89, 157.

HARRISON, R.B. (1966) J. Sci. Fd. Agric. 17, 10.

LITTLEWOOD, A.B. (1962) Gas Chromatography, Academic Press.

LOVELOCK, J.E. (1961) Nature, Lond. 189, 729.

OTT, D.E. and GUNTHER, F.A. (1965) Residue Reviews, 10, 70.

PETERSON, J.E. and ROBISON, W.H. (1964) Toxic. appl. Pharmac., 6, 321.

PHILLIPS, D.D., POLLARD, G.E. and SOLOWAY, S.B. (1962) J. Sci. Fd. Agric., 10, 217.

RADOMSKI, J.L. and FISEROVA-BERGEROVA, V. (1965) Ind. Med. Surg., 34, 934.

RICHARDSON, A. (1967) Paper presented at the VIth International Congress of Plant Protection, Vienna, 30th August - 6th September.

RICHARDSON, A., ROBINSON, J., BUSH, B. and Davies, J.M. (1967) Archs. envir. Hlth., 14, 703.

ROBINSON, J., RICHARDSON, A. and ELGAR, K.E. (1966) Paper presented at the 152nd Meeting of the American Chemical Society, New York, 12th - 16th September.

ROBINSON, J., RICHARDSON, A., CRABTREE, A.N., COULSON, J.C. and POTTS, G.R. (1967) Nature, Lond., 214, 1307.

ROBINSON, J., RICHARDSON, A. and DAVIES, J.M. (1967) Archs. envir. Hlth., 15, 67.

ROBURN, J. (1965) Analyst, 90, 467.

SIMMONS, J.H. and TATTON, J.O'G. (1967) J. Chromat., 27, 255.

YOUNG, I.G. (1961) Gas Chromatography, ed. by H.J. Noebels, Academic Press, New York.

WIDMARK, G. (1967) Paper presented at the VIth International Congress of Plant Protection, Vienna, 30th August - 6th September.

THE ECOLOGICAL EFFECTS OF PARAQUAT AND DIQUAT WHEN USED TO CONTROL AQUATIC WEED

J.F.Newman
Imperial Chemical Industries Limited
Jealott's Hill Research Station, Bracknell, Berks.

Summary This paper considers the ecological investigation of the possible effects of new pesticidal chemicals during the period of their development. Trials on the use of the bipyridyl herbicides for submerged aquatic weed control are taken as examples. The following aspects are considered :-

1. Direct toxic effects on plants and animals in various types of habitat in which the chemicals may find use.
2. The fate of the chemicals in the environment. Level and duration of persistence of residues in water, plants, animals and mud.
3. Indirect effects. Deoxygenation of water following decay of weed.
4. Importance of ecological work in formulating recommendations for safe use. Code of Practice. Recommendations in relation to different types of water use.

INTRODUCTION

The general strategy underlying the ecological investigation of new pesticides has been discussed elsewhere (Newman, J.F., 1965). Before a new chemical is brought into extensive commercial use the possible impact of it must be investigated in each type of habitat in which it is likely to be used. Special problems are presented by aquatic conditions in that it is difficult to localise the effect of the chemical. Whereas on arable land the influence of a chemical can sometimes be localised by placement treatment, this is less practicable in water. It has to be accepted that the applied chemical will be carried in currents or will diffuse beyond the immediate treatment area.

It is well established that concentrations of between 0·5 and 1·0 mg/litre of paraquat or diquat (1 to 2 gal/acre ft. of "Reglone" or "Gramoxone S") will kill most submerged weed, while the more susceptible species can often be killed at rates below these.

EXPERIMENTAL SITES

Observations on the effects of the treatments on the animal life of the water have been made at 10 sites of varying ecological type. These comprised 4 lakes (1·8, 4·4, 8, and 50 acres in area), 3 small ornamental ponds, a section of 15 ft wide canal and narrow drainage channels in Lincolnshire, Romney Marsh and West Sussex.

RESULTS

Toxic Effects on Fauna

Data for the Romney Marsh site have been published (Newman, J.F., 1965). At none of these sites was any evidence obtained of any direct effects of the treatments on the invertebrate fauna.

No direct toxic effects on fish were seen at any site. This finding is to be expected, as laboratory toxicity tests on fish (Carter, L., 1965) show a wide margin of safety at herbicidally effective rates. The median survival time of rainbow trout in a 10 mg/litre solution of paraquat is 13 days, and in a 1 mg/litre solution of diquat is 15 days. In these tests the concentrations of the solutions were maintained continuously throughout the exposure times.

As distinct from direct toxic effects, it is necessary to consider changes in the ecological balance of different groups of animals consequent upon the use of the herbicides. After treatment the breakdown of the killed weed is rapid, and it disintegrates and sinks to the bottom within about two weeks. Most of this breakdown is effected by bacterial activity but some part of it can be assisted by the activity of larger detritus feeding animals. This may be the cause of the increased numbers of larvae of Diptera, mainly Chironomidae, recorded in the treated areas of the Romney Marsh channels.

Fate of the chemicals in the environment

Before a chemical can be used widely with confidence it is necessary to establish adequate information on the duration of its persistence and on its ultimate fate in the ecosystems in which it is to be used. In relation to the aquatic use of paraquat and diquat this has involved analyses of samples of water and weed and of bottom mud cores. The results of these analyses show that the concentration of herbicide in the water falls to half the initial concentration in 36 hrs and to undetectable levels within 16 days. This removal is mainly by absorption into the weed. As the weed sinks, the remaining undecomposed residue is incorporated into the bottom mud. At Oxton Lake, about 30% of the total applied paraquat could be accounted for in the bottom mud after 5½ months. (Newman, J.F. and Way, J.M., 1966). At some sites a reduction in weed growth has been noted in the year following herbicide application, and the question arises as to whether or not the residue in the bottom mud is exerting any persistent effect. Mud cores were taken from a treated lake and transferred undisturbed to plant pots. After some drying, barley seeds were planted. No signs of damage were apparent in the barley seedlings, and it is concluded that bipyridyl herbicide residues in the mud are inactive. The effects on the vegetation in the year following treatment are no doubt due to the weakening of the rootstocks following the killing of the photosynthetic parts in the middle of the preceding summer.

These field observations are compatible with results of laboratory experiments on the breakdown and inactivation of paraquat. Experiments on microbiological degradation of paraquat (Geoghegan, M.J., 1967) have shown that a number of micro-organisms commonly present in soil can metabolise paraquat to give simple compounds. Experiments on physical inactivation (Knight, B.A.G. & Tomlinson, T.E., 1967) have shown that any free paraquat in the soil is rapidly inactivated by firm adsorption on clay minerals.

De-oxygenation of water

When submerged weed is killed the cessation of photosynthesis, together with the oxygen demand of the decomposition processes, results in a fall in the concentration of dissolved oxygen in the water which in severe cases can be damaging to fish. The experiments at the various sites have shown that the natural oxygen balance of the water varies widely in relation to the type of site and the quantity and distribution of the aquatic vegetation. A large lake in an open site and with a moderate growth of weed is likely to be well mixed and well oxygenated throughout. At the other extreme, a narrow but deep drainage channel, with a dense surface growth of weed which shades out vegetation below it, is likely to have low dissolved oxygen concentrations even a few inches below the surface.

Application of ecological work

The practical exploitation of the properties of novel chemicals such as paraquat and diquat in aquatic weed control could not be attempted without a wide investigation of their total impact in the aquatic environment. The work here outlined has formed a basis for recommendations for safe use.

The recommendations necessarily vary in relation to the use which is to be made of the body of water concerned. In lakes and ponds where fishing and the preservation of wild life are important the need is for some control of submerged vegetation rather than its complete elimination, and it is essential that the water should not become severely deoxygenated. Both these considerations indicate the desirability of treating only part of the lake at any one time and of treating fairly early in the summer before weed growth becomes too dense. If such activities as sailing or water ski-ing are important, there is a need to keep the area free of weed throughout the summer months. The treatment is then best applied just before the weed becomes dense enough to interfere with the passage of boats, usually in early June. If the weed is killed at this time it is unlikely to grow again to a sufficient extent to impede boats in the same year.

In drainage channels an important requirement is that there should be weed free conditions in the autumn and winter, so that flood water can flow freely. Fishing is not in general of great importance in such channels, where low dissolved oxygen conditions often exist naturally during the summer. In these circumstances herbicidal applications may be made later in the year, and de-oxygenation is of less importance.

The conclusions of the ecological and other work here described have been embodied in a "Guide to water weed control" which is available to users of "Reglone". The work has also been of value to those concerned with the production of the Code of Practice relating to chemical weed control in aquatic situations.

References

CARTER, L. (1965) I.C.I. Paints Division, Brixham. Private communication.

GEOGHEGAN, M.J. (1967) I.C.I. Jealott's Hill. Private communication.

KNIGHT, B.A.G. & TOMLINSON, T.E. (1967) J. Soil Sci., 18, 233

NEWMAN, J.F., (1965) Proc. 3rd. Br. Insecticide & Fungicide Conf. 342.

NEWMAN, J.F. and WAY, J.M. (1966) Proc. 8th Br. Weed Control Conf., 2, 582.

CHANGES IN SOIL INVERTEBRATE POPULATIONS CAUSED BY SOME ORGANOPHOSPHORUS INSECTICIDES

by C. A. Edwards
Rothamsted Experimental Station, Harpenden, Herts.
A. R. Thompson
"Shell" Research Ltd., on secondment to Rothamsted Experimental Station.
J. R. Lofty
Rothamsted Experimental Station.

Summary.

Results are given of the effect of the organophosphorus insecticides parathion, diazinon, phorate and disulfoton on soil invertebrates. Numbers of the animals and the biomass were decreased less than by aldrin and dieldrin. Numbers of parasitic mites were greatly lessened but, as in response to treatment with DDT, the numbers of springtails, trombidiform and oribatid mites often increased. The effects on these groups often remained longer than the residues of the insecticides. Only phorate lessened numbers of earthworms significantly. All four lessened numbers of pauropods. Beetles were affected only by parathion. No insecticide controlled symphylids economically.

INTRODUCTION

The partial ban of chlorinated hydrocarbon insecticides in England (M.A.A.F. Report 1964) has led to the greater use of organophosphorus, and, more recently, of carbamate insecticides to control soil-borne pests. Little attention has been paid to the possible effects of these less persistent compounds on invertebrates that are not pests. For example, the only significant effect of menazon at 2, 4 and 8 lb/acre was to lessen the numbers of onychiurid springtails (Collembola) (Raw 1965), and phorate and thionazin at 250 p.p.m in the top 4 inches of soil killed almost all springtails, mites and earthworms (Way & Scopes 1965). Other information is scanty, and the present work aimed to study the effects of some commonly used organophosphorus insecticides on populations of soil invertebrates.

Methods

Four field experiments were done at four widely dispersed sites. Plots were taken at random. The insecticides tested, and the size of the plots, differed from site to site. The appropriate insecticide was sprayed on to the surface of each plot which was then rotovated in two directions as soon and as evenly as possible.

Four, two-inch diameter soil cores, six inches deep, were taken from each plot just before treatment, and at regular intervals afterwards. They were placed in screw-top aluminium cans and then placed in modified Tullgren funnels either during the same, or next day. The sieves above the funnels were six inches in diameter and each broken-up soil sample filled a sieve to a depth of two inches. The temperature above the soil sample was kept near 30°C., but the room was maintained at 20°C. to give a gradient through the soil of 10°C. Animals dropped into vials containing a mixture of 70% aqueous alcohol and 5% glycerol and were stored until sorted under a binocular microscope.

Numbers of earthworms and macroarthropods were estimated once at each site from three two-ft.sq. quadrats in each plot, by hand-sorting the top three to four inches in the field. The surface of the shallow pit was then levelled and a gallon of dilute formalin (25 cc in 1 gallon of water) poured on to it. All earthworms and macroarthropods that came to the surface during the next fifteen minutes were removed and counted.

The sites and experimental details were as follows:

A. Bethersden (Vine Farm, Kent)

This site had been pasture for many years and bore wheat during the experiment. The soil was a heavy clay loam with a high water table and a large invertebrate fauna. Plots were 4 yards square in a random layout with one yard between plots. The insecticides were applied at the following rates:

a) Parathion; 8 lb a.i./acre
b) Diazinon; 8 lb a.i./acre
c) Disulfoton; 8 lb a.i./acre
d) Phorate; 4 lb a.i./acre

Soil samples were taken at approximately monthly intervals.

B. Sittingbourne (Woodstock Agricultural Research Centre, Kent)

A field that had been pasture for more than ten years was made available by courtesy of the Shell Chemical Co. The soil, a clay loam with flints, was ploughed in autumn, thoroughly cultivated and the insecticides applied soon after. The whole site was then planted to winter wheat. Plots were four yards square in a random layout with one yard between plots. The insecticides applied were:

a) Parathion; 4 lb a.i./acre
b) Diazinon; 4 lb a.i./acre

There was a very diverse fauna including many wireworms, earthworms and millipedes. Soil samples were taken at approximately monthly intervals.

C. Alton (Manor Farm, Hampshire)

The site was old pasture with very heavy clay subsoil, but well drained and covered with good clay loam surface soil. The whole site was planted with potatoes during the period of the experiment and soil samples were taken from the rows of plants. Plots were 20 feet x 10 feet. The insecticides used were:

a) Parathion; 4 lb a.i./acre
b) Diazinon; 4 lb a.i./acre

Soil samples were taken 4 weeks, 18 weeks and 24 weeks after treatment.

D. Harpenden (Highfield, Rothamsted, Herts.)

This had been a 300-year old pasture and had recently been ploughed. The soil was a silty loam with good texture in the top 6 inches. The plots were kept fallow for the period of the experiment and then grassed down. Plots were 8 feet x 9 feet. Of the insecticides applied the only one considered here is parathion at 4 lb a.i./acre. Soil samples were taken one month, four months and ten months after treatment.

RESULTS

The invertebrates were identified only to suborders and families. All results were analysed statistically and Table 1, which summarises the analyses of variance, shows that all treatments produced highly significant changes in numbers of animals, and that numbers changed significantly between sample dates. The interactions between treatments and sampling dates were also highly significant. Space does not allow all the results to be given, so the more important are summarised in Tables 2, 3 and 4.

Table 2 summarises the changes in numbers of soil invertebrates averaged over all sampling dates. Each figure is the mean number of animals per soil core averaged over the whole sampling period, expressed as a percentage of the corresponding mean number for the untreated plots. Numbers in treated plots usually differed more from the untreated ones soon after treatment than later. The sum of all the means per sample for the different groups of invertebrates for a particular site and insecticide, expressed as a percentage of a similar total for the untreated control plots, gave the total percentage survival. Because such totals and percentages combine numbers of very small and very large invertebrates, it is best to express the results as the wet weight of animals,or, that is to say, the biomass. Biomass was estimated by multiplying the mean number for each group of invertebrates by the average weight for the group. Tables of such average weights are available (Edwards 1967). Mean biomasses were calculated for plots treated with each insecticide, for every site, and each group of animal, and the percentage change from the use of the insecticide was calculated.

The mean change in numbers of animals during the season does not give the whole picture, so the numbers in plots treated with insecticide, expressed as a percentage of numbers in control plots, when populations were at their peak, are summarised in Table 3. Peak populations, which depended on the particular group of animals, occurred at different times after the insecticides were applied but were usually in spring or autumn. Data expressed as above usually illustrate the maximum effect of the insecticide because numbers increase or decrease at different rates in treated and untreated soil.

The mean number per sample of each group of animals for every sampling date, and insecticide treatment, was plotted on graphs from which the time that the effects persisted, whether increases or decreases, were estimated (Table 4). None of the experiments lasted more than one year and effects still detected then are shown in the table as >52.

Table 1.

Summary of Analysis of Variance for experiments

Site	Insecticide	F		
		Treatment	Date	Treatment X Date
Bethersden	Diazinon	156.5**	72.2**	5.28**
	Disulfoton	176.0**	67.9**	4.02**
	Phorate	205.0**	37.6**	7.04**
	Parathion	282.5**	69.8**	7.07**
Woodstock	Diazinon	324.5**	168.0**	4.91**
	Parathion	143.0**	100.0**	13.23**
Alton	Diazinon	107.3**	48.8**	4.18**
	Parathion	98.7**	63.2**	5.21**
Highfield	Parathion	86.5**	32.3**	3.87**

Table 2

Percentage survival averaged from all sampling dates over one year after insecticide treatment.

Insecticide Site Animal Group	Parathion A	 B	 C	 D	Diazinon A	 B	 C	Phorate A	Disulfoton A
Earthworms	96.3	96.0	83.0	-	73.9	108.1	100.0	21.4	70.0
Symphylids	79.5	61.4	34.8	92.5	46.5	78.6	-	58.2	110.0
Pauropods	3.0	18.2	20.0	82.4	5.0	26.7	4.0	21.0	3.0
Parasitic Mites	25.0	38.2	77.5	49.1	52.8	39.0	69.8	62.1	41.1
Trombidiform Mites	47.2	34.8	265.0	125.0	26.6	41.3	282.0	62.3	87.5
Oribatid Mites	69.5	145.0	213.0	66.4	87.5	68.0	167.6	39.0	55.2
Surface-Living springtails	67.7	80.5	207.0	78.8	46.9	72.0	80.0	42.5	17.9
Deep Soil-Living springtails	48.8	58.2	301.5	145.6	136.0	33.1	102.4	72.2	69.7
Fly Larvae	34.4	80.2	23.3	38.6	27.9	53.3	51.7	21.4	41.4
Beetles	59.0	49.2	43.5	90.8	91.0	90.3	71.4	84.5	86.0
Total % Survival (Numbers)	53.7	72.8	166.8	94.0	74.0	70.5	104.8	52.4	49.5
Total % Survival (Biomass)	52.4	91.2	117.7	83.5	44.8	58.7	92.1	46.5	44.6

Table 3

Numbers of animals expressed as percentage of control at peak population

Insecticide	Parathion				Diazinon			Phorate	Disulfoton
Site Animal Group	A	B	C	D	A	B	C	A	A
Symphylids	50.0	26.6	-	37.8	50.2	87.6	-	40.0	30.1
Pauropods	2.1	11.1	-	7.1	3.8	16.5	-	36.8	7.2
Parasitic Mites	32.2	19.2	18.1	64.3	41.6	20.8	24.4	51.0	60,0
Trombidiform Mites	13.3	18.6	444.0	135.0	19.2	46.6	334.0	50.0	65.0
Oribatid Mites	42.5	336.2	201.0	57.2	262.0	60.2	179.0	31.2	137.2
Surface-living springtails	48.8	57.3	392.0	35.1	52.6	53.5	268.0	43.3	34.4
Deep-living springtails	33.9	63.7	267.8	174.0	194.5	49.6	109.8	29.2	171.0
Fly larvae	7.8	59.8	7.7	34.0	7.2	35.2	32.6	31.4	33.1
Beetles	5.2	28.0	21.4	25.0	36.3	39.2	36.8	40.7	43.8

Table 4

Minimum time for effects of insecticide to disappear (weeks)

Insecticide	Parathion				Diazinon			Phorate	Disulfoton
Site	A	B	C	D	A	B	C	A	A
Time for insecticide to disappear 50%		6 weeks				9 weeks			
Time for insecticide to disappear 95%		26 weeks				37 weeks			
Symphylids	16	10	18	16	16	10	18	14	12
Pauropods	>52	>52	-	40	>52	>52	-	>52	>52
Millipedes	-	>52	-	40	-	>52	-	-	-
Centipedes	-	20	-	40	-	>52	-	-	-
Parasitic Mites	50	45	28	41	37	43	32	38	39
Trombidiform Mites	35	>52	>52	>52	40	>52	>52	34	34
Oribatid Mites	42	>52	>52	40	>52	48	28	39	52
Deep-living springtails	36	50	>52	>52	>52	>52	>52	34	>52
Surface-living springtails	41	36	>52	32	38	36	>52	40	44
Fly larvae	21	18	22	36	38	25	25	42	38
Beetles	20	12	19	16	16	12	24	12	8

DISCUSSION

The four organophosphorus insecticides did not lessen numbers of soil invertebrates as much as did aldrin and dieldrin (Edwards 1965a, 1965b, 1967). The invertebrates most susceptible to the chlorinated hydrocarbons are pauropods (Pauropoda), isotomid springtails and fly larvae (Diptera). Pauropods are also easily killed by organophosphorus insecticides (Table 2); fly larvae are killed less easily. The surface-dwelling springtails (Isotomidae, Entomobryidae) seem to be more resistant to organophosphorus insecticides than to the chlorinated hydrocarbons. The parasitic mites (Gamasina), not very easily killed by the chlorinated hydrocarbons except DDT, are more easily killed by the organophosphorus insecticides. Symphylids seemed to be a little more susceptible to organophosphorus insecticides than to chlorinated hydrocarbons, but the differences were small and transient. The response of springtails, trombidiform and oribatid mites to organophosphorus insecticides varied (Tables 2 and 3), and often resembled the response of springtails to DDT, which greatly lessened numbers of parasitic mites and increased numbers of springtails. This also happened occasionally with lindane but with none of the other chlorinated hydrocarbons (Sheals 1956; Edwards, Dennis and Empson 1967). Oribatid mites, entompbryid and onychurid collembola also increased in plots treated with chlorfenvinphos (Edwards, Thompson and Beynon 1967). Our experiments show that the insecticides cause these invertebrate groups to increase in numbers almost as often as to decrease. These increases are so similar to those found with DDT that it is reasonable to suspect that they are caused by killing parasitic mites, or other predators (Edwards, Dennis and Empson 1967). Beetles (Coleoptera), both adults and larvae, are less susceptible to the organophosphorus insecticides but different species differ greatly in their susceptibility.

Parathion and diazinon do not persist long in soil (Table 4), but their effects can last longer than the residues. The effects were least on symphylids, fly larvae and beetle adults and larvae, and lasted little longer than the life of the chemical. For all the groups of animals mean numbers in treated plots often differed significantly from those in untreated plots even after one year. When an insecticide increased numbers of a particular group, the effect usually lasted longer than did concomitant decreases in other groups (Table 4), probably because of the lag of a month or more after the insectidide is applied, before numbers begin to increase. If the increase occurred soon after applying the insecticide, some stimulating cause rather than death of predators could be postulated.

There were few outstanding differences between the four insecticides. Over the whole season, never more than 50 per cent of the animals were killed, and parathion and diazinon increased the total numbers at the Alton site. Changes in biomass did not differ greatly from changes in numbers. Similar results were obtained with chlorfenvinphos in four experiments (Edwards, Thompson and Beynon 1967). Only phorate, applied at 4 lb a.i./acre, lessened numbers of earthworms significantly. Experiments other than those described here have confirmed that phorate is very toxic to earthworms. No insecticide controlled symphylids economically, even though parathion and diazinon are recommended. All four insecticides easily killed pauropods but parasitic mites and fly larvae were killed variably though all insecticides considerably lessened their numbers. Numbers of beetles were not much lessened by any of the insecticides except parathion.

These organophosphates, which are becoming commonly used, do not seriously alter populations of soil invertebrates, and beyond the season of application may either increase or decrease numbers. Most of the pests formerly treated with persistent insecticides can now be controlled with these less persistent ones.

Acknowledgements

The cooperation of the "Shell" Chemical Co., Woodstock Agricultural Research Centre, in providing sites at Woodstock, Bethersden and Alton, in help with the insecticide applications, and in other ways, is gratefully acknowledged. The assistance of A. E. Whiting, D. J. Holdaway and D. R. Jordan in sorting the invertebrates into taxonomic categories is gratefully acknowledged.

References

EDWARDS, C. A. (1965a) Vth Symp. Brit. Ecol. Soc., 239.
EDWARDS, C. A. (1965b) Ann. appl. Biol. 55, 329.
EDWARDS, C. A. (1967) "Progress in Soil Biology". Proc. Coll. Dynamics of Soil Communities 1966. Graff and Satchell (Eds.) North-Holland Publishing Co. Amsterdam.
EDWARDS, C. A., DENNIS, E. B. and EMPSON, D.W. (1967) Ann. appl. Biol. 60, 11.
EDWARDS, C. A., THOMPSON, A. R. and BEYNON, K. I. (1967) In press.
M.A.F.F. (1964) "Review of the persistent organochlorine pesticides" H.M.S.O. London 68 pp,
RAW, F. (1965) Ann. appl. Biol. 55, 342.
SHEALS, J. G. (1956) Bull. ent. Res. 47, 803.
WAY, M. J. and SCOPES, N. E. A. (1965) Ann. appl. Biol. 55, 340.

SOME FACTORS AFFECTING THE EFFICACY OF METHYL BROMIDE FUMIGATIONS OF GLASSHOUSE SOILS

D.J. Galley & N.G.M. Hague
Zoology Dept., Imperial College, London, S.W.7

Summary Methyl bromide has many advantages over other halogenated hydrocarbon fumigants. The dosage can be regulated, planting can be done very soon after treatment and it can be used at relatively low soil temperatures. Methyl bromide is applied under polythene sheets and after the removal of the sheet measureable concentrations are found at various depths in the soil for several days after fumigation.

Commercially methyl bromide is applied by 1lb cans but results have shown that vaporisation through a heated coil gives quicker and more efficient penetration into soil especially at low temperatures. Methyl bromide has not been reported to be very fungicidal but recent experiments in glasshouses in Great Britain have showed that it controls the brown root rots of tomatoes when vaporised into the soil.

INTRODUCTION

Methyl bromide is considerably more expensive than other chemical soil sterilants but it has one major advantage, shared with steam sterilisation, that planting can follow within a few days of treatment, thus enabling growers to increase the turnover of crops in any one year.

Commercially, there have been some failures to control nematodes and weeds when methyl bromide is applied to soil in 1lb cans under polythene sheets. Hague et al (1964) showed that methyl bromide could be vaporised into the air-space under the polythene sheet resulting in good nematode control in the soil. Using the same method of application James & Hague (in press) reported excellent control of both nematodes and weeds while tomatoes gave greatly increased yields in soil infested with brown root rot although the fungus itself was not controlled.

The experiments described in this paper aim to:

1. Compare the distribution of methyl bromide applied by the vaporiser and can methods.
2. Extend previous observations of the dissipation of methyl bromide during the aeration period following removal of sheet.
3. Investigate the effect of methyl bromide on the brown root rot (G.S.F.) of tomatoes.

METHODS

Application of Methyl Bromide

Fumigations were carried out under 12 ft wide polythene sheets (500 gauge) the edges of which were dug into the soil to obtain a good gas-tight seal. The sheet was held above the soil by a wire supported on posts down the centre of each plot. The methyl bromide was measured and vaporised by an apparatus described by Hague et al (1964) and distributed under the sheet through a metal T-piece connected to tubes perforated at 18" intervals.

It is commercial practice to introduce methyl bromide in one lb cans, resting in small containers, which are placed in position before the polythene sheet is sealed. A sharp blow through the sheet is sufficient to pierce the cannister and allow the liquid methyl bromide to escape into the trough from where it evaporates to spread over and penetrate the soil.

All treatments were made at the dosage rate of 1 lb/100 sq ft.

Determination of Methyl Bromide

Hague et al (1964) measured the distribution of methyl bromide using a thermal conductivity meter. Although adequate for the high concentrations normally experienced during fumigations this meter was unable to follow methyl bromide concentrations below about 1 mg/l during the final phase of airing and moreover tended to overestimate low concentrations.

In these experiments greater sensitivity was achieved by using a gas chromatographic technique with a halide sensitive detector (Goulden et al 1963) coupled in the more sensitive circuit with amplifier and recorder. The column was a short length of stainless steel capillary 45 mm long x 2 mm internal diameter packed with one part silica gel to 9 parts Celite 545, both components being between 100-200 mesh : with nitrogen as a carrier gas at a temperature of 20°C the flow rate was set at 100 ml/min. Air samples as large as 15 ml could be accommodated by this method though in general smaller volumes of 1-2 ml suffice.

Gas samples were withdrawn from spears set at 6, 9 and 18 in. The open ends of the spears, above ground, were sealed with a short length of silicone rubber tube stoppered at the open end with a short length of glass rod. The dead space was thus kept to a minimum and samples were taken directly from the spear by piercing the rubber with the syringe needle.

The heights of the methyl bromide peaks derived from these samples were compared with standards obtained by the injection of various volumes of a known concentration of methyl bromide contained within a 100 l glass flask.

RESULTS

Variation in Methyl Bromide Distribution with Method of Application

Hague et al (1964) showed that methyl bromide vapour, applied at one end of a polythene sheet, gave a poorer distribution of the gas in soil than when it was introduced uniformly down the length of the treated area through a perforated distribution pipe.

The commercial practice of introducing methyl bromide in 1 lb cans could also give a poorer distribution, similar to the release of vapour at the end of the sheet, in that, close to the canisters, the soil may receive higher doses than the more distant areas lying halfway between the points of application.

Several fumigations were carried out at different temperatures using both methods of application. During winter and early spring conditions, when the soil temperature at 6 inches was about 7°C, distribution of methyl bromide concentrations both near to the dishes containing the 1 lb cans and about 5 ft away were measured (Fig.1). The difference in the concentration-time products (C.T.P.) was very marked and it was noted that the methyl bromide took about 10 hours to evaporate completely. The distribution of methyl bromide introduced as vapour through a perforated tube gave a more normal curve.

In a later series of experiments when the soil temperature at 6 inches was 18°C and the air temperature under the polythene sheet was 47°C, no significant differences could be detected between the C.T.P.'s obtained from the two methods of application (Fig.2). However, even at these temperatures the methyl bromide from the 1 lb cans took about 1 hr to evaporate completely.

Fig. 1 Methyl bromide concentrations at 6" in soil fumigated under polythene with 1 lb cans. Soil temperature -7°C at 6", nominal dose 1 lb/100 sq ft.

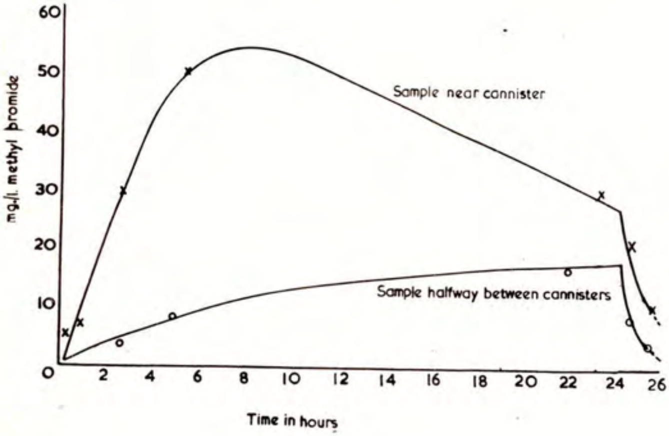
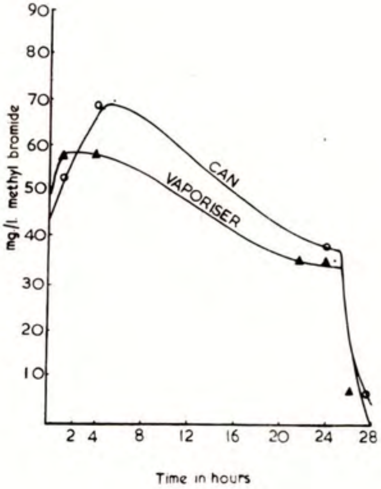


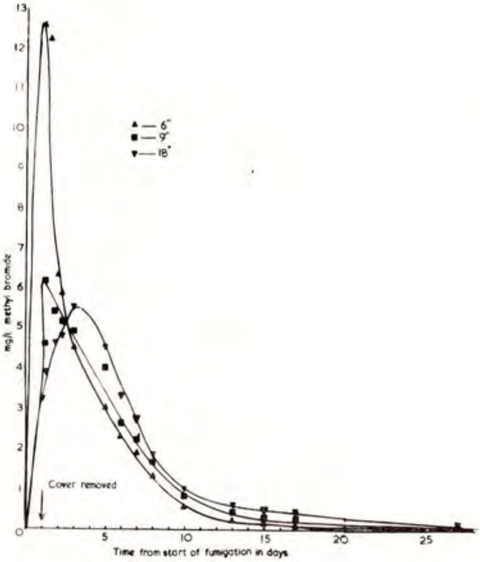
Fig. 2 Comparison of methyl bromide concentrations at 6" during fumigation by can and vaporizer technique. Soil temperature 18°C at 6", nominal doses 1 lb/100 sq ft.



Dissipation of Methyl Bromide during aeration

In another low temperature fumigation, concentrations were taken at 6", 9" and 18" - see Fig.3. The rate of penetration and aeration were greatly reduced producing much broader and flatter C.T. curves. Further, the maximum concentrations were lower than those found at higher temperatures. However the C.T.P.'s were similar to those obtained at higher temperatures since the lower concentrations were maintained for much longer periods during the aeration period. In the experiment illustrated in Fig.3 the C.T.P.'s, taken over a 20 day period, were 1000 mg hour/l and 780 mg hour/l at 6" and 18" respectively.

Fig. 3 Methyl bromide concentrations during fumigation and aeration, showing persistance of the gas at low temperatures. (7° at 6") Nominal dose 1 lb/100 sq ft.



Using the gas chromatographic technique described above it was possible to follow the dissipation of methyl bromide from soil after concentrations had fallen below the minimum detectable limit of the thermal conductivity meter. At low soil temperatures a concentration of 50 µg/l was reached after 17, 24 and 30 days at 6", 9" and 18" in the soil respectively : 1 µg/l was reached after about 35 days. At higher temperatures 50 µg/l was reached at 8, 12 and 15 days at the same levels in the soil.

Biological Effects of Methyl Bromide

The distribution of methyl bromide in the soil over a period of 4 days was estimated by Hague *et al* (1964) and a summary of the results are shown in Table 1.

Table 1

Cumulative concentration-time products (mg hour/l) obtained with a polythene sheet in position for 4 days

Depth in soil	24 hrs.	48 hrs.	72 hrs.	96 hrs.
Free-space	1710	2700	3390	3890
6"	760	1580	2130	2620
9"	500	1180	1750	2210
18"	120	250	390	530

Except at 18" these concentration-time products should control nematodes in soil - Hague & Sood (1963), Peachey et al (1963) and James & Hague (in press).

Although methyl bromide is not a particularly efficient soil fungicide it has recently been shown that tomatoes can grow satisfactorily in brown root rot infested soil treated with the gas - Read (1964) and James & Hague (in press), the latter authors reporting greatly increased yield of tomatoes at a dose of one lb per 100 sq ft for 24 hrs although G.S.F. was not controlled.

To estimate the effect of methyl bromide on the brown root rot of tomatoes infested soil was fumigated at a range of concentrations in a chamber. In the 1966 season tomatoes were grown in pots of soil artificially inoculated with G.S.F. and the resultant infested soil was bulked, sieved and mixed to provide a source of material for the present experiment. This infested soil, placed in flower pots, was fumigated at 20, 40 and 80 mg/l for 48 hrs to give a range of C.T.P.'s spanning those shown in Table 1 i.e. 960-3840 mg hour/l.

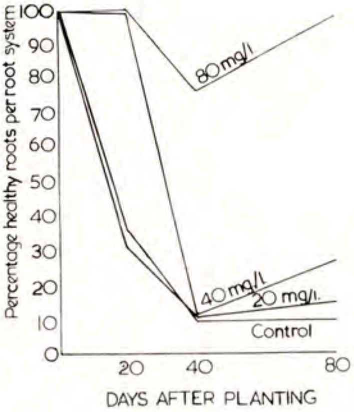
Tomato seedlings, variety Money Maker, of approximately equal size were planted one to each pot; replication was threefold for each of the three dates of assessment of the percentage of roots infected with G.S.F.

At 20, 40 and 80 days after planting the roots of each plant were carefully removed from the pots and washed free of all soil particles. They were laid on a cutting board and 1 cm bands of root were cut out every 4 cm down the length of the root system. The root pieces were separated into those which were healthy and those with brown root rot lesions. The numbers of each type were counted and the percentage of healthy roots plotted against time of treatment at each dosage (Fig.4)

It is clear from the results that only after exposure to a C.T.P. of nearly 4000 mg hour/l was the degree of infestation kept down to a low level.

In recent years many workers have assessed the effect of various sterilants on G.S.F. using different varieties of tomato. In the present experiment six tomato varieties, Sutton Leader, Ailsa Craig, Money Maker, Potentate, Eurocross A and Eurocross B were planted in G.S.F. infested soil and the roots assessed as previously for lesions. No significant differences were detected in the incidence of disease in the roots of the six varieties.

Fig.4 The development of fungal attack on tomato root systems growing in soils fumigated for 48 hrs at different concentrations of methyl bromide.



DISCUSSION

These studies on the distribution of methyl bromide in soil reveal that penetration will become more uneven at low temperatures. It seems clear that distribution is more likely to be uneven when methyl bromide is applied by can which may well explain some of the failures experienced in practice. The slower penetration and aeration of soil shown in Fig.3 also indicate that phytotoxicity may be a problem if fumigations are done at low temperatures unless there is a considerable time interval between treatment and planting - the main disadvantage one is trying to avoid in methyl bromide treatment.

No significant differences could be detected in C.T. curves for the two methods of application at high temperatures which suggests that commercial fumigation should be done immediately <u>after</u> the tomato crop and not just before planting when the temperatures may be quite low. The temperatures at which distribution of methyl bromide becomes uneven probably also depend to a large extent on soil type.

The results of studying the dissipation of methyl bromide at low concentrations confirmed the expected behaviour of the gas during the aeration period i.e. that the concentrations continue to decay approximately exponentially with time though concentrations below about 0.5 mg/l contribute little to the overall C.T.P. even when spread over a number of days at low temperatures.

The fumigation of G.S.F. infested soil confirmed that it is extremely difficult to kill the fungus with methyl bromide.

Fumigation at 1 lb/100 sq ft for 48 hrs only produces C.T.P.'s in the range of 1100-1600 mg hour/l (See Table 1) while it is clear that a C.T.P. of about 4000 mg hour/l is required to effectively control the fungus. Although treatment for 24 hrs gives a good growth response (James & Hague, in press) much higher

C.T.P.'s are required to give complete control which may persist for more than one year.

Thus, it is recommended that, for control of G.S.F., fumigation should be done at the end of cropping under the highest possible temperatures and that to obtain a C.T.P. of approximately 4000 mg hour/l down to about 12" in the soil, the time of exposure should be at least 4 days.

Acknowledgements

The authors would like to thank Miss L. Burdett-Clark who carried out the experiments concerned with the fumigation of G.S.F. infested soil.

References

GOULDEN, R., GOODWIN, E.S., & DAVIES, L. (1963) The Analyst 88, 951.
HAGUE, N.G.M., LUBATTI, O.F., & PAGE, A.B.P. (1964) Hort.Res.3, 84.
HAGUE, N.G.M. & SOOD, U. (1963) Pl.Path.12, 88.
JAMES, G.L., & HAGUE, N.G.M. (1967) Pl.Path.16, (in press)
PEACHEY, J.E., RAO, G.N., & CHAPMAN, M.R. (1963) Ann.appl.Biol.52, 19.
READ, W.H. (1965) Rep.Glasshouse Crops Res.Inst. 1964, 98.

OBSERVATIONS ON THE CHEMICAL CONTROL OF PESTS ON YEAR-ROUND CHRYSANTHEMUMS

C.R. Worthing
Glasshouse Crops Research Institute, Littlehampton

Summary Thirty-seven pesticides were examined on year-round chrysanthemums as protectants against *Myzus persicae* and *Tetranychus urticae*. In further trials of the more promising compounds demeton-S-methyl, Du Pont 1179, oxydemeton-methyl, and Union Carbide 21149 gave consistently good results against *M. persicae*; several other insecticides were effective but too phytotoxic. Binapacryl, dinobuton, Du Pont 1179, Du Pont 1642, quinomethionate, Union Carbide 20047A and Union Carbide 21149 gave excellent kills of *T. urticae*; Du Pont 1179, Du Pont 1642 and Union Carbide 21149 also controlled *Phytomyza atricornis*.

Sprays and soil drenches of demeton-S-methyl and oxydemeton-methyl were studied at the commercial rates using bioassay with *M. persicae* and chemical analyses of the foliage and buds. Whichever compound was applied the toxicant was present as the sulphoxide (85 to 90%) and the sulphone (15 to 10%). Some factors influencing the persistence of the toxicants were noted. With drenches demeton-S-methyl persisted longer in the plants than oxydemeton-methyl; with sprays the reverse was true. By either route oxydemeton-methyl was less effective in controlling aphids on the flower buds. Satisfactory kill of *M. persicae* by drenches required about 24 p.p.m. of toxicant in the foliage.

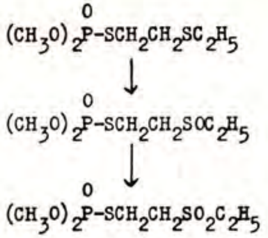
INTRODUCTION

The continuous glasshouse culture of year-round chrysanthemums has predictably led to a number of pest problems. Growers have encountered difficulty in the control of the peach-potato aphid (*Myzus persicae*), the glasshouse red spider mite (*Tetranychus urticae*) and the chrysanthemum leaf miner (*Phytomyza atricornis*). On this host under glasshouse conditions *M. persicae* continues to reproduce throughout the year, and *T. urticae* does not enter diapause. *M. persicae* has only become a pest of chrysanthemums since the introduction of year-round culture, and although several other aphid species occur on this crop they are easily controlled by most aphicides. Resistance to organophosphorus pesticides is widely encountered in *T. urticae* and has also been demonstrated in *M. persicae* on chrysanthemums (Gould, 1966; Wyatt, 1966). The difficulty of obtaining adequate coverage of foliage when applying H.V. sprays of contact pesticides in this densely-planted crop (planting distances 5 inches by 6 inches) grown with supporting wires has been stressed (Read, 1966); suitable systemic compounds would therefore seem to be the simplest answer for the control of these pests.

Thirty-seven commercial and experimental compounds were tested on the chrysanthemums Bluechip, BGA Dawn Star and BGA Tuneful. Observations on *M. persicae* and *T. urticae* were made on BGA Tuneful since populations of both pests are known to increase rapidly on this cultivar.

Two aphicides, demeton-S-methyl and oxydemeton-methyl, found to be of value against *M. persicae*, are of interest in that the former is known (Mühlmann and Tietz, 1956) to be oxidized readily to the latter in plants (Fig.1.). However, there seem to be some differences in the behaviour of the two compounds in chrysanthemums (Worthing, in press), and the biological performances of the two insecticides were therefore compared with the persistences of the toxic sulphoxide and sulphone, as determined by chemical analyses, in the foliage and buds.

Fig. 1. Oxidation of demeton-S-methyl in plants
(Mühlmann and Tietz, 1956)



$(CH_3O)_2\overset{O}{P}-SCH_2CH_2SC_2H_5$ demeton-S-methyl (thioether)

$(CH_3O)_2\overset{O}{P}-SCH_2CH_2SOC_2H_5$ oxydemeton-methyl (sulphoxide)

$(CH_3O)_2\overset{O}{P}-SCH_2CH_2SO_2C_2H_5$ dioxydemeton-S-methyl (sulphone)

METHODS

Materials The compounds examined included : *S*-(2-acetamidoethyl) *O,O*-dimethyl phosphorodithioate, arprocarb, binapacryl, carbaryl, demeton-S-methyl, dinobuton, disulfoton, Du Pont 1179 [*S*-methyl *N*-(methylcarbamoyloxy)thioacetamidate], Du Pont 1642 [*S*-methyl *N*-(carbamoyloxy)thioacetamidate], ethoate-methyl, fenthion, formothion, GC 6506 (dimethyl 4-methylthiophenyl phosphate), isolan, mecarbam, menazon, methiocarb, morphothion, naled, NC 1493 (4-dimethylamino-2-isopropyl-5-methylphenyl *N*-methylcarbamate), nicotine, oxydemeton-methyl, phorate, phosphamidon, PSP 204 (*S*-ethylsulphinylmethyl *O,O*-diisopropyl phosphorodithioate), quinomethionate, schradan, sophamide, tetradifon, thionazin, trichlorphon, Union Carbide 20047A [3-chloro-6-cyano-2-norbornanone *O*-(methylcarbamoyl)oxime] Union Carbide 21149 [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] and vamidothion. All dosages are given in terms of the active ingredient. The trials with demeton-S-methyl and oxydemeton-methyl were made using the emulsifiable concentrates available commercially containing 26% and 56% a.i. (w/v), respectively.

Plant culture For the preliminary examination BGA Tuneful was used with three plants per 9 inch pot. Compounds formulated as granules were mixed into the top three inches of compost just before, or sprinkled over the surface just after, the rooted cuttings were planted. Most pesticides were applied as soil drenches and foliar sprays (avoiding contact with the leaves and soil, respectively) three to four weeks after the cuttings were planted. Further trials with the more promising materials were made on BGA Tuneful or Giant No. 4 White Indianapolis, cuttings of which were planted in the border soil at the normal density (5 inches by 6 inches). Drenches were applied at 2 gal/yd^2, granules incorporated as before and sprays applied with a pressure sprayer (2 kg/cm^2).

Aphicidal properties The aphicidal activity of the treatments was assayed by caging adult apterous *M. persicae* on the undersides of leaves near the top of the plants (Worthing, in press). A strain of this aphid from a local chrysanthemum nursery and which was known to show resistance to organophosphorus and organochlorine contact insecticides, was used. In the trials with border-planted chrysanthemums the plots were infested with *M. persicae* about ten days after the cuttings were planted, and routine counts of this and other aphid spp. were made on sample plants.

Acaricidal properties The plants were infested with *T. urticae* about ten days after the cuttings were planted. Counts of dead and live mites were made about a week after the treatments had been applied (later in the case of granules) if some of the mites appeared to have been killed. Studies of egg viability were also made.

Effects on leaf miner The incidence of feeding marks and mines was noted in trials where *P. atricornis* was present either as a deliberate or a natural infestation.

Statistics Five *M. persicae* were used per cage in the bioassays. The numbers of live aphids in each cage were recorded after four days, and transformed to square roots for statistical analysis. The pooled results for each trial were shown to be homogeneous by the Bartlett test.

Chemical analysis for demeton-S-methyl, oxydemeton-methyl and dioxydemeton-S-methyl
The foliage for chemical determinations of the toxicants was taken from the same level of the plants as that used for the simultaneous bioassays. The surface insecticide deposit on the foliage was removed by immersing the leaves in benzene, and the internal content isolated by a method similar to that of Tietz and Frehse (1960). The leaves were macerated in acetone and the toxicants separated from the plant materials by liquid-liquid partition followed by "clean-up" on a column containing charcoal. The thioether, sulphoxide and sulphone were separated by thin-layer chromatography and determined by estimating the phosphorus contents of the appropriate zones of the chromatograms compared with those derived from untreated plants. Details of the method will be reported elsewhere.

RESULTS

Preliminary trials As fuller details of the preliminary trials are being published elsewhere (Worthing, in press) only the essential points will be mentioned here. Union Carbide 21149 was active against M. persicae throughout the life of the crop, whilst Union Carbide 20047A, which was phytotoxic at the high rate used in the drench treatment, merits examination at lower dosages. However, the durations of protection shown in Table 1 must be interpreted with care (see Discussion). Good aphicidal activity was also shown by arprocarb, ethoate-methyl, formothion and isolan, but these compounds were too phytotoxic on the cultivars under test to warrant further work at present.

Table 1.

Preliminary trials on BGA Tuneful - performances of some compounds effective against M. persicae

Treatment	Rate	Approximate duration of protection (days)
Granules	(mg/pot)	
PSP 204	100	30-35
Union Carbide 21149	50	> 70
Drenches		
Demeton-S-methyl	50	17-25
Du Pont 1179	50	10-17
Du Pont 1642	50	< 9*
Oxydemeton-methyl	50	14-20
Union Carbide 20047A	200	> 27*
Sprays	(%)	
Demeton-S-methyl	0.02	14-16
Du Pont 1179	0.02	8-12
Du Pont 1642	0.04	< 9*
Oxydemeton-methyl	0.02	10-14
Union Carbide 20047A	0.04	9-18*

* Result obtained on Giant No. 4 White Indianapolis, so not strictly comparable with the other figures

Trial August-November 1966 BGA Tuneful cuttings were planted in the border soil on 3 August. The brick earth soil had a pH of 7.3 and 3.4% organic carbon. Two plots (8 x 8 plants) were used for each treatment in a randomised block layout. The mean numbers of M. persicae per plant (Table 2) show definite trends, but the numbers were too low to justify statistical analysis. Hence the efficiencies of treatments were also assessed by caging this aphid on the plants, the results for the soil applications are in Table 3. Of the granule treatments only Union Carbide 21149 gave a long duration of protection, lasting almost until the flowers were cut. Demeton-S-methyl and Du Pont 1179 were the only drenches active against M. persicae;

these two compounds and oxydemeton-methyl were also effective as sprays. It is interesting to note that all drenches and sprays completely eradicated a natural infestation of the recognised chrysanthemum aphid (Macrosiphoniella sanborni), and that this aphid did not appear on plots where granules had been applied.

Table 2.

Aphicides - effect on populations of M. persicae

Cuttings (BGA Tuneful) planted 3 August 1966

Treatment and date applied	Dosage	Mean number of aphids/plant on				
		August		September		October
		24	30*	7	20	6
Untreated	-	2.6	4.6	8.5	10.9	5.1
Granules (2 August)	(lb/acre)					
PSP 204	20	0.5	-	0	1.1	0.5
PSP 204	25	1.2	-	1.6	4.5	0.1
Union Carbide 21149	15	0	-	0	0.2	0
Drenches (31 August)						
Demeton-S-methyl	12.6	-	5.1	1.8	1.4	-
Du Pont 1179	15	-	8.8	0	0	0
Menazon	10	-	6.0	8.0	9.9	-
Menazon	20	-	3.6	5.9	2.1	-
Vamidothion	25	-	6.8	4.7	3.8	-
Sprays (31 August)	(%)					
Demeton-S-methyl	0.02	-	4.5	0.2	2.2	-
Du Pont 1179	0.04	-	2.6	0	0.7	0.7
Oxydemeton-methyl	0.02	-	3.2	0.9	0.8	-

* Pretreatment count

It was noticed that Union Carbide 21149 and the drench of Du Pont 1179 kept the plants completely free from T. urticae and P. atricornis that had been introduced into the glasshouse. The spray of the latter carbamate was also effective against these two pests, though the duration of protection was shorter than for the drench. The untreated and the majority of the other treated plots had very high infestations of these pests. PSP 204 and the higher dosage of menazon also gave some protection against P. atricornis.

Trial June-August 1967 In view of the excellent results obtained with Union Carbide 21149 this compound was examined at much lower dosages, namely 1, 2 and 4 lb/acre. Cuttings of Giant No. 4 White Indianapolis were planted in the border soil on 1 June, granules (2% a.i.) of Union Carbide 21149 having been pricked into the soil (pH 6.6; 3.3% organic carbon) with the fertilizer base the previous day. The crop was infested with M. persicae a week after planting by placing in the glasshouse other plants on which there were many alate forms. Heavy infestations developed on the plots where no granules had been used; there were few live aphids but some dead alatae on the carbamate-treated plots (Table 4).

The untreated plots were sprayed with demeton-S-methyl and oxydemeton-methyl on 28 June. These two compounds were equally effective in reducing the aphid populations in this trial. Caging of M. persicae confirmed that the aphicidal potency persisted longer with oxydemeton-methyl than with demeton-S-methyl. No general build-up of M. persicae populations took place (Table 4) when the protective effect of the sprays had declined (as judged by caging assay). A contributory factor here was the effect of the fungus, Entomophthora coronata, where the populations had been dense (Hussey, 1967; Worthing, 1967b). At the time the flower buds were opening the only treatment to have an appreciable number of M. persicae was the lowest dosage of Union Carbide 21149.

There was also a natural infestation of another aphid (Aphis sp.) which attacked the opening buds a few days after sprays of several carbamates had been applied. The blooms from the granule-treated plants showed no toxicity to this Aphis sp., neither did those where the sprays listed in Table 5 had been applied.

Table 3.

Duration of protection by soil treatments - challenge by caging M. persicae

Cuttings (BGA Tuneful) planted 3 August 1966

Treatment and date applied	Dosage (lb/acre)	Mean square root of number of aphids per cage*							
		Aug. 26	Sept. 6	Sept. 13	Sept. 23	Sept. 30	Oct. 18	Oct. 21	Oct. 28
Untreated	-	3.85^a	3.38^b	3.05	4.22	4.17	2.45^a	2.93^a	2.47^a
Granules (2 August)									
PSP 204	20	3.75	-	2.78	-	-	-	-	-
PSP 204	25	3.85	-	3.01	-	-	-	-	-
Union Carbide 21149	15	0.17	-	0.50	0.74	1.91	1.33	-	2.51
Drenches (31 August)									
Demeton-S-methyl**	12.6	-	1.54	3.00	-	-	0.95	1.19	2.16
Du Pont 1179**	15	-	0.46	1.30	3.04	3.70^c	1.50	2.50	2.34
Menazon	10	-	2.74	2.44	-	-	-	-	-
Menazon	20	-	2.85	2.46	-	-	-	-	-
Vamidothion	25	-	3.41^c	2.77	-	-	-	-	-

*Five aphids per cage exposed for 4 days;
6 replicates unless otherwise stated, a = 8, b = 7, c = 5 replicates

No. of replicates	(8 + 6)	(7 + 6)	(6 + 6)	(7 + 5)	(6 + 5)
LSD 5%	0.88	0.91	0.94	0.96	0.99
LSD 1%	1.19	1.21	1.27	1.29	1.33
LSD 0.1%	1.57	1.62	1.68	1.71	1.77

** 2nd application made 12 October

A natural infestation of P. atricornis occurred part way through the trial. Sprays of carbamates were applied on 21 July and the effect on this pest was observed (Table 5).

Control of T. urticae. In addition to the carbamates for which the results are given in Table 6 a number of trials have been made with contact acaricides on mite-infested plants. Excellent kills (90-100%) were obtained with sprays of binapacryl (0.005%), dinobuton (0.05%) and quinomethionate (0.0125%). Thus sprays of these compounds and of the carbamates in Table 6 show promise for the control of this pest. Cases of phytotoxicity with binapacryl (0.005%) and with the two Du Pont carbamates (0.04%) were sometimes observed, however.

Chemical observations on demeton-S-methyl and oxydemeton-methyl Using the commercially recommended rates drenches and sprays of these two aphicides have been compared in several trials. It is clear that demeton-S-methyl was rapidly oxidised in the chrysanthemum plant (cf Fig.1). Of the toxicants isolated the main component was the sulphoxide (85 to 90%) together with some sulphone (15 to 10%); when demeton-S-methyl was used only traces (<3%) of the thioether were found.

These toxic oxidation products persisted longer in the foliage when a drench of demeton-S-methyl (12.6 lb/acre) (Fig. 2a) was applied than was the case with an equivalent quantity of oxydemeton-methyl (13.5 lb/acre) (Fig. 2b). The figures show the rapid decline in the concentration of toxicants (expressed as µg total insecticidal phosphorus/g fresh foliage) in the rapidly developing growth of young plants in summer. The mean numbers of live aphids per cage following four days' exposure on the treated plants are included in Fig. 2. The higher toxicant content in the case of demeton-S-methyl (Fig. 2a) is seen to be reflected in a greater toxicity to <u>M. persicae</u> than is the case with oxydemeton-methyl (Fig. 2b). These results were obtained on Giant No. 4 White Indianapolis, the cuttings of which were planted in the border soil (pH 6.8; 2.4% organic carbon) on 7 April, 1966, the drenches being applied 27 days later.

Table 4.

<u>Aphicides - effect on populations of</u> M. persicae

Cuttings (Giant No. 4 White Indianapolis) planted 1 June 1967

Treatment	Aphid category*	% of plants** in each category on				
		June 22	July 7	July 12	July 19	August 3
Untreated	0	0	0	17	75	-
	1-3	0	25	25	8	-
	4-8	0	0	8	0	-
	>8	100	75	50	17	-
<u>Granules</u> (applied 31 May)						
Union Carbide 21149 1 lb/acre	0	46	59	-	79	79
	1-3	33	25	-	8	4
	4-8	13	8	-	0	0
	>8	8	8	-	13	17
Union Carbide 21149 2 lb/acre	0	87	63	-	88	79
	1-3	13	29	-	4	4
	4-8	0	8	-	4	13
	>8	0	0	-	4	4
Union Carbide 21149 4 lb/acre	0	79	96	-	100	92
	1-3	21	4	-	0	8
	4-8	0	0	-	0	0
	>8	0	0	-	0	0
<u>Sprays</u> (applied 28 June)						
Demeton-S-methyl 0.02%	0	0	17	71	83	-
	1-3	0	46	29	17	-
	4-8	0	29	0	0	-
	>8	100	8	0	0	-
Oxydemeton-methyl 0.02%	0	0	12	83	79	-
	1-3	0	52	17	21	-
	4-8	0	24	0	0	-
	>8	100	12	0	0	-

* No. of aphids/plant, top 10 leaves examined

** 24 replicates per treatment

Foliar sprays (0.02%) of these two insecticides were also compared (Fig. 3). The chemicals were measured as the foliar surface deposit, as removed by 30 seconds' immersion in benzene, and the internal content by maceration in acetone of the pre-dipped leaves. The toxicants from oxydemeton-methyl (Fig. 3b) were more persistent than those from demeton-S-methyl (Fig. 3a), as was also reflected in the bioassays. These results were obtained on BGA Tuneful plants sprayed on 12 October, 1966, the cuttings having been planted on 3 August. The plants were making relatively little vegetative growth when treated, hence the longer life of the toxicants in the foliage.

Table 5.

Activity of some carbamates against P. atricornis

Cuttings (Giant No. 4 White Indianapolis) planted 1 June 1967

Treatment and date applied	Rate	Assessment on 7 August of infestation of *P. atricornis*
Granules (30 May)	(lb/acre)	
Union Carbide 21149	1	Control failed at end of crop
Union Carbide 21149	2	Complete control, no mines or feeding marks
Union Carbide 21149	4	
Sprays (21 July)	(%)	
Du Pont 1179	0.02	Complete control, only dead larvae found
Du Pont 1179	0.04	
Du Pont 1642	0.02	
Du Pont 1642	0.04	
Union Carbide 20047A	0.02	Partial control, some live larvae and pupae found
Union Carbide 20047A	0.04	

Table 6.

Activity of some carbamates against T. urticae

Cuttings (Giant No. 4 White Indianapolis) planted 1 June 1967

Treatment and date applied	Rate	Assessment on 7 August of activity against *T. urticae*
Granules (30 May)	(lb/acre)	
Union Carbide 21149	1	Not infested artificially, very few mites present
Union Carbide 21149	2	
Union Carbide 21149	4	Infested artificially, very few mites present
Sprays (21 July)	(%)	
Du Pont 1179	0.02	Kill of adults (16%) and young (72%)
Du Pont 1179	0.04	Kill of adults (95%) and young (94%)
Du Pont 1642	0.02	Poor kill of adults(15%) and young (0%)
Du Pont 1642	0.04	Kill of adults (89%) and young (93%)
Union Carbide 20047A	0.04	Kill (100%) of adults, young and eggs

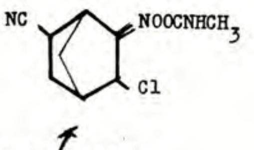
DISCUSSION

It is important to obtain complete control of all pests before the flower buds open. It was thought that this would be achieved most readily by using a compound at an earlier stage that rendered the plants toxic for a reasonable period. Surprisingly, most of the insecticides examined showed a short duration of protection (if any), as judged by the caging assay with the resistant strain of *M. persicae*. This result does not mean that the compounds would be useless against this aphid present on the plants at the time of treatment, especially in the case of sprays, or against susceptible strains of this or other aphid spp. - note, for instance, the effects of the treatments in Table 2 against M. persicae and M. sanborni. The caging assays were made as high on the plant as practicable, so that newer growth was examined at each successive caging. This will select those compounds that are translocated to the top of the plant, especially the growing tip, and that persist on those expanding leaves in concentrations toxic to M. persicae. The translocation of some of the aphicides examined to the growing tip may not be sufficient to continue to protect the new growth. Unfortunately, a number of the effective compounds proved to be too damaging to the cultivars examined to justify further work.

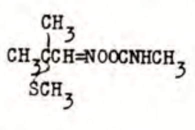
It is clear that the durations of protection given to plants grown in pots in the preliminary examination were far higher than those for plants grown in the border soil, hence the results in Table 1 should not be taken as applicable to commercial conditions. The difference could be due to the ease in obtaining a complete coverage of the foliage when spraying individual pots compared to the difficulty of spraying satisfactorily the dense canopy of foliage of a plot in the border soil. The pots may also represent a semi-artificial environment when soil treatments are used for the toxicant might be stored on the pot surface and released slowly, or be less liable to biological breakdown in the sterilised compost.

The most effective aphicides of those commercially available are demeton-S-methyl and oxydemeton-methyl, but four experimental carbamates have shown great promise in the trials to date. The latter group of compounds are chemically similar in that they are derivatives of O-carbamoylhydroxylamine (Fig. 4). These carbamates are potentially of considerable value for the control of pests on chrysanthemums because they are effective against T. urticae and P. atricornis in addition to M. persicae. This broad spectrum of activity would enable a grower to control all of these pests with one pesticide application, but might be a disadvantage in integrated chemical and biological control programmes. It is to be hoped that these carbamates will still prove effective should M. persicae and other pests become more resistant to the organophosphorus compounds in use at present. Union Carbide 21149, incorporated in the soil before planting at rates of about 2 to 4 lb (a.i.)/acre, would seem to offer the possibility of protecting the crop from several pests throughout its life, and may be of particular value to producers of cuttings. This compound has previously been reported to control pests on chrysanthemums (Worthing, 1967a; Baranowski, 1967).

Fig. 4. Some experimental carbamate insecticides

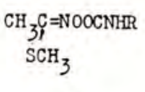


Union Carbide
20047A



$$CH_3-\underset{SCH_3}{\overset{CH_3}{\overset{|}{C}}}-CH{=}NOOCNHCH_3$$

Union Carbide
21149



$$CH_3\underset{SCH_3}{C}{=}NOOCNHR$$

Du Pont 1179, R=CH_3
Du Pont 1642, R=H

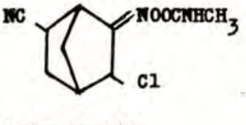
Its failure to control a very late infestation of aphids in the flowers is not surprising, since few systemic insecticides would be translocated to blooms. Union Carbide 20047A, Du Pont 1179 and Du Pont 1642 all show good activity against M. persicae, P. atricornis and T. urticae, but slight marginal scorch has sometimes occurred with the Du Pont materials as sprays (0.04%) or drenches (15 lb/acre). Clearly all these compounds will require examination on a wide range of chrysanthemum cultivars before their use can be recommended.

The non-systemic acaricides, binapacryl, dinobuton and quinomethionate, have against susceptible strains of this or other aphid spp. - note, for instance, the effects of the treatments in Table 2 against M. persicae and M. sanborni. The caging assays were made as high on the plant as practicable, so that newer growth was examined at each successive caging. This will select those compounds that are translocated to the top of the plant, especially the growing tip, and that persist on those expanding leaves in concentrations toxic to M. persicae. The translocation of some of the aphicides examined to the growing tip may not be sufficient to continue to protect the new growth. Unfortunately, a number of the effective compounds proved to be too damaging to the cultivars examined to justify further work.

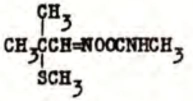
It is clear that the durations of protection given to plants grown in pots in the preliminary examination were far higher than those for plants grown in the border soil, hence the results in Table 1 should not be taken as applicable to commercial conditions. The difference could be due to the ease in obtaining a complete coverage of the foliage when spraying individual pots compared to the difficulty of spraying satisfactorily the dense canopy of foliage of a plot in the border soil. The pots may also represent a semi-artificial environment when soil treatments are used for the toxicant might be stored on the pot surface and released slowly, or be less liable to biological breakdown in the sterilised compost.

The most effective aphicides of those commercially available are demeton-S-methyl and oxydemeton-methyl, but four experimental carbamates have shown great promise in the trials to date. The latter group of compounds are chemically similar in that they are derivatives of O-carbamoylhydroxylamine (Fig. 4). These carbamates are potentially of considerable value for the control of pests on chrysanthemums because they are effective against T. urticae and P. atricornis in addition to M. persicae. This broad spectrum of activity would enable a grower to control all of these pests with one pesticide application, but might be a disadvantage in integrated chemical and biological control programmes. It is to be hoped that these carbamates will still prove effective should M. persicae and other pests become more resistant to the organophosphorus compounds in use at present. Union Carbide 21149, incorporated in the soil before planting at rates of about 2 to 4 lb (a.i.)/acre, would seem to offer the possibility of protecting the crop from several pests throughout its life, and may be of particular value to producers of cuttings. This compound has previously been reported to control pests on chrysanthemums (Worthing, 1967a; Baranowski, 1967).

Fig. 4. Some experimental carbamate insecticides

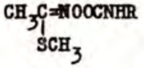


Union Carbide 20047A



$$CH_3\overset{CH_3}{\underset{SCH_3}{C}}CH{=}NOOCNHCH_3$$

Union Carbide 21149



$$CH_3\underset{SCH_3}{C}{=}NOOCNHR$$

Du Pont 1179, R=CH_3
Du Pont 1642, R=H

Its failure to control a very late infestation of aphids in the flowers is not surprising, since few systemic insecticides would be translocated to blooms. Union Carbide 20047A, Du Pont 1179 and Du Pont 1642 all show good activity against M. persicae, P. atricornis and T. urticae, but slight marginal scorch has sometimes occurred with the Du Pont materials as sprays (0.04%) or drenches (15 lb/acre). Clearly all these compounds will require examination on a wide range of chrysanthemum cultivars before their use can be recommended.

The non-systemic acaricides, binapacryl, dinobuton and quinomethionate, have shown excellent kills of T. urticae, although complete coverage of the foliage is essential if this pest is to be eliminated. Binapacryl has shown complete kills of all stages of T. urticae even at 0.005%; phytotoxicity has been observed at higher rates and, on one occasion, at 0.005% during very hot weather (cf Anon, 1967). Of these acaricides only quinomethionate is at present approved for use on chrysanthemums, but it is liable to damage the petals on open blooms of some cultivars.

Although the use of demeton-S-methyl gives rise within the plants to the same toxicants, namely the sulphoxide and sulphone, as does oxydemeton-methyl there are clearly important differences between the performances of these two insecticides on this crop. Drenches of these insecticides gave a longer duration of protection than sprays, but the former require a greater quantity of toxicant and so cost more. If drenches are to be used demeton-S-methyl is the compound of choice. Under winter conditions, however, it does not reach a maximum concentration in the foliage until about four days after application, so it may be slow to kill aphids under these conditions. Its superiority, as a drench, to oxydemeton-methyl could be due to more selective translocation to the growing tip of the plant or to greater stability in the soil.

In contrast, when these two materials were applied as H.V. sprays (0.02%) the deposit with oxydemeton-methyl was greater and more persistent than that with demeton-S-methyl. A similar result is known with sprays on apples (Maier-Bode, 1965), but if the nature of the leaf surface is a factor this difference may not apply to all plant spp. However, sprays of oxydemeton-methyl are virtually useless against M. persicae on flower buds, whilst sprays of demeton-S-methyl give effective control. Possibly demeton-S-methyl shows a greater contact toxicity. If so, sprays of oxydemeton-methyl, whilst showing a greater persistence of toxicity, might be inferior to demeton-S-methyl in their initial contact action and overall kill of aphids. In a few trials to date there does not appear to have been much difference between the relative control given by the two compounds before the flower buds are clearly visible. Oxydemeton-methyl is a less phytotoxic material on a number of sensitive chrysanthemum cultivars.

With both soil drenches and foliar sprays, the toxicants persist longer in the foliage when it is growing more slowly. Thus their life is longer in old leaves at the bottom than in young new ones at the top of a plant. They are more persistent in plants coming into flower and so making less vegetative growth, and in winter compared with summer. This is possibly due to two effects: the toxicants will be less readily diluted in the slower-growing foliage, and also may be metabolised more slowly to non-toxic compounds.

From a number of trials with drenches it seems that effective toxicity to the resistant strain of M. persicae used in this work requires about 3 p.p.m. of total insecticidal phosphorus, that is 24 p.p.m. expressed as sulphoxide. The corresponding value for sprays is somewhat greater; this suggests that less of the toxicants penetrating into the leaf are available to aphids caged thereon than is the case with soil applications where translocation may occur only to specific sites in the foliage.

Acknowledgements

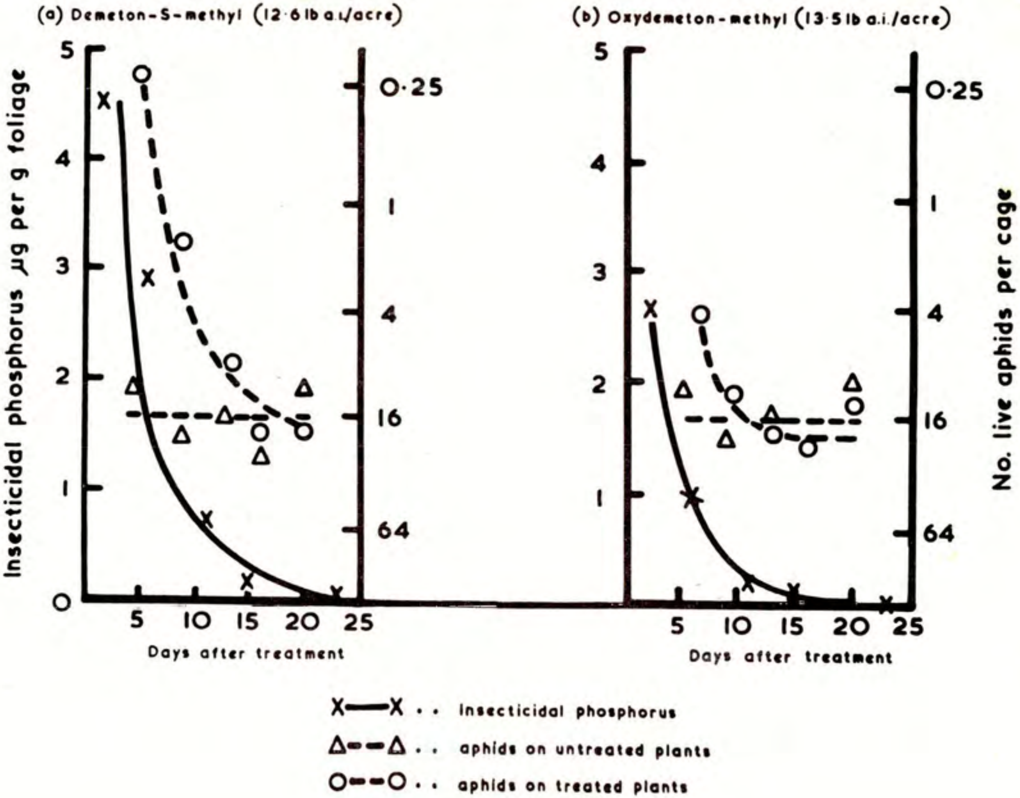
The author wishes to thank the many firms, too numerous to list here, for gifts of pesticides and their metabolites, Miss B. Gurney and Dr. N.W. Hussey for making the assessments on leaf miner, P. Bocion and Mrs. E.S. Walker for technical assistance, W.E. Betts for the culture of the plants, and W.H. Read for his advice and encouragement.

References

Anon. (1967) DCK Information, No. 23, May, p. 2.
Baranowski, R.M. (1967) Proc. Fla St. hort. Soc. 1966, 79, 478.
Gould, H.J. (1966) Pl. Path. 15, 109.
Hussey, N.W. (1967) Rep. Glasshouse Crops Res. Inst. 1966, in press.
Maier-Bode, H. (1965) Mitt. biol. BundAnst. Ld.-u. Forstw. 115, 91.
Mühlmann, R. and Tietz, H. (1956) Höfchenbr. Bayer PflSchutz-Nachr. 9, 116.
Read, W.H. (1966) Proc. 3rd. Brit. Insecticide & Fungicide Conf. 1965, p. 377.
Tietz, H. and Frehse, H. (1960) Höfchenbr. Bayer PflSchutz-Nachr. 13, 212.
Worthing, C.R. (1967a) Comml Grow. 24 March, p. 617.
Worthing, C.R. (1967b) Rep. Glasshouse Crops Res. Inst. 1966, in press.
Worthing, C.R. (in press). J. hort. Sci.
Wyatt, I.J. (1966) Proc. 3rd Brit. Insecticide and Fungicide Conf. 1965, p. 52.

Fig. 2

PERSISTENCE OF INSECTICIDE IN FOLIAGE AFTER SOIL APPLICATION

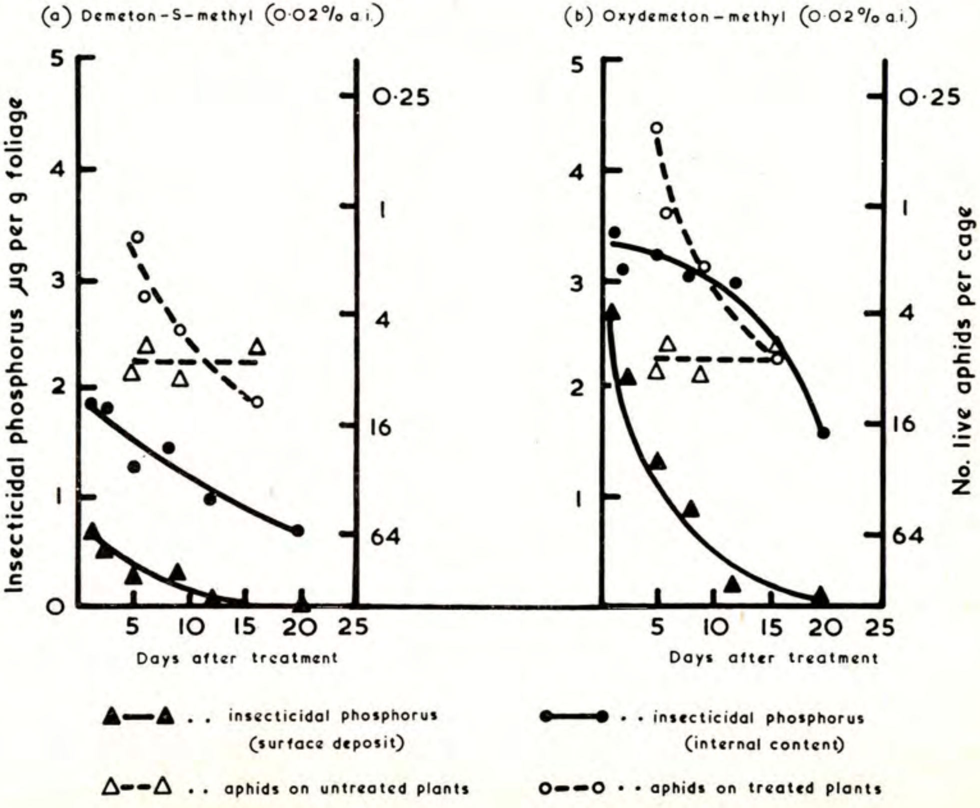


(a) Demeton-S-methyl (12·6 lb a.i./acre)

(b) Oxydemeton-methyl (13·5 lb a.i./acre)

Fig. 3

PERSISTENCE OF INSECTICIDE IN FOLIAGE AFTER SPRAY APPLICATION


(a) Demeton-S-methyl (0·02% a.i.)

(b) Oxydemeton-methyl (0·02% a.i.)

CONSIDERATIONS IN THE USE OF PESTICIDES IN MUSHROOM CULTURE

N.W. Hussey
Glasshouse Crops Research Institute, Littlehampton

Summary

From a consideration of the life-cycles of the principle groups of mushroom pests it is concluded that, by incorporating pesticides in the compost or casing, control can be achieved through the elimination of the developmental stages.

Experimental data shows that admixture of up to 100 p.p.m. of most pesticides will not affect cropping, though treatment of the casing may be more hazardous. At these concentrations residue problems are unlikely to arise in the harvested mushrooms.

INTRODUCTION

More pesticides are applied to the mushrooms crop than to any other grown in a protected environment. This situation arises from the mechanisms by which many of the pests find their host. Most pests of glasshouse vegetable crops have only limited capacity for locating host plants so that infestations arise, for the most part, entirely adventitiously. Normally, therefore, pesticide applications at 10-14 day intervals suffice to contain pest populations. The most important insect pests of mushrooms, however, are often attracted to the crop over considerable distances. Prevention of infestation is consequently difficult to achieve so that some pests almost inevitably become established. This situation is aggravated by the fact that the normal habitat of these pests is within the compost which cannot be adequately treated with pesticides once the beds are made up.

Additional complications are caused both by the fact that the mushroom itself is unusually sensitive to many chemicals and also that it must be sold to the consumer as rapidly as possible after harvest to prevent premature deterioration.

These considerations demand adherance to certain principles if efficient pest control is to be achieved.

TIMING OF APPLICATIONS

Efficient pest control depends on the application of the pesticide at the most vulnerable stage of pest development. This stage naturally varies with different insects and we must therefore examine each of the important groups of pests separately. For this purpose we can recognise four such groups namely phorids, sciarids, cecids and mites. Only the Worthing Phorid fly (Megaselia halterata) among the Phoridae is sufficiently important to occupy our attention but this species is almost universally regarded as the dominant insect pest of the mushroom crop. In a series of papers (Hussey, 1961 & 1962 and Hussey & Wyatt, 1963a) we have emphasized the vital fact that the fertilised females are attracted only to growing mycelium. The large numbers of flies commonly seen during the six-week picking period all develop from eggs laid during the first 3-4 weeks after spawning. Since it is the nuisance value of the adult flies emerging from the compost that the grower is attempting to prevent, large quantities of pesticides are directed against them. Such applications are often regarded as ineffective. This opinion is usually an artefact since the mortality achieved is often masked by the large numbers of flies maturing after the application was made. More permanent control follows if early infestation of the crop can be prevented by killing the flies within spawn-rooms before they can oviposit. It is most important to appreciate the fact that the speed of mycelial colonization, and hence the period of oviposition, is dependent on the quality of the compost. In many cases, growth may be delayed and so continue for some days after 'casing' the beds. Failure to give additional protection at this stage may enable unexpectedly large numbers of flies to mature later in the crop.

We have, during the past year, witnessed a steady increase in the importance of sciarids in the mushroom industry. During the pre-war years this group of flies was the dominant pest of the industry which, at that time, was largely practised on outdoor beds. During the past twenty years hardly any cases were reported but recently at least two species have become established on many farms.

Preliminary observations suggest that these flies are also attracted to the crop at, or soon after, spawning but for very different reasons than phorids. Sciarids are primarily saprophagous insects which normally feed on decomposing vegetable matter. They do not feed on mushroom mycelium specifically but only indirectly as they chew the compost straws over which the web of hyphae spread. Experiments have shown that female sciarids do not readily lay eggs in compost in which mycelium has started to run. Evidently they are attracted to freshly 'peak-heated' compost and hence become established within a few days of spawning before any significant degree of mycelial colonization has occurred. The larvae are "dirty-feeders" in the sense that their colonial feeding, after hatching from groups of up to 100 eggs, leads to a gradually extending area of fouled compost into which mushroom mycelium seems unable to grow.

Once established they differ from phorids in their ability to continue oviposition throughout the life of a crop in response to increasing bacterial activity. This situation is exaggerated by disease such as the virus 'die-back' and combined infestations of fly and virus have frequently been observed.

Hence, while early infestations must be prevented it may also be necessary to continue control throughout the crop.

Cecids, a remarkable group of insects by virtue of their unique paedogenetic reproduction, cause commercial damage merely by the swarming of the larvae over the sporophores. However great the numbers of larvae become there is no effect on the number of mushrooms produced.

Our observations have revealed that mushroom beds may become infested at any time during their existence - such infestations are almost always caused by larvae since the adults, although abundant, are rarely fertilised and have only a very weak capacity for flight. Successful control must therefore be directed against these larvae so that the pesticide must persist throughout the cropping period.

The fourth group of pests - the mite complex - is, despite its size, almost entirely saprophagous feeding either on weed moulds or on diseased sporophores. Apart from one species, <u>Tarsonemus myceliophagus</u>, chemical control is pointless as no cure can be achieved except by removal of the cultural faults which have led to the development of competitors and disease. Even in the case of <u>T. myceliophagus</u> the effective use of acaricides is impossible because the whole life of the mite is spent within the casing or compost which surface applications of pesticides cannot penetrate.

PROBLEMS ASSOCIATED WITH EFFICIENT APPLICATION

From the foregoing account of the tactics necessary if lasting results are to follow pesticide applications, it is apparent that either the casing or the compost must be made lethal to the immature stages of the pests.

In the case of incorporation within compost there are two possible points in the cultural cycle at which chemicals may be added. The most obvious is at the end of the composting operation - incorporation is commonly made during the last turn thereby relying both on the passage of the manure through the composting machines and the filing procedure into the cropping trays to obtain a uniform distribution of the chemical. Even if the pesticide were evenly distributed by this process, which from the physical movements of the machinery is unlikely, the chemical would have to survive peak-heating. This pasteurization process involves heating the compost to 130°-140°F for several days, often in the presence of large quantities of ammonia. In the case of diazinon, used to control first-instar phorid larvae, this breakdown is of the order of 50% so that twice the recommended rate must be incorporated to obtain satisfactory control (Hussey, 1964). This is not necessarily a serious matter as only very small quantities of concentrate - of the order of 1 fl.oz. of concentrate per ton of compost - are commonly required.

Incorporation through the spawning-machines, after peak-heat, is the most attractive technique as these machines are designed to distribute the grains of spawn evenly throughout the compost and one would expect a similar distribution of pesticide. Future developments in this machinery should include equipment for the metering of pesticides, and current research at G.C.R.I. is directed towards determining the most efficient application procedure. The only foreseen complication is the phytotoxic effect of pesticides coming into direct contact with the spawn.

Similar mixing problems arise in connection with the incorporation in peat used for casing the beds. There is no universal technique for this admixture, but addition of the pesticide to the water used to soak the bales is particularly effective. Use of concrete mixers for adding the pesticide to wet peats has also proved successful.

At present, all too few growers have considered pesticide incorporation when developing cultural equipment but this should be an essential future development.

PHYTOTOXICITY OF PESTICIDES

The industry is normally extremely apprehensive about the use of oil-based insecticides which, when applied in gross amounts, can cause rose-comb disorders as illustrated by Snetsinger (1963). This worker however used as much as 30 gallons of 4% diazinon per 1000 sq.ft of bed surface (≡ 5.6g per sq.ft) to obtain these effects and in our experience, using much lower quantities of pesticides (< 100 mg/sq.ft) we have found no growth abnormalities. It should be emphasized that these large amounts were studied, in America, to investigate the effects of dripping from structures treated with high concentrations of diazinon.

From the tactical considerations on pest control already outlined it should be evident that aerial application against flying adults is, at best, a palliative which does not get to the heart of the problem and we will therefore concentrate our attention only on casing and compost treatments.

As early as 1955 Moreton showed that 89 p.p.m. of malathion in compost would depress yield while 22 p.p.m. had no such effect. He also found that aldrin at 17 p.p.m. and gamma-BHC at 10 p.p.m. did not affect yield. Shanahan (1948), in Australia, applied up to 24 p.p.m. of gamma-BHC without harm. Over the past few years a long series of cropping experiments have been made at G.C.R.I. and the main results are summarised in Table 1. Statistically significant losses occurred only when dosages of chlorinated hydrocarbons in excess of 100 p.p.m. were used.

Table 1.

Phytotoxic effects of pesticides incorporated in compost

Chemical	Maximum concentration tested (p.p.m)	Minimum concentration at which any crop reduction recorded (p.p.m)	Minimum concentration at which yield reduction significant at 5% level (p.p.m)
aldrin	500	10	100
BHC	500	100	500
DDT	500	100	500
demeton	10	-	-
diazinon	200	100	-
dieldrin	10	-	-
dimethoate	10	-	-
fenchlorphos	100	-	-
malathion	10	-	-
menazon	100	100	-
TDE	100	-	-
tetradifon	50	50	
thionazin	80	-	
trichlorphon	10	-	

Table 2.

Phytotoxic effects of pesticides incorporated in the casing material

Chemical	Maximum concentration tested (p.p.m)	Minimum concentration at which any crop reduction recorded (p.p.m)	Minimum concentration at which yield reduction significant at 5% level (p.p.m)
aldrin	50	-	-
BHC	100	100	-
carbophenothion	100	100	-
chlordane	100	100	-
chlorvenfinphos	100	-	-
diazinon	50	-	-
dicofol	50	50	50
dioxathion	50	-	-
endosulfan	50	-	-
fenchlorphos	100	100	100
fluoreacetamide	1000	100	100
malathion	100	-	-
menazon	100	-	-
phosphamidon	100	100	-
pyrethrin	50	50	-
TDE	100	100	100
tetradifon	50	-	-
thionazin	40	10	10

Similar data (Table 2) for pesticides incorporated in the casing material (peat and chalk in equal ratio by volume) indicate that several chemicals are more dangerous where they may interfere with the initiation of sporophores than if used in compost - e.g., thionazin, fenchlorphos and TDE.

San Antonia and Lambert (1963) showed that, while the overall crop weight was unaffected, aldrin, dieldrin and DDT caused fewer, larger mushrooms when used at 20 p.p.m. while at 50 p.p.m. DDT, BHC and chlordane reduced the total yield by 17%, 22% and 90% respectively. Contrary to our experience 50 p.p.m. of dicofol had no effect on yield or quality though this difference may, in some way, have been due to the use of clay loam for casing in the American experiments.

From all this work one can conclude that incorporation of most pesticides at concentrations up to 50 p.p.m. in casing or compost is unlikely to affect the total yield.

There has always been some interest in the application of insecticides as a layer on the casing surface before the first mushrooms begin to form. Snetzinger and Frear (1966), using about 320 mg of diazinon per sq.ft, greatly reduced the number of the mushrooms produced while those that did develop were excessively large and malformed. Similar effects of this nature were shown by Hussey et al (1960) where 104 mg of gamma-BHC were applied per sq.ft. Table 3 reveals the great differences in concentration of different pesticides which can be tolerated.

Table 3.

Phytotoxic effects of insecticides applied to surface of casing

Chemical	Rate of application mg/sq. ft	% crop reduction	Significance at 5% level
aldrin	20	12	-
BHC	720	10	-
fenchlorphos	20	100	-
malathion	36	-	-
pyrethrin	44	50	-
"	14	-	-
"	11	-	-
"	4	-	-

Another type of damage that may occur, even when the total yield of mushrooms is unaffected, is a delay in fruiting. Hussey & Wyatt (1960) showed that malathion or BHC incorporated in the casing at 100 p.p.m. delayed the appearance of the first flush for 3 days.

PESTICIDE RESIDUES IN MUSHROOMS GROWN ON TREATED COMPOST OR CASING

Residues on mushrooms, where pesticides are applied directly to the surface of cropping beds, can be quite considerable. Pigatti & Amaral (1960) showed that parathion, applied at a rate equivalent to 9 mg/sq.ft, left residues in the mushrooms, two days after application, of 0.1 p.p.m. while malathion, applied at the same rate, left 0.5 p.p.m. Hussey & Wyatt (1963b) using 20 mg/sq.ft of aldrin recorded residues of 0.4 p.p.m. dieldrin in the mushrooms six days later. Snetsinger & Frear (1966) recorded residues of diazinon as high as 6 p.p.m. in first-flush sporophores after 5.6g/sq.ft had been applied soon after casing. William & Martens (1967), on the other hand, found that they could only detect 0.7 p.p.m. residues of BHC after experimental applications in which that chemical, aldrin, DDT, diazinon & dimethoate had been incorporated in the casing at up to 200 p.p.m.

Table 4.

Residues in sporophores following incorporation of pesticides in casing or compost

Chemical	Incorporation in compost (p.p.m)	Incorporation rate in casing (p.p.m)	Residue (p.p.m)
aldrin	-	5	.02
BHC	200	-	0.8
"	40	-	0.3
DDT	500	-	0.6
diazinon	50	-	Nil
"	10	-	Nil
"	-	10	Nil
dimethoate	10	-	Nil
fenchlorphos	100	-	0.4
"	50	-	0.4
malathion	85	-	Nil
thionazin	20	-	Nil

Our own data (Table 4), based on determination from a long series of experiments, confirm that residues tend to be absent, or very low, if pesticides are mixed with casing or compost.

CONCLUSIONS

The life-cycles of the principal mushroom pests are such that the most efficient control techniques must be based on pesticide incorporation in the casing and/or compost. No serious problems are likely to arise either from phytotoxicity or persistence of toxic residues. Further progress in this field is, however, dependent on improvements in cultural equipment so as to ensure an even distribution of applied pesticides.

One important complication lies in the persistence demanded by the method. For most purposes materials should remain effective for about ten weeks and such properties are generally at variance with the policies being adopted for outdoor crops. In general, the trend away from persistence is designed to prevent contamination of the environment but the mushroom industry is in a special position and, provided undue residues do not persist in spent compost used for horticultural purposes, the pesticide usage outlined should develop rapidly.

REFERENCES

HUSSEY, N.W. (1961) M.G.A. Bull. 144, 495.

HUSSEY, N.W. (1962) Mushr. New. 8, 144.

HUSSEY, N.W. (1964) Rep. Glasshouse Crops Res. Inst. 1963, 76.

HUSSEY, N.W., WYATT, I.J. and HUGHES, J.T. (1960) Ann. appl. Biol. 48, 336.

HUSSEY, N.W. and WYATT, I.J. (1960) Ann. appl. Biol. 48, 347.

HUSSEY, N.W. and WYATT, I.J. (1963a) Mushr. Sci. 5, 509.

HUSSEY, N.W. and WYATT, I.J. (1963b) Ann. appl. Biol. 50, 423.

MORETON, B.D. (1955) Grower 44, 1537.

PIGATTI, O. and AMARAL, J.F.D. (1960) Sao Paulo Inst. Biol. Ang. 27, 35.

SAN ANTONIA, J.P. and LAMBERT, E.B. (1963) Mushr. Sci. 5, 327.

SHANAHAN, G.J. (1948) Agric. Gaz. N.S.W. 59, 184.

SNETSINGER, R. (1963) A.M.I. Mushr. News. 11, 11.

SNETSINGER, R. and FREAR, D.E.H. (1966) J. econ. Ent. 59, 1292.

WILLAM, A. and MARTENS, P. (1967) Mushr. Sci. 6, 507.

THE USE OF FUNGICIDES AND BACTERICIDES IN MUSHROOM GROWING

L. Jacobs
University of Bath

Summary

The use and limitations of chemicals for controlling the most important fungal and bacterial diseases of the cultivated mushroom is surveyed. The importance of hygiene and fly control is stressed.

INTRODUCTION

The chemical control of fungal and bacterial diseases of mushroom crops is difficult due to the close phylogenetic relationship of parasites and host, and the marked sensitivity of the mushroom to a wide range of fungicides and other materials. Unfortunately, no truly selective fungicides are available, materials in common use are merely being employed below the dosage level which would adversely affect sporophore development. The comparative complexity of commercial crop production, the rapid growth rate of sporophores and the short interval of time between flushes adds significantly to the problems involved.

As most of the microbial diseases of the cultivated mushroom are associated with the casing layer the desirability of wide-spectrum, persistent, materials which could be mixed with casing need hardly be stressed. In practice however, the grower is only able to treat the surface layer of casing with a very few unstable protectants, the application of which is restricted to the periods when rapidly maturing mushrooms are absent from the beds. These periods are the initial 12 - 16 days of the growing cycle and the intervals between the four flushes, which normally develop during a total growing period of 8 - 10 weeks. Furthermore, attention must be paid to any infected sporophores occurring, in spite of protective chemical treatment, at any time during the growing cycle, and must take preference to cutting and watering of mushrooms. This procedure poses difficulties for growers but is essential if epidemics, which can easily develop in such an intensively grown crop as the mushroom, are to be avoided.

CONTROL OF PATHOGENS ASSOCIATED WITH THE CASING LAYER

The limitations associated with the use of fungicides is illustrated by the work of Stoller *et al.* (1956) on the control of Mildew (*Dactylium dendroides*) using Pentachloronitrobenzene (P.C.N.B.). Laboratory experiments employing mushrooms inoculated with *Dactylium* spores indicated inhibition of growth at 50 ppm. In practice concentrations ten times as great were required to eradicate the mildew established on mushroom beds. Spraying the surface of beds with P.C.N.B. (above 100 ppm, level) immediately after casing resulted in significant reductions in yield. Mixing 50 or 100 ppm. of P.C.N.B. with casing peat delayed cropping and reduced yields. Mushrooms were normal in all aspects however, when the beds were sprayed with P.C.N.B. at concentrations up to 2000 ppm. after the first flush had been harvested. No yield records were determined but as far as could be observed there was no decrease in yields, except possibly a small reduction at the 2000 ppm. level. The use of this fungicide is thus restricted to the time when mushroom mycelium has colonised the casing and its usefulness as a protective fungicide is therefore considerably reduced, but it still serves a valuable role as a curative chemical. In commercial practice, early, localised outbreaks of *Dactylium* are controlled by covering with common salt. Frequent applications of P.C.N.B. dust are used only to limit spread and prevent severe outbreaks developing. It is of interest to note that even 14000 ppm. of available chlorine did not inhibit the growth of this pathogen.

Brown spot (*Verticillium malthousei*) and Bubble (*Mycogone perniciosa*) are particularly destructive fungal pathogens in growing crops. Unlike *Dactylium*, which is sporadic in occurrence, both organisms can attack the crop continuously throughout the year. Following the work of Yoder *et al.* (1950) many growers use zineb dust as a protective fungicide against both pathogens at application rates of ¼ lb of 15 or 20% zineb dust /1000 ft^2 of bed area. Levels in excess of this can reduce cropping yield. Zineb application normally begins 3 or 5 days after casing, treatment being discontinued a few days before "pinning" occurs. As the active life of zineb on the beds is only 3 days (Kneebone-personal communication) frequent dusting is necessary to achieve maximal effect. In severe attacks zineb application is continued throughout the whole cropping period. Chlorine also controls both these pathogens (Ayers and Lambert, 1955) but unfortunately it is incompatible with zineb. This is an important consideration since chlorine is also employed to control several bacterial pathogens of mushrooms. Both materials can, however, be employed providing that zineb is applied one day after chlorine watering and a further 3 day interval elapses after the fungicide application before another watering is given. Information on the possible interaction of dithiocarbamates and other fungicides, with insecticides such as dichlorvos and diaxinon is wanting. It is therefore possible that growers unwittingly nullify the effect of some of the materials they use.

The value of zineb treatment is difficult to assess. Last and Gandy (1965) reported that answers given by growers to a *Mycogone* questionnaire suggested that zineb, as a protective fungicide, afforded very little control. Possible reasons for this situation could include faulty application, the restricted area of activity in the casing or the development of resistent strains of the disease organisms. Good control can be obtained by other means, omitting zineb treatment. Thus the restriction *Verticillium* and *Mycogone* attack to the sporophore allows the physical isolation of diseased mushrooms by covering them with plastic cups which remain in situ throughout the cropping period. This technique, if properly applied, limits disease spread, without resorting to other means. *Dactylium*, which spreads extensively over casing materials, cannot be controlled in this way.

The facility of an experimental disease house to enable investigators to evaluate fungicides for these, and other disease problems, would be essential in order to provide guidance for growers. It would appear that very little stimulus for the use of chemical protectants has been forthcoming from fungicide manufacturers, possibly because of the lack of good trial facilities.

Another disease encountered quite frequently in growing houses is bacterial blotch attributed to *Pseudomonas tolaasi*. These bacteria are readily controlled by roguing and regular application of chlorinated water. Concentrations of 150 ppm. available chlorine are permissible, higher concentrations causing mushroom discolouration. Bacterial rot and bacterial watery stipe are less frequently encountered and are controlled in the same way.

CONTROL OF PATHOGENS ASSOCIATED WITH COMPOST

The foregoing examples of mushroom diseases are the most important ones associated with casing. Some pathogens or harmful competitive fungi are associated with the compost. e.g. Truffle (*Pseudobalsamia microspora*), Mat disease *Myceliophthora lutea*), and Lipstick disease (*Geotrichum sp.*). These organisms are thermoduric and so survive composting at peak heating temperatures. As chemical control is difficult, prolonged cook-out at high temperatures is necessary to eradicate organisms of this type. Truffle, which is extremely heat resistant, is fortunately a rare occurrence, being controlled by removing any source of soil contamination from compost, and carrying out the cultivation cycle at 60°F when ascopore germination is inhibited. *Myceliophthora* and *Geotrichum* are both eradicated by prolonged cook-out at 160°F for at least 6 hours. Methyl bromide sterilisation of compost is an important new development in mushroom culture which could eradicate or markedly reduce the incidence of these organisms.

With the very limited amount of chemical control at their disposal growers rely greatly on pasteurisation procedures. Steam pasteurisation in the final preparative stages of composting is standard practice, and steam treatment of casing materials is also done by some growers.

Cooking out, i.e. steaming of spent houses, with or without addition of formaldehyde, is frequently practised. Where cooking-out is impracticable disinfectant spraying is employed. However, there is a great need for an efficient, easily applied sporicidal agent active against the wide spectrum of disease and competitive organisms encountered in mushroom growing. Otherwise, it will remain difficult to attain the high standards of hygiene necessary to minimise and effectively control those organisms.

As an additional protective measure some growers have installed air filtration facilities in spawn running, peak heat and growing rooms, in an effort to frustrate many of the maladies which can befall them.

Evidence of the overall importance of farm hygiene in disease control is frequently found. Disease incidence is low on farms where high standards of hygiene are maintained. Poor hygiene standards make disease control difficult in spite of intensive chemical prophylaxis. The use of isolation cups, where applicable, has minimised the spread of disease organisms and has reduced the role of fungicides here. Until such time as effective preventative chemicals are available to growers it would appear that efficient hygiene procedures offer the best method of control. The cost of such practice can be offset against the reduced expenditure on fungicides. This does not imply that chemicals in current use have no part to play in disease control, but experience suggests that the frequency of their application can be reduced if backed up by good farm management.

In conclusion it should be stressed that control of fungal pathogens in particular cannot be divorced from fly control on farms. Flies are efficient disseminating agents and must not be overlooked in any disease control programme.

References

Ayres and Lambert (1955) Plant. Dis. Reptr. 39, p. 829.
Last and Gandy (1965) M.G.A. Bull. 186, p. 258.
Stoller et al (1956) Plant Dis. Reptr. 40. p. 193.
Yoder et al (1950) Mushroom Science I p.100.

DISEASE INCIDENCE AND YIELD REDUCTION OF WHEAT AND BARLEY FOLLOWING ARTIFICIAL SOIL INFESTATION WITH OPHIOBOLUS GRAMINIS

P.C. Cunningham

An Foras Taluntais, Oak Park, Carlow, Ireland

Summary: A technique was devised for preparation of inoculum of Ophiobolus graminis and artificial infestation of field plots. The method consists of colonising beet clusters of uniform size with the pathogen and drilling the inoculum through the fertiliser box of the corn drill at seeding.

Disease incidence and yield reduction of spring-sown wheat and barley were compared in a replicated field experiment having controls and one level of soil infestation with the pathogen. Take-all was assessed at two stages of crop growth. At the early assessment take-all was measured by calculating, a disease index, percentage of plants with severe and with slight take-all, and number of infected roots/sample, per plant and per gram of total root. Disease assessment of the grown crop was based on, a disease index, percentage of straws with severely diseased root systems and percentage of straws with crown infections. While the various disease measurements revealed no pronounced or consistent differential resistance between the two cereals, grain yields indicated that barley was considerably more tolerant of take-all than was wheat. Take-all reduced wheat yields of infested plots from those of the controls by 50 percent whereas it reduced barley yields by approximately 24 per cent only.

INTRODUCTION

Precise yield losses in cereals caused by soil-borne fungal pathogens are generally difficult to assess. This is so because of numerous variables associated with disease levels in field surveys, and because of fertility and other factors allied to infestation levels in rotational experiments. Thus, the pathogen has to be introduced into the plots to be infested and in a manner not to expose the controls to infection. This type of approach is essential to measure effects of disease on yield and quality as well as to screen varieties and selections for tolerance and field resistance.

More than thirty years ago Glynne (1935) observed that the percentage infection with take-all caused by Ophiobolus graminis was usually higher in wheat than in barley; more recently this was confirmed in Australia (Chambers 1962). However, other workers found the reverse to be true (Pedersen and Jorgensen 1960).

In this contribution a technique is described for infesting field plots with O. graminis and a comparison is made of the reactions of wheat and barley as measured by disease incidence and yield response in a replicated field experiment laid down in 1964.

METHOD AND MATERIALS

Three isolates of O. graminis were obtained from the roots of three wheat plants and similarly three from the roots of three barley plants. The isolation was achieved by surface sterilizing diseased roots (5 per cent sodium hypochlorite for 30 seconds) and allowing the fungus to grow out on potato dextrose agar (PDA). Sugar beet clusters between 3/16 and $\frac{1}{4}$ inch in diameter (got by sieving) were added in lots of 150 g. to 1 litre flasks. Water was added and the clusters were allowed to steep for 24 hours. Free water was drained off and 40 ml. of water was added to each flask and the plugged flasks were autoclaved for one hour at 20 psi. When cool 25 ml. of 2 per cent glucose solution was added to each flask. The contents were shaken and the flasks reautoclaved for 1 hour at 15 psi. Disks of culture from the PDA plates were added to the flasks subsequently. An equal number of flasks was infested with each of the six isolates and all flasks were incubated at 20°C for 6-7 weeks in darkness. The cultures were shaken up every two weeks to obtain uniform colonisation of substrate within each flask. After incubation the inoculum was removed from the flasks, thoroughly mixed and was thinly spread on newspapers over an expansive floor area. Warm air blowers were used to help the drying process which continued for 48 hours with intermittent mixing of the inoculum to ensure fairly uniform drying out. This resulted in free flowing beet clusters devoid of surface moisture.

The site of the field experiment was a medium loam which was in pasture for the previous 20 years. The site was not very fertile and ploughing was deliberately delayed until mid-March to help establishment of the pathogen. The experiment consisted of six randomised blocks each containing eight plots or four treatments replicated twice in each block. The four plot treatments comprised Atle wheat and Hunter barley infested and non infested with the take-all fungus. Sowing was done on April 8. The seeding rates for wheat and barley were 11 and 8 stone/acre respectively. Plot dimensions were 37 ft x 7 ft 6 in with a 2 ft 6 in space between plots. A fertiliser dressing of 3 cwt. 8-8-13 granulated compound/acre was applied broadcast to the experimental site.

The fungal infestation process was carried out at seeding. When sowing the appropriate plots the partially dried colonised beet clusters were placed in the fertiliser box of the compound corn drill and this ensured that the inoculum was placed beside the grain. The level of soil infestation was that obtained at a granulated fertiliser setting of 140 lbs/acre. Sterilised (by autoclaving) and partially dried non colonised beet clusters were drilled with the control wheat and barley plots at the same setting.

Plots were sampled for take-all assessment on June 10 and again on July 22. Samples were lifted with a garden fork and most of the adhering soil was shaken off. In the early or braird sampling a 6 in sub-sample was taken at random from each of the 12 corn drill rows in each plot, the sub samples being bulked subsequently, tied with string and labelled. The samples were placed in a refrigerator at approximately 5°C and diagnosed within 3 days. Each sample taken per plot from the grown crop (July) comprised a 6 in sub-sample of the 12 corn drill rows, each corn drill row sub-sample being selected at random. For samples from the grown crop the straw was cut to within about 8 in of the crowns and the upper portion discarded. Each individual straw with its complement of roots was taken apart from the parent plant and the leaf sheaths were removed. Samples were then placed in a greenhouse to dry out until examined.

The root systems of all samples for disease assessment were thoroughly washed with water under pressure and diagnosis was carried out against a white enamel background. A number of isolations was done initially to correlate symptoms with the pathogen. Different disease assessments were done on the braird samples. In one measurement of disease, plants having roots with take-all were placed in one of four categories as follows - (a) $>$ 2 in total lesioned roots, (b) 1-2 in, (c) $\frac{1}{2}$ - 1 in and (d) $<$ $\frac{1}{2}$ in. A disease index was calculated by giving values of 4, 3, 2, and 1, respectively to each category and the infection was expressed as a percentage of the maximum possible (i.e. all plants having $>$2 in of lesioned root system). Plants falling into the two highest categories of this rating were considered severely diseased (i.e. $>$1 in of lesioned root per plant). Other measurements of take-all disease made at the braird stage consisted of (i) number of diseased roots/plant, (ii) number of diseased roots/sample, and (iii) number of diseased roots/g. of total root. These measurements were made from counts of the infected main seminal and crown roots (crown roots were developing at this stage), and from root weight of samples (air dried on filter paper at laboratory temperatures for 48 hours). For take-all assessment of the grown crop a disease index was formulated in which straws having $>\frac{3}{4}$, $\frac{1}{2}$-$\frac{3}{4}$, $\frac{1}{4}$-$\frac{1}{2}$, and $<$ $\frac{1}{4}$ of the total root system with take-all lesions were given values of 4, 3, 2, and 1 respectively and infection was expressed as a percentage of the maximum possible rating. The percentage of severely diseased straws was also considered a useful measurement of disease and comprised the percentage of straws falling within the two highest infection ratings (straws having more than 50 percent of the root system with take-all lesions). The percentage of straws with crown infections was also recorded.

Observations were made on the plots throughout the growing season. All plots were sprayed with MCPA at the rate of 3 pints in 25 gal of water per acre on June 7 for weed control. Barley plots were harvested on September 3 and wheat on September 17. The plots were cut by Mayfield mower and the produce was tied in small sheaves and threshed indoors in a small mill. Yields were calculated in cwt/acre at 20 percent moisture; bushel weights and 1,000 corn weights were determined.

In the statistical analysis of the disease ratings of the braird and grown crops, because of a difference in the variance between the infested treatments and the controls, the standard errors were calculated for the means of the artificially infested plots only.

RESULTS

Field observations: Yellowing of the foliage was evident in the infested wheat plots by early June. Inoculum appeared to be more abundantly placed in some lines than in others as indicated by the yellowing symptoms. Such symptoms were scarcely noticeable on barley in the infested plots at this stage. By early July noticeable stunting was very obvious in the diseased barley plots, but the vigour differential between infested and non infested plots was always much greater for wheat than for barley plots. In the case of infected wheat there was noticeable killing off of shoots by late braird stage. With barley most shoots in the diseased crops appeared to form ears as in the controls though in some cases the ears were quite small and barely emerged from the sheaths. Ear emergence in diseased and healthy wheat plots occurred about the same time. Diseased barley crops eared two days later than healthy plots.

By July 20 profuse weed growth developed in the diseased wheat plots whereas the controls were almost completely weed free. The infected barley crops were relatively free from weeds although not quite so free as the barley controls. By mid-August weed growth in diseased wheat plots had worsened. The following were the weeds present in order of prevalence, black bindweed (Polygonum convolvolus), redshank (Polygonum persicaria), sow thistle (Sonchus oleraceus), poppy (Papaver rhoes) and silverweed (Potentilla anserina)

In diseased wheat crops while many shoots failed to form ears many formed small ears while in other cases normal sized ears formed on normal sized plants. Ears of normal size generally whitened prematurely as invariably did the undersized ears. With barley there was only very little premature whitening of ears and this was not very obvious. In general, in the diseased wheat crops, the whiteheads were scattered throughout the plot but occasionally intense patches of whiteheads occurred and sometimes the disease gave extremely stunted take-all patches as often happens with natural infestation in the field. There was no darkening of the ears by saprophytes in the case of barley as happened with wheat. For barley diseased crops ripened about 5 days earlier than healthy plots. However, there were late tillers in the infected barley crops which were considerably later in ripening than the control plots. In the case of wheat the great majority of infected plants in diseased crops were ripe 2-4 weeks before the controls and by the time of ripeness of the latter, the prematurely ripened plants were discoloured by mould growth.

Disease and Crop Assessments: Various counts were made on the braird samples apart from disease measurements (Table 1).

Table 1

Effect of take-all on growth and development of wheat and barley crops at braird stage

	Wheat		Barley		
	Diseased	Control	Diseased	Control	S.E. per mean
No. of plants/6 in. row	9.7	11.1	8.8	9.8	1.3
No. of shoots/ 6 in. row	12.7	14.7	16.8	18.1	1.3
No. of shoots/plant	1.4	1.4	1.9	1.9	0.2
Wt. of roots(g.)/sample	2.2	2.9	2.4	2.7	0.3

Root disease tended to reduce the amounts of plants and shoots at braird stage but these differences were not significant. Similarly disease reduced root weight but the reduction was short of significance.

Table 2

Effect of artificial infestation of soil on take-all levels of wheat and barley at braird stage

	Plots Infested			Control Plots	
	Wheat	Barley	S,E. per mean	Wheat	Barley
Percentage take-all (Disease Index)	56.3	54.0	5.0	2.9	3.2
Percentage of plants with severe take-all	47.4	51.2	5.0	0.9	0.4
Percentage of plants with slight take-all	33.6	19.1	3.6	8.0	9.8
Number of infected roots/sample	187	250	26.8	9.8	9.3
Number of infected roots/plant	3.3	4.4	0.4	0.2	0.2
Number of infected roots/g. of total root	93.9	112	16.4	3.6	4.4

Take-all assessments at braird indicated no great difference in susceptibility between wheat and barley (Table 2). The data indicate that the numbers of infected roots, per sample, per plant and per unit weight of total root were somewhat greater for barley but such difference were not significant. Measurements based on the amount of total diseased root per plant did not show the same trend but the only significant difference was for percentage of plants with slight take-all where the infection rating was greater on wheat ($P<0.05$).

Data on disease assessment of the grown crops are presented in Table 3.

Table 3

Effect of soil infestation on take-all levels of wheat and barley (fully grown crops)

	Plots Infested			Control Plots	
	Wheat	Barley	S.E. per mean	Wheat	Barley
Percentage take-all (per disease index)	66.5	68.5	2.26	10.5	8.6
Percentage of straws with severe take-all	62.0	63.9	2.53	5.5	3.8
Percentage of straws with crown infections	41.5	49.5	1.52	1.6	2.4

In the grown crop take-all ratings based on the percentage of straws with root systems having severe take-all and on percentage take-all derived from a disease index revealed no significant difference between wheat and barley. However, barley had a significantly greater ($P < 0.01$) percentage of straws with crown infections.

Table 4

Effect of take-all disease on straw density of wheat and barley crops (No of straws per 6 ft row sample)

Crop	Take-all infected crop	Control	Mean
Wheat	106.8	136.8	121.8
Barley	182.6	195.3	188.9
Mean	144.7	166.0	155.4

Standard error for crop and disease treatment means ± 5.82
Standard error for individual treatment means ± 8.23

While the straw density was very significantly greater in the case of barley than wheat ($P < 0.001$) the effect of disease in reducing overall straw density was also significant ($P < 0.05$) (Table 4). However, in the case of the individual crops disease reduced straw density significantly in wheat only ($P < 0.05$). There was no significant interaction between crop and disease for this value.

Table 5

Effect of take-all on wheat and barley grain yield measurements

	Wheat		Barley		Standard error per mean
	Diseased crop	Control	Diseased crop	Control	
Grain Yield cwt/acre at 20% moisture	12.8	25.6	20.6	27.2	0.78
1,000 corn weight (g)	30.0	35.1	39.4	42.4	0.54
Bushel Weight (lb)	60.3	64.1	53.8	55.1	0.22

The effect of disease in reducing grain yield, bushel weight and 1,000 corn weight was highly significant ($P<0.001$) (Table 5). There was a highly significant interaction ($P<0.001$) between crop and disease for yield and bushel weight. The interaction, which arose because of the much greater differential between the diseased and control wheat crops by contrast with barley, was not evident for 1,000 corn weight.

DISCUSSION

The reduced foliar symptoms observed on barley by comparison with wheat provides a good measure of the tolerance of take-all by the former crop species. Greater stress on the wheat than on the barley crop is also indicated by the effect of disease on root production. It is probable that the weed regrowth may be more a direct consequence of the malfunctioning wheat root systems than the reduced shading effect of a less vigorous and more stunted crop. A partially defunct root system will absorb less water and nutrients. Furthermore, it has been shown that where plants grow closely there is competition between overlapping roots system long before plants shade one another (Pavlychenko and Harrington 1935). It is also true that some of the most abundant weed species present are resistant or moderately resistant to MCPA and that these were probably present from early on, and only grew rapidly when competition became more favourable. This emphasises the need for particular care in choosing a herbicide that will control the minor as well as the major weed species in a site infested with the take-all pathogen. A weed species resistant to a particular herbicide and present to a lesser degree only may, on elimination of more prevalent species, proliferate extensively when crop growth is checked.

Since the total percentage of plants with take-all at braird (sum of percentages with severe and slight take-all) (Table 2) was greater for wheat than for barley it indicates that the number of infected roots (somewhat greater for barley) was distributed over a greater proportion of plants in the case of wheat. Thus, while the number of infected roots per plant was slightly greater (just short of significance) in the case of barley the number of infected roots per infected plant if calculated would be considerably greater for barley. Since the percentage take-all calculated by means of a disease index was approximately equal for wheat and barley and since this value is related to total length of lesioned root, it would seem that the fewer infected roots per diseased wheat plant were more extensively lesioned. No information was collected on numbers of healthy roots in the two crops although root weights did not vary much (Table 1). The ideal in measuring relative susceptibility of the two crops would appear to be the length of lesioned root per unit length of total root. The number of infected roots/g of total root probably gives a good comparison of relative susceptibility since it takes into account a measure of the disease as well as total weight of roots. However, number of infected roots as a measure of susceptibility is not very precise, and resistance to a pathogen may be as much a function of limitation of lesion size as of initial foci of infection.

Disease assessment of the grown crop paralleled to an extent disease ratings of the braird samples. Whereas the percentage take-all (disease index) and percentage of straws with severe take-all were about equal in wheat and barley, crown infections were more prevalent in barley (Table 3). This may be because of the considerably greater number of straws arising from a single barley plant compared with wheat. Once the pathogen reaches the crown of a plant it will probably colonise the stem base of all straws. It seems that as the crop tillers and matures the root system must proliferate more extensively with barley than with wheat, because of the greater number of ear bearing straws in the case of barley (Table 4). The chance of getting crown infection will depend largely on root infections adjacent to the crown. The greater the number of roots infected the greater will this possibility be, and the greater the total number of roots formed the greater will be the number of roots that will become infected by contact with inoculum. A greater number of infected roots in barley than in wheat would not tend to give more total or severe take-all of samples from the grown crop as these measurements are based on proportion of diseased to healthy roots at this stage. It could, however, affect the disease differential for wheat and barley based on straws with crown infections.

Whereas soil infestation with O. graminis reduced wheat grain yields by 50 percent below those of the controls the same level of infestation reduced barley yields by approximately 24 percent. The interaction between crop and disease represents a high degree of tolerance in barley, particularly when it is considered that the various disease assessments indicated no appreciable difference in susceptibility of the two crops in the early or later phases of growth. There is little doubt that the stress on the plant caused by take-all infection of the root system is far greater in the case of wheat than with barley. While an analysis of variance showed a similar interaction in the case of bushel weight as for grain yield there was no such interaction for 1,000 corn weight. This suggests at least that, as in the case of yield, grain quality was not so adversely affected with barley as with wheat.

It could be contended, because of the soil infestation process, that the resulting effects of disease incidence and yield reduction would differ greatly from what might happen with natural infestation. It seems possible that the artificial infestation technique by concentrating the inoculum in one zone might not affect the two cereals in the same way as would natural infestation because of different rooting habits of the two crops. However, studies done recently under conditions of natural infestation by means of rotational experiments partly concurred with these results (Cunningham 1964, 1965). It was found by comparing disease levels and yields of wheat and barley in first and fourth year rotations that intensive cropping had a considerably more depressing effect on wheat than on barley yields although take-all ratings of wheat compared to barley were appreciably greater than in the current experiment.

Acknowledgments

The author is indebted to the research staff of the Plant Pathology Department, Oak Park, for advice and criticism when writing this paper; Mr. D. Cunniffe for the statistical analysis; and Mr. A. Shannon and Mr. C. Sheehy for assistance with the experimental work.

REFERENCES

CHAMBERS, S.C. (1962) West Australian Dept. Agr. J. 3: 521.

CUNNINGHAM, P.C. (1964), (1965) Plant Sciences and Crop Husbandry Division, An Foras Taluntais, Research Reports.

GLYNNE, M.D. (1935) Ann. Appl. Biol. 22: 225.

PEDERSEN, P.M. and JORGENSEN, J. (1960) Tidsskr Planteavl, 64: 369. Abstract from Rev. Appl. Mycol. 40: 408.

THE EFFECT OF DIRECT-DRILLING ON THE FOOT-ROTS OF CEREALS

D.H.Brooks
Imperial Chemical Industries Limited
Jealott's Hill Research Station, Bracknell, Berks.

Summary The drilling of winter wheat, without mechanical cultivation, into stubble or grassland which has been treated with paraquat to kill the vegetation has been found to cause a reduction of take-all and eyespot. The reduction of disease appears to be due, not to fungicidal action of paraquat, but to biological factors limiting spread of the fungus in the undisturbed soil.

Ophiobolus graminis is a facultative parasite; it is able to invade living roots of cereals and grasses. It is not able to colonise dead tissues in competition with other soil fungi. Thus following death of the host at harvest, the fungus enters upon a period of decline during which it is able to survive for a time on tissues on which it is already ensconced, but is not able to compete for new substrates. Its survival time therefore depends on the rate of distruction of the stubble by microbial activity, and any cultural practice which favours this activity will hasten its decline. Early ploughing to achieve a loose, well aerated soil favourable to microbial activity as long as possible before sowing the subsequent crop has been found to be effective. After sowing, compaction of the soil by rolling builds up carbon dioxide levels and restricts the spread of O. graminis in the crop (Garrett, 1956).

Cercosporella herpotrichoides survives the winter on stubble on which it produces spores which infect the bases of the new cereal plants. Since the incidence of eyespot infection has been found to depend on the number of potentially infective propagules lying on the soil surface (Cox & Cock, 1962), a complete inversion of the stubble by ploughing is important.

With these well tried and proven techniques in mind, it might be expected that take-all and eyespot would be aggravated by direct-drilling. Although various forms of minimal cultivation have been practiced in America and Australia for many years, it was only with the discovery of the herbicide paraquat that the abandonment of soil inversion as a method of weed control became a practical proposition in the United Kingdom. Paraquat rapidly desiccates all green foliage, but has no residual soil action; it can therefore be used as an alternative to ploughing (Boon, 1965). Special drills are used to penetrate the undisturbed soil. Direct-drilling was first attempted at Jealott's Hill in 1961 when plots of winter wheat were drilled into an old (at least 100 years) pasture which had been killed with paraquat (Hood, Jameson & Cotterell, 1963). In plots alongside, wheat was drilled after normal cultivation, and in a third series plots were sprayed with paraquat prior to ploughing. The experiment has continued each year using the same plots. In the second wheat crop, the direct-drilled wheat was observed to be more vigorous than that on both ploughed series. In 1964 the effect was more pronounced, and plants removed from direct-drilled plots were found by Mr. D.B.Slope to have significantly less take-all and eyespot than those from ploughed plots. In each year since then, take-all has been found to be less severe in the direct-drilled plots (Table 1). Differences in eyespot have been less marked, but except in 1965 when take-all was too severe on plants from the ploughed plots to allow an accurate eyespot assessment, there has been a tendency towards some control by direct-drilling. In 1965 the direct-drilled plots out yielded the ploughed ones by ½ ton/acre, and this difference was almost certainly caused by take-all. In 1966 take-all declined somewhat in importance, but in 1967 it was rather more severe. In each of these years there was more disease on the ploughed plots.

Table 1

Incidence of take-all and eyespot on experiment CP1

Each figure is the mean of eight plots

% tillers diseased

	Take-all				Eyespot			
	June 1964	August 1965	June 1966	June 1967	June 1964	August 1965	June 1966	June 1967
Ploughed	20	64	48	69	38	30	64	93
Sprayed/ploughed	-	77	62	63	-	30	68	93
Sprayed/direct-drilled	3	14	24	35	27	42	49	88

Thus for four consecutive years there has been a marked reduction in take-all following direct-drilling, and similar reductions have been noticed in several other trials. The reduction in disease does not appear to be due to the slight fungicidal properties of paraquat. In the plots sprayed prior to ploughing, the pattern of disease development was similar to that in conventional plots, not to that in direct-drilled plots (Table 1, Fig. 1). Also a closer study of the pattern of disease development in the 1965 crop (Fig. 1) shows that the differences in take-all between treatments are not marked until May inspite of differences in distribution in the previous crop. The factors retarding disease development in the direct-drilled crop seem to be operative, not immediately after treatment with paraquat, but when rapid plant growth begins. Further, similar reductions in take-all have been noticed in trials where weed control was achieved by burning instead of by chemical treatment.

Table 2

Incidence of take-all on direct-drilled wheat, Micheldever, Hants

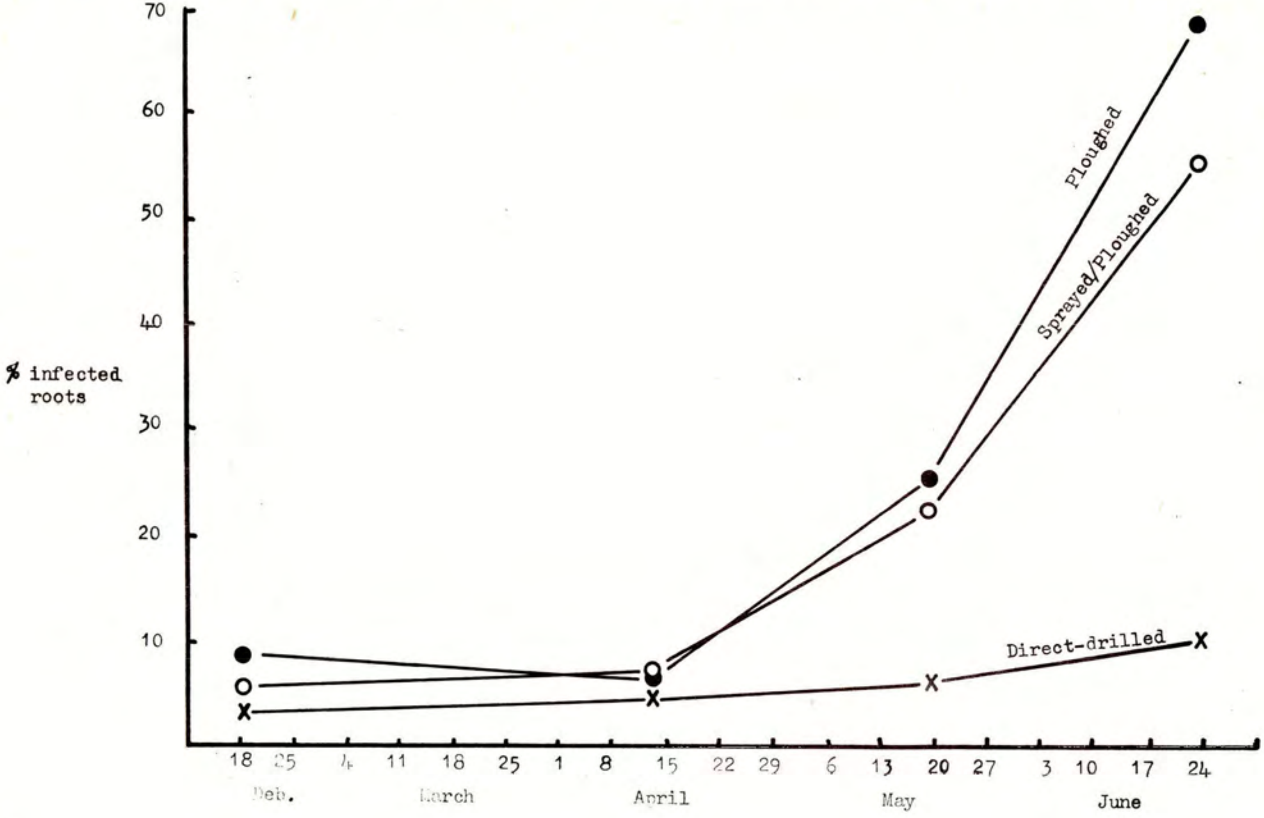
Each figure is the mean of six plots

% tillers diseased

	1966	1967
Ploughed	83·8	50·6
Flamed/direct-drilled	70·6	26·7
Sprayed/direct-drilled	67·5	23·0

At the site referred to in Table 2, there was a uniform and very severe attack of take-all in the wheat crop previous to the trial. In the first year of the trial take-all was severe throughout, but most severe on ploughed plots. In the second year (1967), take-all declined in importance throughout the trial, but most markedly on the direct-drilled plots. In many others, but not all, of our trials direct-drilling has caused some reduction in take-all. In one trial on spring barley there was an increase in disease on the direct drilled plots and here paraquat had failed to control grass weeds (Table 3). There was a decreasing amount of take-all and grass weed infestation with increasing rates of paraquat application: in the

Fig. 1. Experiment CP1: percentage roots infected with take all, 1965



ploughed plots, which were almost free of grass, take-all was least. Presumably take-all was carried over into the experimental crop on the grass roots. Even here as the season advanced the disease spread more rapidly on the ploughed plots than on the direct drilled plots and by August there was little difference between treatments.

Table 3

Incidence of take-all and eyespot in spring barley, Bury St. Edmunds

Each figure is the mean of ten plots

	% plants infected		% grass weed cover
	Take-all	Eyespot	
Paraquat 1 lb a.i./acre	26·2	34·0	3·1
Paraquat 2 lb a.i./acre	19·9	31·3	2·3
Ploughed	14·5	26·6	0·2

Thus in a large number of trials in this country, direct-drilling has given some control of take-all, and to a lesser extent, of eyespot. Similar reductions in both diseases have been noticed in France and Germany. The reduction seems to be due, not to direct effect of paraquat, but to establishment of crops in undisturbed soil, made possible by the use of paraquat. Experiments have shown that this undisturbed soil contains more organic matter and more available nutrients in the surface layers than the ploughed soil. Coincident with this there is a higher rate of carbon dioxide production in the top 2 in. of unploughed soil than at any level in the ploughed soil. This suggests that the unploughed soil supports a higher microflora, and it is possible that this may limit the growth of fungal pathogens, and this would account for the fact that O. graminis spreads more rapidly along wheat roots in soil removed from ploughed plots than in soil removed from direct-drilled plots.

Acknowledgement

Data for Table 1 and Fig. 1 are reproduced with the permission of the Editors of The Annals of Applied Biology.

References

BOON, W.R. (1965) Chemy Ind. 1965. 782

COX, J. and COCK, L.J. (1962) Pl. Path. 11, 65

GARRETT, S.D. (1956) Biology of Root Infecting Fungi. Cambridge University Press

HOOD, A.E.M., JAMESON, H.R. and COTTERELL, R. (1963) Nature, Lond., 197, 748

TAKE ALL DECLINE

P. J. Shipton
National Agricultural Advisory Service, Reading, Berkshire.

Summary

Evidence is presented to show the widespread nature of Take All Decline, recognised by an improvement in yield concurrent with a fall in severity of attack by the Take All fungus Ophiobolus graminis (Sacc.) Sacc., as cropping continues beyond four to six successive susceptible cereal crops. It is suggested that the phenomenon is a natural consequence of monoculture of wheat or barley and appears to be necessarily preceded by a severe attack of the disease. The use of the Added Inoculum technique in the detection and measurement of Decline is discussed and it is suggested that Decline is a result of the development of a soil or rhizosphere flora antagonistic to O. graminis.

INTRODUCTION

Economic and social changes in British Agriculture since the last war have resulted in fundamental alterations in the pattern of farming and particularly in cereal production. Cereal growers in the major arable areas have been forced to adopt simpler and cheaper farming systems at the sacrifice of traditional rotations. The number of successive cereal crops has been increased to 4, 5, or 6 before the introduction of the break, now often only of one year's duration. On some farms this intensive system has been replaced by continuous cereal growing or monocropping which is usually of spring barley especially on the chalklands of southern and eastern England. Continuous barley cropping was successfully practised many years before any scientific investigation of its consequences was undertaken. Monoculture has long been regarded as a hazardous operation because of increasing soil-borne disease. In the case of cereals it was considered that Take All (Ophiobolus graminis (Sacc.) Sacc.) in particular would develop to such severity that economic production would cease. This view has been held in spite of evidence to the contrary from the classical long term cereal fields at Rothamsted where Broadbalk has been cropped with wheat for 124 years and Hoosfield with barley for 115 years. Recently evidence has been accumulating from experiments and from observations on farms on the levels of yield and disease in shorter-term continuous barley growing, which substantially agrees with Rothamsted experience.

Yield and Take All in Continuous Cereal Cropping

Slope and Cox have examined the changes in levels of yield and of Take All in continuous winter wheat experiments at Rothamsted and have summarised (Etheridge and Slope 1967) their results over the period 1963-1966 (table 1).

Table 1

Summary of Take All Decline experiments
Rothamsted 1963-1966

	Continuous wheat (mean of 5th 6th 7th 8th crops)	2nd and 3rd crops
Grain yield (cwt./acre)	32.2	29.6
% tillers with Take All (July)	64	75

They concluded from their experiments over nine successive years that for each 1% Take All the yield was decreased by 0.6%.

They first pointed out in 1962 (Slope and Cox 1963) that the yield of the 4th consecutive winter wheat was higher in each of three years than the yield of the 3rd or 2nd consecutive crops, while the level of Take All was slightly lower in the 4th crop. They referred to this reduction in disease as the "Decline" of Take All (Slope and Cox 1964) and the term has now been accorded general acceptance.

Investigations were begun in 1964 by Lester and Shipton to see if the Take All Decline phenomenon could be demonstrated elsewhere in this country and to what extent, if at all, it was a factor in successful continuous barley growing practised on the chalkland.

Until 1963 no evidence on levels of Take All and yields in continuous spring barley or in spring wheat had been collected, which could be compared with that for winter wheat at Rothamsted. In that year a series of trials was laid down at several N.A.A.S. Experimental Husbandry Farms including Bridget's E.H.F. on the chalk downland near Winchester, Hampshire. The trials provide a phased intake of barley or wheat in each of the years 1963 to 1968 enabling within-season comparisons of yield, disease etc. between the first to the fifth successive crop in 1967. The plots are split for four levels of nitrogen designed to provide a nitrogen response curve each season.

In the spring wheat trial over the years 1963 to 1967 the highest yield obtained in the trial was invariably from the plots in their first year of wheat and usually at an optimum level of 80 units of nitrogen in the range of 0, 60, 80 or 100 units. Until 1967 yields fell progressively with increasing numbers of successive crops.

In 1966, the fourth year crop was severely attacked by Take All, while a year later these plots showed a recovery in yield and a marked reduction in Take All level compared with the fourth year crop in the same year (Table 2).

Table 2

Comparative yields and Take All levels in Spring Wheat
Bridget's E.H.F. 1967

	Yield*	Take All**
1st crop	100	12.1
2nd crop	74.7	45.5
3rd crop	54.5	89.3
4th crop	47.5	95.5
5th crop	55.1	74.9

*Yields are expressed as a percentage of maximum yield (= 100) each year

**Take All figures are the percentage of plants with more than 25% of the roots infected, examined in July.

Take All Decline was observed on a field scale at Bridget's E.H.F. in a field of continuous spring barley. The field was harvested as plots for calibration purposes and detailed Take All measurements were made in 1964 and 1965 (Table 3).

Table 3

Take All Decline in Spring Barley
Bridget's E.H.F. 1964-5

Year	Units of Nitrogen	% Take All	Yield cwt./acre
1964 (4th crop)	60	90	19.8
1965 (5th crop)	90	33	32.9

Whilst there was an increase in the level of nitrogen applied in 1965 it is doubtful whether this could have accounted for more than a part of the yield increase and the fall in Take All. Laboratory tests confirmed that the Decline factor was operating in 1965.

A further well-documented example of Decline occurred in a farm trial at Crux Easton, Hampshire in 1967 on winter wheat. After a very severe attack of Take All in 1963 part of the field was devoted to an unreplicated trial to assess the value of break crops of differing duration. This year, the whole trial area was sown to winter wheat, providing a comparison between first crop after three year break, second crop after two year break, third crop after one year break and continuous susceptible cropping (Table 4).

Table 4

Take All Decline in continuous winter wheat
Crux Easton, Hampshire

Yields of winter wheat cwt./acre at 85% D.M.

1961		Woodland reclaimed			
1962		Spring barley			
1963		Winter wheat (18.7)			
1964	Break	Break	Break	Spring Barley	15.1
1965	Break	Break	32.2	?	26.6
1966	Break	33.8	27.4	26.7	27.2
1967	36.5	28.7	28.5	38.7	40.5
			% Take All		
1967	16.1	87.6	42.9	11.4	28.0

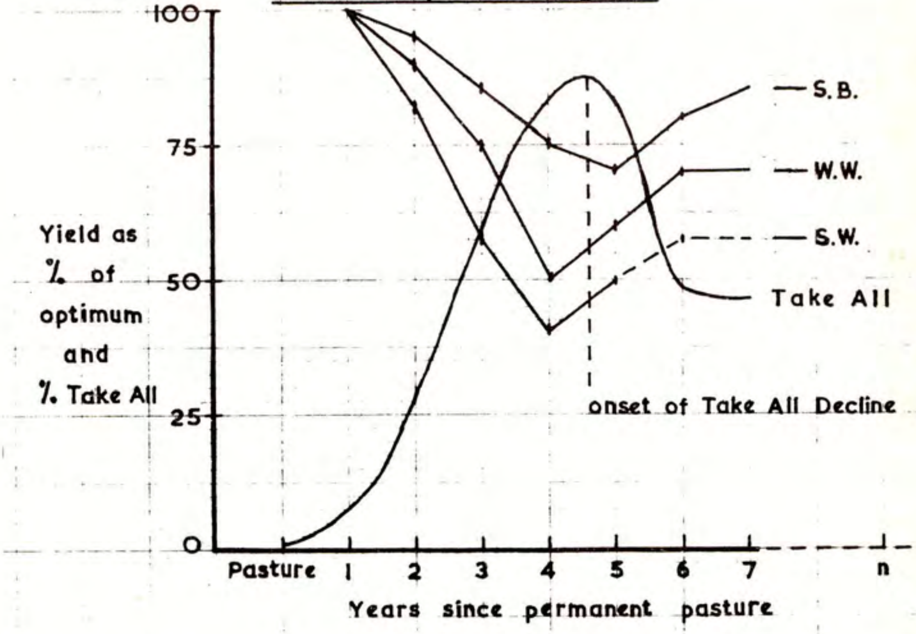
The similarity between these results and those from Rothamsted experiments is quite clear, though the high yield in the continuous cereal plots must be regarded as exceptional.

The available evidence suggests that there may be a general pattern of yield related to Take All during the early stages of a continuous cereal sequence (Figure 1).

It is not suggested that the pattern outlined is absolute or constant. Marked seasonal variations in the intensity of Take All attack occur and individual fields will be carrying different initial levels of take all at the time of their introduction to continuous cropping, depending on their previous cropping history and their degree of infestation with weed-grass carriers. The concept is nevertheless a valuable one in advisory work, enabling a forecast of probable crop performance to be made given a knowledge of the farm and detailed cropping history for several

Figure 1.

Generalised pattern of yields of wheat and barley grown continuously in England



Key S.B.– Spring Barley, W.W.– Winter Wheat, S.W.– Spring Wheat.

years.

Observations on farms and results so far available from Bridget's E.H.F. indicate that the year of peak Take All and the onset of Decline may be delayed in barley compared with wheat. The loss in yield of barley is less than that of wheat, while the level of Take All is usually much lower. This accords with the greater tolerance of barley to Take All widely recognised by farmers and pathologists.

Figure 1 may be compared with that of Zogg (1951) who gave a hypothetical yield curve for good crop rotation, bad crop rotation and continuous cereals in Switzerland. (Figure 2). It will be seen that the period of continuous cropping before a substantial yield recovery is obtained is much longer than in this country and Zogg (personal communication) considers the recovery to 80% may take as long as 30-70 years.

Zogg (1951) considered that the improvement in yield from Section II to section III in his hypothetical situation (figure 2) which occurs at some time or other after a series of years illustrated merely the passing away of a (disease) epidemic.

Gerlagh (personal communication) at Wageningen has also recognised Take All Decline in continuous winter wheat on the newly reclaimed East Polder in Holland where the fourth crop shows substantial yield recovery compared with the third crop, accompanied by a fall in the level of Take All.

Yield patterns in farm crops

Until recently accurate field yields have been almost unobtainable. Growing recognition, on the part of farmers, of the value of such data in management decisions has led to greater use of satisfactory methods of yield measurement and over the years 1965-67 advantage has been taken of this development to carry out field yield surveys in Hampshire, Berkshire and Oxfordshire (Figure 3). In spite of the obvious fact that very many variables other than Take All have a bearing on the final yield of cereals, the trends shown in these surveys are remarkably similar to the yield trends postulated in Figure 1.

Disease assessments have been made in 1967 on some of the fields included in the survey but these have not yet been analysed. Cook at Imperial College, London (personal communication) however has carried out an independent survey, examining Take All levels in fields of different cropping history with results shown in Table 5.

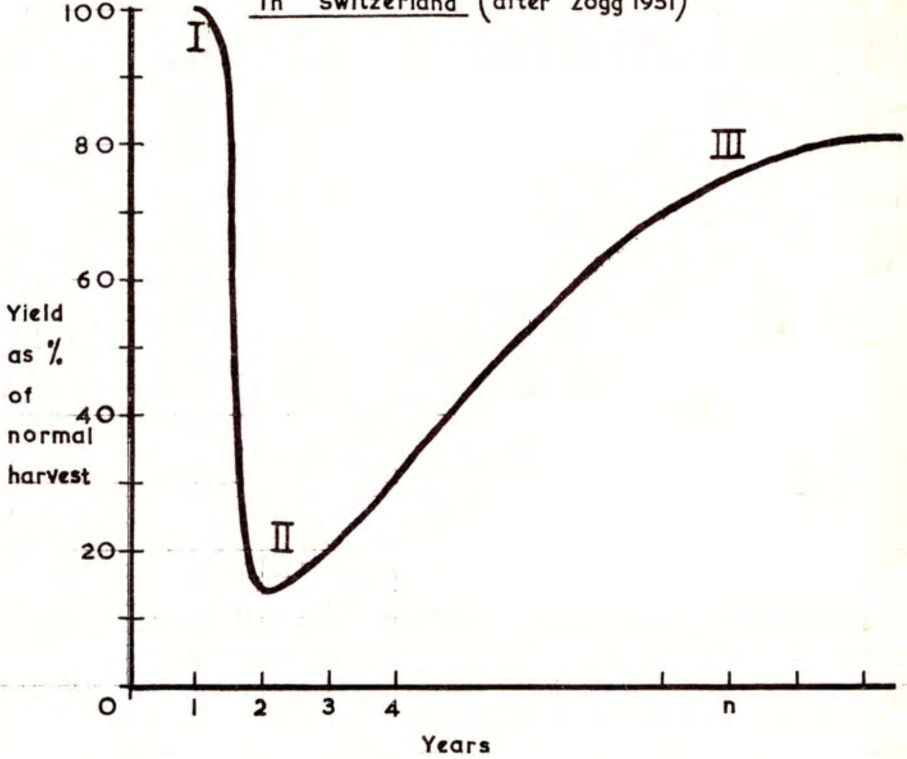
Table 5

Decline of Take All in continuous spring barley fields on farms in Hampshire and Berkshire 1967 (after Cook)

Number of years of barley	mean Take All		No. of fields
	% plants	% roots	
1 - 3	50.1	7.0	4
4 - 6	76	22.8	11
7 - 11	64	11.9	6

Figure 2.

Generalised pattern of yields of wheat grown continuously in Switzerland (after Zogg 1951)



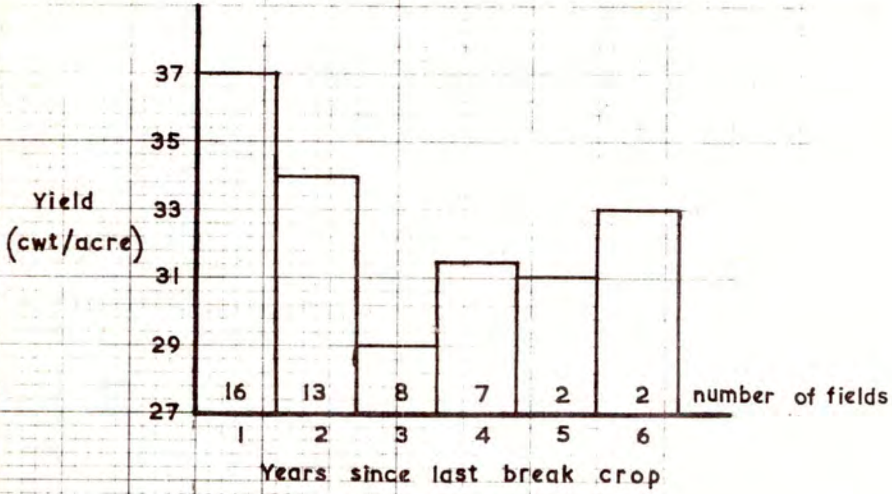
Key: Section I Good crop rotation, yield 100%

Section II Poor Crop rotation (susceptible cereals planted several years in succession) yield about 10%.

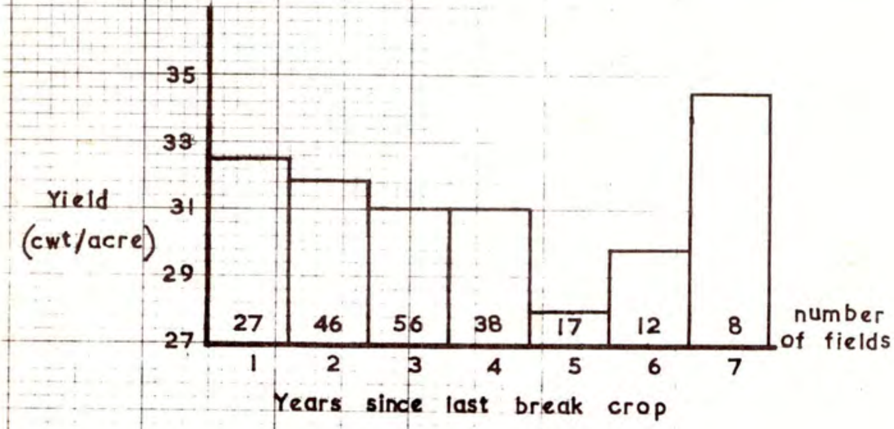
Section III Continuous cereals (mountain situations) yield about 80%.

Figure 3.

Yields of cereals and numbers of years of consecutive cropping.



(a) Survey of 85 fields of wheat and barley, Hampshire 1965.



(b) Survey of 204 fields of spring barley Hampshire and Berkshire 1966

Fields were chosen only on the basis of the length of cropping with barley and there was no information available on the amount of Take All in previous barley crops.

The figures support the observations that the peak of Take All occurs between the 4th and 6th year of continuous cropping.

Mechanism of Take All Decline

While there is now an accumulation of experimental and field evidence for Take All Decline in wheat and barley, work has only recently been started to investigate its mechanism.

Lester and Shipton (1967) considered it possible that Take All Decline may be the result of the development of a soil microflora, induced by continuous cereal cropping, which is antagonistic to O. graminis.

They devised a method based on the work of Zogg (1959) involving the addition of artificial inoculum of O. graminis to field soils and, after a period of incubation, measuring the survival of the fungus by bio-assay of the roots of test wheat plants. The technique has been applied to soil samples collected from many fields of differing cropping history and a selection of typical results is presented in Table 6.

Table 6

Survival of O. graminis added to field soils with different cropping histories

(Lester & Shipton 1967)

Year	Location	No. of previous cereal* crops	Root infection of wheat seedlings grown in soil	
			with added inoculum (per cent)	with sterile medium (per cent)
1964	Hampshire	1	59	3
		6	12	3
1965	Somerset	none	80	7
		9	19	4
1965	Devon	4	90	1
		9	42	nil
	Yorkshire	none	82	2
		6	36	3
	Hampshire	4	76	1
		8	30	4
1966	Hampshire	none	49	nil
		10	11	1
	Gloucestershire	none	58	nil
		8	14	1
	Hampshire	1	92	nil
		5	48	9

* wheat or barley

In the majority of comparisons made, soils from fields with long runs of cereals have reduced the survival of O. graminis to less than 50% of that of fields with short runs (6 crops or fewer). This technique has been used and might well be used more extensively in advisory work concerned with cropping problems.

The Added Inoculum technique has been used for the last two years to study the development and expression of antagonism in the phased cereal trials at Bridget's E.H.F. Results from the application of the technique to soil samples from the spring wheat trials in September 1966 are shown in table 7.

Table 7

Added Inoculum test results on phased Continuous Spring Wheat trials, Bridget's E.H.F. Sept. 1966
(% test plant infection)

Units of nitrogen	4th year wheat crop		3rd, 2nd, 1st year wheat crops	
	I	II	I	II
60	92	46.5	100	100
80	87.5	96	100	100
100	36.2	100	100	100

This indication of the onset of antagonism followed a severe attack of Take All in the 4th year plots only. In the barley trial, Take All was at a low level and no indication of the onset of antagonism was found.

It will be observed from Table 2 that those plots exhibiting antagonism in September 1966 were those showing Take All Decline in July 1967.

Studies on Take All Decline in the minimal cultivations trial at Jealott's Hill Research Station by Shipton and Brooks (in lit) using the Added Inoculum technique in 1965 and 1966 have indicated a strong association between level of nitrogen and antagonism (Table 8).

Table 8

Added Inoculum test results on Minimal Cultivation trial Jealott's Hill 1965

	Depth of sampling (ins)	% Plants	Take All % Roots	Number of lesions	p.p.m. phosphate	p.p.m. potash	% organic matter
N_3	0-2	8	1.9	4	20	432	10.9
180	2-4	41	3.2	46	17	317	9.4
units of	4-6	71	10.6	180	8.6	185	7.3
nitrogen	6-8	37	15.5	338	5.5	170	6.6
N_0	0-2	100	85.9	511	31	330	10.6
20	2-4	100	84.8	743	29	215	9.4
units of	4-6	100	85.5	847	23	150	8.0
nitrogen	6-8	100	77.9	626	11	135	6.3

The very strong manifestation of antagonism in the minimally cultivated plots is associated with the highest levels of applied nitrogen in the surface layers of the soil profile and also with the high level of phosphate potash and organic matter built up as a result of the minimal cultivations technique.

The spring wheat trial at Bridget's E.H.F. and the winter wheat trial at Crux Easton provide information on the change in level of Take All and yield before and after the onset of Take All Decline. There appear to be considerable differences in magnitude of the change between the two crops, which are summarised in Table 9.

Table 9

Changes in Take All and Yield in Cereals before and after Take All Decline

	Change in First to second crop			Change in Fourth to fifth crop		
	% Take All	% Yield	Ratio of Yield to Take All	% Take All	% Yield	Ratio of Yield to Take All
Spring Wheat	+ 33.4	- 25.3	1:9.76	- 20.6	+ 7.5	1:0.27
				Third to sixth crop		
Winter Wheat	+ 71.5	- 21.5	1:0.3	- 14.9	+32.9	1:2.2

Before Take All Decline, for every 1% increase in Take All the yield of spring wheat fell about twice as fast as that of Winter wheat. After Take All Decline the yield response to a 1% reduction in Take All the yield was nearly ten times as fast with winter wheat (but over a longer time scale).

Explanations for these differences are being considered and the possibility that there may be changes in pathogenicity of <u>O. graminis</u> is being investigated.

Experiments are planned to examine changes in the soil microflora particularly in the cereal root rhizosphere, before and after Take All Decline. There has been a number of papers published in which soil-borne micro-organisms have been examined for direct antagonism to <u>O. graminis</u> or in which the effect was antibiotic. None of the results except those of Cox (1963) were relate specifically to field soils where there was any evidence of Take All Decline nor was there any attempt to relate them to this phenomenon. The implications of the results however are being considered afresh in attempts to explain the mechanism of Take All Decline.

Zogg (1951) examined the pathogenicity of mixtures of stem base pathogens of cereals and found many that altered the pathogenicity of <u>Ophiobolus graminis</u>. In another paper (1959) he compared the influence of the microflora of different soils with differing crop rotations on the pathogenicity of <u>O. graminis</u> but his work did not include fields with a long enough run of continuous cereals to be showing Take All Decline. He considers that over many years of continuous cereal cultivation that in spite of stubble residues it is possible that a specific microbial association establishes itself which most certainly develops a strong soil disinfecting action (against <u>O. graminis</u>) which allows near-normal crop yields. Lal (1939) was able to group soil organisms into three groups according to their antibiotic effect against <u>O. graminis</u> in pot experiments.

Cox (1963) studied the relationship between the microflora associated with wheat roots and Take All development. Distinct differences in the actinomycete flora were found between the rhizosphere soil of the cereal-bean experiment and Broadbalk and several isolates were antagonistic to *O, graminis* in culture. However Slope and Cox (1964) suggested that Take All might be partially suppressed by an inhibitory factor, similar to the one that appears to control Take All in Broadbalk. Ehle (1966) also found in pot trials that antibiotically active actinomycetes could reduce infection of wheat seedlings by *O, graminis*.

Mangan (1967) in studies on the wheat rhizosphere fungi isolated *Gliomastix numcorum* (Cda) Hughes var. *felina* (Marsh) Hughes which killed *O. graminis*.

The role of any of these organisms in the promotion of Take all Decline has yet to be demonstrated but their discovery indicates a fruitful and interesting field of investigation.

DISCUSSION

The existence of Take All Decline as a widespread phenomenon can no longer be in doubt. There is a considerable weight of evidence on the changes in yields and levels of Take All in continuous cereals both from experimental and commercial sources.

Work by Slope and Cox at Rothamsted which first lead to the recognition of the phenomenon, has showed the consistent appearance of Take All Decline in each of nine successive years. The phenomenon appeared regularly in the same cropping sequences but its extent has dependent on season factors. Subsequent investigations by Lester and Shipton have shown that Take All Decline is not confined to winter wheat or to Rothamsted. There is detailed evidence at present to confirm it experimentally in spring wheat and widespread circumstantial support from hundreds of commercial fields of spring barley in Hampshire and Berkshire Surveys during the last three years, which have shown that yields follow the predictable pattern according to length of cropping.

Whilst the phenomenon is recognised it is not yet understood. On the assumption that it may be due to a cereal induced soil borne antagonism the Added Inoculum technique has been used to measure the presence and extent of soil borne antagonism in fields before and after Take All Decline. There is widespread agreement between the absence of such antagonism before Take All Decline in fields with a short run of cereal crops, and its presence once Take All Decline appears in those fields with a long history of cereals. Further work is needed to show that Take All Decline is caused by the development of the soil borne antagonism.

Work at Reading has been concentrated mostly on the chalkland soils and with spring barley and spring wheat. Insufficient evidence exists for other soil types except so far as the use of the Added Inoculum technique has been concerned. There is evidence from other soil types that continuous cereal cropping can be commercially successful but that this is not directly relatable to Take All Decline. On a boulder clay soil in East Anglia winter wheat has been grown seventeen years in succession with yields of nearly two tons. There is very little Take All present in the crops and there is no evidence either for a build up of Take All or for Take All Decline at any time in the sequence of crops. More work is required to investigate the properties of such soils with regard to levels of Take All and soil borne antagonism.

There is considerable information already published on the ability of soil borne organisms to affect the pathogenicity of *O, graminis*. As yet there has not been enough work carried out on Take All Decline soils to indicate that any of these organisms are present in sufficient numbers to account for the antagonism demonstrated by the Added Inoculum technique. It may be that there is a Take All Decline soil microflora typical of each separate soil type.

The widespread occurrence of Take All Decline is now well recognised by farmers and pathologists and its value is repeatedly apparent in the very satisfactory yield of fields with long runs of cereals when allied to a high standard of husbandry.

Acknowledgements

The author is indebted to Mr. E. Lester of the N.A.A.S. Reading for his valuable comments and criticisms in the preparation of this paper.

References

COX, Miss J. (1963) Rep. Rothamsted exp. Stn. for 1962 (1963) 118
EHLE, H. (1966) Z.f. Pflkrankh. 73. 321
ETHERIDGE, Mrs. J. and SLOPE, D.B. (1967) Rep. Rothamsted exp. Stn. for 1966 (1967) 123

LAL, A. (1936) Ann. Appl. Biol., 26, 247
LESTER, E. and SHIPTON, P. J. (1967) Pl. Path, 16, 121
MANGAN, Miss AEDINE, (1967) Ir. J. agric. Res. 6, 9

SLOPE, D.B. and COX, Miss J. (1963) Rep. Rothamsted exp. Stn. for 1962 (1963) 116
SLOPE, D.B. and COX, Miss J. (1964) Rep. Rothamsted exp. Stn. for 1963 (1964) 108

ZOGG, H. (1951) Phytopath. Z., 18, 1
ZOGG, H. (1959) Phytopath. Z., 34, 432.

EXPERIMENTS ON THE CHEMICAL CONTROL OF BARLEY LEAF DISEASES

J. E. E. JENKINS
National Agricultural Advisory Service, Trawscoed, Aberystwyth.

J. L. JEMMETT
National Institute of Agricultural Botany, Seale Hayne Agricultural College, Newton Abbot.

Summary

Up to 12 fungicide sprays gave an excellent control of several barley leaf diseases. Tank mix zineb and lime sulphur gave a good control of leaf blotch, halo spot, brown rust and yellow rust. Dichlofluanid controlled brown rust and drazoxolon controlled brown rust and mildew. The effects of the fungicides on yield and possibilities of chemical control of leaf diseases are discussed.

Introduction

In the past ten years the barley acreage in the west of England and in Wales has increased rapidly and during the latter five years it became apparent that leaf diseases were common and often severe. In 1965 a series of experiments were started in Devon and Cornwall to assess the effects of these diseases on yield. The effect of mildew on the yield of spring barley has been investigated on a national scale (Large & Doling, 1962) and our experiments were designed to assess the effects of other diseases. The most important was leaf blotch (*Rhynchosporium secalis*) and varieties susceptible to this disease were used in most of the experiments. Other leaf diseases which occurred in the experiments, and which were recorded, included halo spot (*Selenophoma donacis*), brown rust (*Puccinia hordei*) and yellow rust (*Puccinia striiformis*). Mildew resistant varieties were used in most of the experiments but in one experiment the effects of mildew were investigated using a susceptible variety.

The effects of cereal leaf diseases on yield have been assessed in several ways. Batts and Elliott (1952) compared the yields of susceptible and resistant varieties of winter wheat under conditions of severe yellow rust and no disease. Lawes and Hayes (1965) compared the yields of two lines of oats isogenic apart from disease resistance to mildew. Last (1955) used a fungicide to control mildew in spring barley. In the case of leaf blotch and all other barley leaf diseases except mildew, a high level of varietal resistance does not occur and isogenic lines were not available so in our investigations fungicides were used. Skoropad (1960) showed that tank mix zineb (nabam + zinc sulphate) gave a good control of leaf blotch and this fungicide was used in most of the experiments. Lime sulphur which Last (1955) showed did not effect the yield of barley in the absence of disease was also used and later some other fungicides were tested. This paper reports the effect of these fungicides on leaf diseases.

Methods

The plots were 15 or 13 drills wide (about 9 ft.) and 30 ft. long. The fungicides were applied with a pneumatic sprayer at a pressure of 50 lb/in² and at a rate of 40 gal/acre. The first spray was applied at the early tillering stage and subsequent sprays at 7 to 10 day intervals until the crops were mature. About 10 to 12 sprays were applied.

Disease assessments were made on the lamina of the flag leaf (leaf 1) and of the leaf below (leaf 2). The percentage leaf area affected was assessed for each of the various diseases separately. Records were made at growth stage 11.1 when the developing grain was very small and watery.

Results

Table 1

The effect of fungicides on barley leaf diseases recorded at growth stage 11.1. Each column is a separate experiment.

Fungicide, a.i./acre	% Leaf area affected (flag leaf)					
	Leaf Blotch	Halo Spot	Yellow Rust	Brown Rust		Mildew*
				A	B*	
Zineb, 26 oz. nabam + 20 oz. zinc sulphate	1.54	0.05	0.72	0.88	-	-
Lime sulphur, 1 gal. (2½%)	9.10	0.25	1.91	1.90	-	-
Dichlofluanid, 16 oz.	-	-	-	2.50	-	-
Dinocap, 2 oz.	-	-	-	-	5.93	4.53
Drazoxolon, 16 oz.	-	-	-	-	1.55	0.54
Control	48.5	19.77	6.30	13.70	7.64	8.28
S.E.	2.77	0.47	0.85	0.52	1.58	0.73

*assessments on leaf 2

The results in Table 1 have been taken from a series of experiments and show that a substantial reduction in the severity of leaf diseases is given by several fungicides. Tank mix zineb and lime sulphur gave a good control of leaf blotch and halo spot. These and other experiments indicated that zineb gives the better control. In the case of brown rust all the fungicides used except dinocap gave a good control. In experiment A in Table 1 dichlofluanid gave a slightly poorer control than zineb or lime sulphur when brown rust was recorded on the leaf lamina. Subsequent records on the leaf sheath showed that zineb was much the best fungicide and dichlofluanid was better than lime sulphur. In the mildew experiment the crop was late sown (in mid May) and the disease developed very late, after ear emergence. One spray at growth stage 10.5.4 was omitted and under these conditions drazoxolon was superior to dinocap.

Discussion

The experiments have shown that a good control of barley leaf diseases can be obtained with several fungicides. The unsprayed plots were easily distinguished from the sprayed plots especially where diseases were severe; the crop became senescent up to ten days earlier and the straw was a dull grey colour in contrast to the bright yellow straw of the sprayed plots.

Spraying increased yields by 12 to 63 per cent. In the case of leaf blotch a very good correlation between the disease on the flag leaf and loss in yield was established (unpublished data). For the other diseases similar direct correlations have not yet been established but in general a high level of disease control has been associated with a large increase in yield. An analysis of the components of yield - the number of fertile tillers, the size of the ear and the size of the grain, has shown that only the last (as measured by 1,000 grain weights) is substantially affected by controlling the leaf diseases. This agrees with the field observations that the plots were largely free from leaf diseases until ear emergence after which time disease often spread rapidly.

An objection to the use of fungicides in investigations into the effect of leaf diseases on yield is that the fungicides may affect yield in some way other than controlling diseases. The two most obvious ways are by the addition of water to the sprayed plots where the control plots are unsprayed, and by a nutritional effect on the crop. Observations and some experiments suggest very strongly that added water is not responsible for yield increases. We have not found any evidence that the nutrition of the host is affected and the yield increases have been obtained with chemicals of widely varying chemical composition. The very good correlation between the incidence of leaf disease and yield is further evidence that the yield increases in these experiments are very largely, if not entirely, due to the control of the leaf diseases.

Efforts to control cereal leaf diseases are mainly concentrated on the production of resistant varieties. Barley varieties with a high level of resistance to mildew have been used in recent years, but there are no varieties available which show a similar level of resistance to the other leaf diseases - halo spot, leaf blotch, brown rust and yellow rust. The breeding of resistant varieties is a long term project and major gene resistance often breaks down as new races of the pathogen are produced. The possibility of controlling leaf diseases by chemical means has not been feasible mainly because of cost, the difficulties of application without causing damage to the crop and also because the fungicides available are not suitable. In our experiments ten to twelve sprays were applied in an attempt to get maximum disease control. No experiments have been carried out to determine the optimum number of sprays to obtain this level of control but it is very likely that most of the early sprays and some of the very late sprays would not be necessary. One of the striking features of the experiments in the South West is the rapid build up of leaf diseases fairly late in the season, after the emergence of the flag leaf, and the large increases in yield which are obtained when these late attacks are controlled. It has been shown (Thorne 1966) that the flag leaf and the ear contribute a large proportion of the dry matter to the grain in barley so that if these two organs could be protected from leaf diseases, then most of the effects of these diseases would be avoided. The fact that in most leaf diseases (with the exception of mildew) the inoculum in the crop is at a low level until after the emergence of the flag leaf should make such protection possible. The ideal fungicide would be one which could be applied at the time of flag leaf emergence and which by systemic action would protect the flag leaf and the ear for a period of about five weeks. The damage to the crop by tractor spraying at such a late stage would be much less than that sustained through leaf diseases where they are severe as in some areas in the South West of England.

References

BATTS, C. C. V. and C. S. ELLIOTT (1952) Plant Pathology 1, 130.

LARGE, E. C. and D. A. DOLING (1962) Plant Pathology 11, 47.

LAST, F. T. (1955) Plant Pathology 4, 22.

LAWES, D. A. and J. D. HAYES (1965) Plant Pathology 14, 125.

SKOROPAD, W. P. (1960) Commonwealth Phytopath News 6, 25.

THORNE, G. N. (1966) in The Growth of Cereal and Grasses (Milthorpe F.L. and
J.D. Ivins, Eds., Butterworths, London. 1966).

ASSESSMENT OF BARLEY LEAF BLOTCH AND ITS EFFECT ON SPRING BARLEY YIELD

by W. Clive James
Plant Pathology Laboratory, Harpenden.

INTRODUCTION

Although leaf blotch of barley, caused by the fungus Rhynchosporium secalis has been recorded in this country since 1919, it is only comparatively recently that the disease has built up to a significant level. In the late 1950's severe attacks were noted in trial plots in Devon and since then the disease has become of increasing importance throughout England and Wales. In 1965 work started on the development of a disease assessment key so that the development of leaf blotch could be recorded, and the relationship between the incidence of the disease, as recorded by the key, and the loss of grain yield could be established. As Croxall (1965) pointed out, the task of producing a successful key presents a paradoxical situation, because the first essential is a complete knowledge of the life history of the pathogen and its effects on the host plant, but in order to estimate the damage caused by the disease, one must have some means of measuring it. In this paper the development of a key for assessment of leaf blotch will be described, and the effect of the disease on the yield of spring barley will be discussed briefly.

DEVELOPMENT OF THE KEY

A successful key for estimating disease in the field must satisfy two cardinal requirements. The first requirement is that all assessments based on the key must be accurate and uniform, and the second is that they must be achieved simply and quickly. The present keys for assessing foliar diseases on cereals are of the general descriptive type where tillers with varying amounts of disease (usually recorded in percentages) are described. The key for yellow rust of wheat has been described by Manners (1950). This involved assessing the percentage area affected on the total leaf area (using standard area diagrams for guidance) of approximately 50 culms. The field key for cereal mildew described by Large & Doling (1962) is a good example of a descriptive key, where seven levels of disease from (0.1 to 75%) are described in terms of the percentage laminae covered by mildew on the top four leaves. Such general descriptive keys assume that the epidemics always develop according to a standard pattern, and on occasions difficulty is experienced in making the assessment. For example, it is difficult to compute a percentage infection if only a fraction of the tillers are infected, or if the infected tillers are infected to varying degrees. Further complications can arise if the disease pattern on the plant, does not fit any of the disease patterns described for specific infection levels in the key. It is possible therefore that assessments made by general descriptive keys can be subjective under certain conditions.

Most of the earlier attempts to assess Rhynchosporium were made on N.A.A.S./ N.I.A.B. Rhynchosporium Trials in Devon and Cornwall. It was noted that most of the Rhynchosporium lesions appeared on the laminae of the leaves, although lesions could be found on the sheaths especially on the older leaves. However, many lesions were recorded at the junction of the lamina and sheath and the auricles had usually become infected. The lesions could be classified into two categories. During the primary stages of infection, distinct elliptical lesions with pale grey centres and dark brown margins were representative of the majority of lesions recorded. At a later stage in the development of the epidemic, large numbers of smaller lesions coalesced to produce a larger indistinct lesion which in some cases occupied the whole of the lamina.

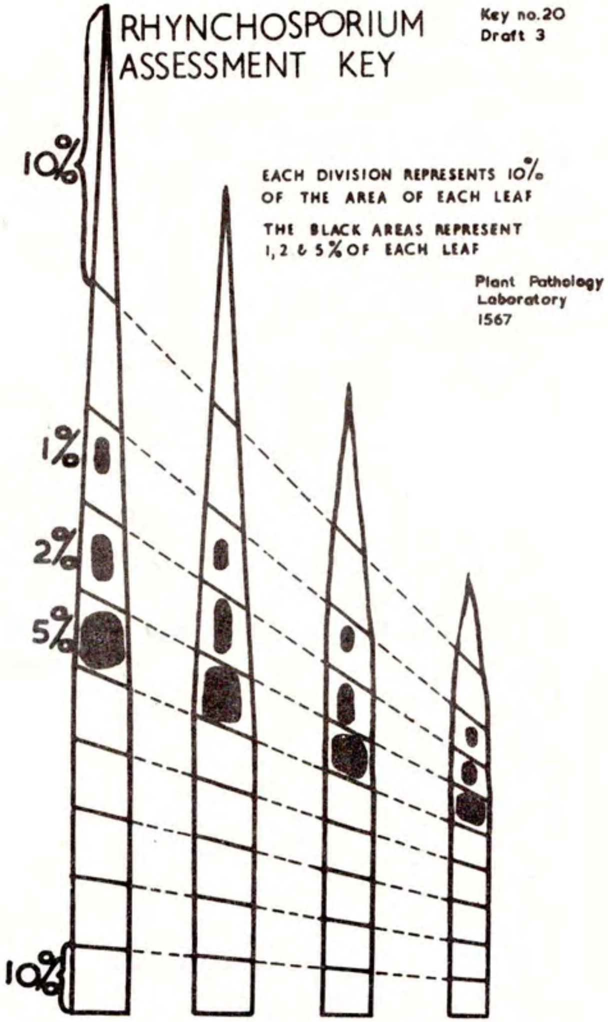
Standard area diagrams depicting the lesions of any particular disease are often used in disease assessment as guides for recorders assessing the area affected by the fungus. It is important that the forms of lesions are represented as accurately as possible on keys, because errors can result due to optical illusions; this is why a separate key is needed to assess yellow rust on barley where the lesions are linearly arranged. Fig. 1 represents the standard area diagram which was accurately drawn to represent the lesions of <u>Rhynchosporium</u>. It will be appreciated that it is impossible to represent all the different forms and distribution of lesions on any one key, but an effort has been made to cover the maximum number of variations without detracting from the value of the key. Variation in size of leaf lamina has been catered for by including four different sizes. Within each of the four laminae, the black areas represent the discreet primary lesions and occupy 1, 2, and 5% of the total area of each lamina. Each lamina has been further divided into ten equal sections by the dotted lines, and these 10% sections can be used individually or in conjunction with contiguous section/s to represent the larger indistinct lesions which develop later in the epidemic. It is therefore possible to use the key to assess any percentage by using the black areas in conjunction with the 10% sections.

The diagram was found to be quite easy to use in the field in assessing the percentage of lamina area affected by <u>Rhynchosporium</u>. (Note that 'affected area' includes the lesions and any yellowing which is clearly associated with the lesion). Before the key was used extensively in the field, tests were carried out to check that there were no significant differences between assessments made by several observers assessing the same sample of leaves, and also that minimal errors occurred between observed and actual affected areas; the latter test was carried out by making assessments in the laboratory using a planimeter. Good agreement was found between observers, and between actual and observed areas. This is probably a reflection of the objective assessments made when individual leaves were recorded as opposed to whole plants or a population of plants. A percentage scale has been used rather than a disease index or categories, because of the many advantages that the percentage scale offers. The upper and lower limits of a percentage scale are always uniquely and strictly defined, and the scale is flexible in that it can be divided and subdivided into meaningful divisions e.g. 10%, 1%, 0.1%. This type of scale is also widely accepted and this will help in standardising methods.

Having developed a satisfactory diagram, there were still three main factors to consider. The first is the time factor, i.e. at what growth stage should the assessments be done, remembering that only one or two visits are usually possible for field assessments. Feekes' Growth Stage key for cereals adapted by Large (1954) provided growth stage diagrams for barley, and it was decided to assess the crops at Growth Stage 11.1 when the grain is milky ripe. This stage is reached approximately 3 weeks after ear emergence when carbohydrate production for the grain is at a maximum. If more than one assessment were possible it was recommended that the first should be done at the start of grain carbohydrate production (Growth Stage 10.5), and the other at Growth Stage 10.5.3. It would have been desirable to have another assessment after Growth Stage 11.1 but assessment difficulties are encountered in the field due to the increasing amount of natural senescence after Growth Stage 11.1.

Secondly it had to be decided which leaf or leaves were going to be assessed for disease. Because the main objective of the key was to establish a relationship between disease incidence and loss in yield of grain, it would obviously be desirable to assess the leaves of barley plant that contributed most to the grain yield. Furthermore, if these leaves were assessed once during the time of maximum grain carbohydrate production (i.e. if only one assessment was possible), the likelihood of obtaining a good correlation with yield should be greater. Of course, if more than one assessment is possible, an additional assessment at the beginning and end of grain carbohydrate production would provide more precise information. Thorne (1965) concluded that "most of the carbohydrate in the grain comes from photosynthesis after the ears emerge", and Watson et al. (1958) showed that only 15% of the final grain weight comes from below the flag leaf node (most of which probably comes from the second leaf) and that the flag leaf and sheath contribute 59%, and the

Fig. 1


RHYNCHOSPORIUM
ASSESSMENT KEY
Key no.20
Draft 3
10%
EACH DIVISION REPRESENTS 10%
OF THE AREA OF EACH LEAF
THE BLACK AREAS REPRESENT
1,2 & 5% OF EACH LEAF
Plant Pathology
Laboratory
1567
1%
2%
5%
10%

ear 26% to grain weight. Most of the grain dry weight of barley can be accounted for by photosynthesis above the flag leaf node (Thorne 1963) of which 55% is contributed by that part of the shoot excluding the ear. This physiological data suggested that leaves 1 (flag) and 2 were important for carbohydrate grain production and it was decided to assess the amount of disease on the laminae of those two leaves. The sheaths were not assessed, because the amount of disease found on them is always very low and furthermore the assessment of percentage area is much more difficult. Similarly, assessment and diagnosis of disease on the ear is difficult and is usually at a very low level.

Finally, a sampling method had to be selected so that a random sample of leaves could be chosen from the plots. The samples of leaves for assessment were selected by choosing main tillers at random from a plot. As it is difficult in practice, to identify the top leaves with their corresponding culms when cutting tillers at ground level, deliberate selection of diseased or healthy leaves is avoided. In the 1/100th acre plots, 5 tillers were taken at random from different drills from each side of the plot, totalling 10 tillers per plot, and the percentage area affected by Rhynchosporium assessed on the laminae of the top two leaves. The number of tillers selected per plot is, in practice, governed to a large extent by the time factor and 10 tillers per plot were found to produce reasonable standard errors for the mean values. For field assessments, reliable estimates of diseases incidence can be achieved if 25 main fertile tillers are selected at random along each of 2 diagonals, totalling 50 tillers.

The relationship between assessments, using the key, and loss in yield is fairly consistent for the results obtain from trials in the south-west; these will be reported in detail in a later paper. The percentage loss in grain yield is equivalent to approximately 2/3 of the infection on the flag leaf lamina or 1/2 of the infection on the lamina of the second leaf e.g. if the percentage infection on the flag leaf was 35% and the 2nd leaf was 60%, the predicted losses in grain yield would be approximately 25% and 30% respectively based on the 1st and 2nd leaf infection. The predicted loss in grain yield is the average of these two values (25 and 30) which is equivalent to 27.5% loss in yield.

References

Croxall, H.E. (1965) Proc. Br. Insectic. & Fungic. Conf., 294.

Jenkins, J.E.E. & Jemmett, J.L. (1967) N.A.A.S. q. Rev., 75, 127.

Large, E.C. (1954) Pl. Path., 3, 128.

Large, E.C. & Doling, D.A. (1962) Pl. Path., 11, 45.

Manners, J.G. (1950) Ann. appl. Biol., 37, 187.

Thorne, G.N. (1963) Ann. Bot. (N.S.), 27, 155.

Thorne, G.N. (1965) The Growth of Cereals and Grasses (Milthorpe, F.L. & Ivins, J.D., Eds., Butterworths, London 1965).

Watson, D.J. Thorne, G.N., & French, S.A.W. (1958) Ann. Bot. (N.S.), 22, 321.

The effect of cereal cyst nematode on the yield of spring barley in S. England

by

C. A. Collingwood

National Agricultural Advisory Service, Coley Park, Reading

High populations of cereal root eelworm occur in intensive corn growing areas on the lighter soils of Southern England. Such soils include chalk, greensand, Cotswold brash, old red sandstone and sandy drifts most suitable for spring barley. Certain fields in these areas may give uneconomic yields of barley except after long breaks from cereals and many of these cases are associated with eelworm populations (Collingwood 1962) estimated at 30 or more eggs per gm. of soil where some effect on barley crops would be expected (Duggan, 1961).

A general survey of 71 spring barley crops in the South West region in 1960/1961 showed 45 to be infested to some degree with cereal root eelworm. The mean yield from these 45 fields based on farmer estimates was 25.3 cwt./acre compared with 30.8 cwt./acre from 26 fields where eelworm was absent or not detectable. In 1962, 39 fields were more critically examined; of these 16 had no detectable eelworm and gave a mean yield of 34.5 cwt./acre, compared with 26.5 cwt./acre from the remaining 23 with varying levels of eelworm, 6 of them (15%) in the high category. There was an indication therefore from these results that eelworm could be associated with general reduction of yields of 5.5 to 8 cwt./acre on infested land. Since field environments were clearly different in a number of variables, confirmation for these indications was sought from within field experiments carried out during 1964-1967 in Hampshire and Berkshire in the South East region.

An attempt to obtain a regression of barley yield on eelworm count from small plots within an infested field, as part of a national exercise, was unsuccessful on a Berkshire site. This was thought to be due to the range of infestation being too small for critical differences to be demonstrated. Therefore trials were carried out using the partially resistant Danish barley Kron (Gair, Price and Fiddian, 1962) compared with the susceptible variety Proctor and also with the soil sterilants methyl bromide ($\frac{1}{2}$ - lb. per 100 sq. ft.) and dazomet (1 lb. per 100 sq. ft.).

Table 1

Comparison of yields of Proctor (susceptible) and Kron (resistant) on high eelworm infestation

Site	Eggs/gm. before drilling	Yields Proctor	cwt./acre Kron	Kron as % Proctor
N.I.A.B. trials 1955-6*	nil	35.0	34.0	98
Aston Tirrold 1967	19.0	30.0	35.0	117
Blewbury 1964	40.0	25.4	36.7	144
Damerham 1967	45.0	15.0	22.0	133
Childrey 1965	80.0	17.0	29.4	173
Rockbourne 1966	90.0	20.5	27.0	132
Mean of infested sites		21.6	30.0	138

*Eade, 1957

On land with low eelworm infestation or none, Kron is normally outyielded by Proctor. Gair, Price and Fiddian (1962) showed that this situation is reversed on high infestations. The series of trials tabled above show large and consistent differences in favour of Kron on infested land with a mean yield difference of over 8 cwt./acre.

In a parallel series of soil sterilant trials, some plots were laid down on low eelworm sites to compare possible soil amelioration or disease control effects with results from plots on high eelworm sites in fields sown to either Impala or Proctor barley.

Table 2

Effects of soil sterilants on barley yields in presence of low and high eelworm counts

Site	Eggs/gm before treatment	Yields cwt./ac. untreated plots	% increase/decrease methyl bromide	dazomet
Hurley 1965	0.0	41.6	+ 5	
Wantage 1966	0.0	22.0		- 5
Buckland 1966	0.2	26.0		- 23
Damerham 1965	3.0	21.0	+ 8	nil
Pasey 1966	4.9	13.0		+ 20
Blewbury 1966	6.8	21.0		+ 5
Mean of low eelworm sites		24.1	+ 6.5	- 0.6
Upton 1966	22.0	24.0		+ 21.5
Ashe 1966	24.0	19.0	+ 71.0	+ 44.4
Damerham 1966	30.0	16.2	+ 69.1	+ 43.8
North Moreton 1966	36.4	18.0		+ 34.5
Damerham 1967	45.0	15.0		+ 92.9
Childrey 1965	215.0	17.0	+ 83.5	
Mean of high eelworm sites		18.2	+ 74.5	+ 47.4

In these trials neither methyl bromide nor dazomet gave yield responses on low eelworm sites suggesting that on the heavily infested sites consistent yield increases could be mainly attributed to the control of eelworm. Yields were increased on the dazomet plots on infested land by an average of over 8 cwt./acre compared with no treatment.

The experimental results shown in tables 1 and 2 therefore confirmed the estimated mean yield loss of 8 cwt./acre that could be attributed to the presence of heavy eelworm infestation from the limited corn yield observations in the South West in 1962. It should be noted however that Kron is only partially resistant to cereal root eelworm sustaining the full larval invasion after germination but reducing cyst production compared with Proctor by about 75%. Similarly none of the soil sterilant trials gave full eelworm control with a mean reduction of eelworms invading roots of about 75%. Thus the true mean yield loss due to eelworm on land with infestations of more than 20 eggs/1 gm. is probably nearer 10 cwt./acre than 8 with the present commercial varieties and husbandry methods used.

An estimate of losses based on large scale random surveys is not available. From advisory cases 1965-1967, in the counties of Hampshire, Berkshire, Oxfordshire and Buckinghamshire, there are at least 70 parishes where one or more farms have fields in the high eelworm category. Most of these are on the chalk areas of West Hampshire and Berkshire and the mean annual loss is estimated at a conservative minimum as 800 tons from 2,000 acres of heavily infested land. In the South West the main areas affected are the chalklands of Wiltshire and Dorset and the limestone brash of Gloucestershire and North Wiltshire. Here from the limited surveys of 1960-1962, 15% of spring barley fields were in the high eelworm category giving approximately 9,000 acres of heavily infested barley crops or average annual loss of 3,600 tons.

The remaining counties of S. England including Cornwall, Devon, Somerset, Surrey, Sussex and Kent have relatively little heavily infested land and an estimate of loss for these counties together would be conservatively estimated as 100 tons per annum. The total annual loss for S. England is here estimated as not less than 4,500 tons due to the effects of cereal root eelworm.

References

Collingwood C.A. 1962

Continuous Corn Growing and Cereal Root Eelworm in the South West
N.A.A.S. Quart. Rev. 58: 70

Duggan J.T. 1961

The effect of cereal root eelworm (Heterodera major O. Schmidt) on its hosts.
Irish Journ. Agric. Res. 1: 7

Eade A.J. 1957

Spring barley variety trials, 1951-1956
J. nat. Inst. agric. Bot 8: 36

Gair R, Price T.J.A. and Fiddian W.E.H. 1962

Cereal root eelworm (Heterodera avenae Woll.) and spring barley varieties
Nematologica 7: 267