

A PRELIMINARY REPORT ON THE GENETICS OF RESISTANCE TO

ORGANOPHOSPHATES IN MYZUS PERSICAE

R. L. Blackman

Department of Entomology, British Museum (Natural History), London SW7 5BD

Summary Preliminary results of a study of the inheritance of organophosphate resistance in Myzus persicae are described. F<sub>1</sub> clones obtained from resistant X susceptible crosses all showed the high level of carboxylesterase activity which is generally associated with organophosphate resistance. In bioassays using a haemocoelic injection technique, F<sub>1</sub> clones all showed a degree of resistance to dimethoate, and at least some of them had a level of resistance comparable to that of the resistant parent clone. It is tentatively concluded that organophosphate resistance is in this case due to a dominant or near-dominant major gene. Present knowledge of the genetics of resistance in M. persicae is discussed.

INTRODUCTION

Resistance to organophosphorus insecticides in the peach-potato aphid Myzus persicae has been known for 20 years (Anthon 1955), yet there has been no published work on the genetic mechanisms involved. Studies of aphid genetics are hampered by many factors such as the single annual sexual generation, and complications of the life cycle including the obligatory host alternation which is found in many important pest species such as M. persicae. Nevertheless, an investigation of the genetics of insecticide resistance is important, in order to understand changes in the frequency of resistance genes in aphid populations, and also to clarify the problem of the varying stability of resistance levels within aphid clones, which has been widely reported (reviewed by Beranek 1974a, and Boness & Unterstenhöfer 1974). Apart from its practical importance, organophosphate resistance is also an ideal marker to use as a starting point for fundamental work on aphid genetics. This report describes and discusses the first year's results of an investigation into the inheritance of organophosphate resistance in M. persicae.

METHOD AND MATERIALS

Techniques for maintaining clones of M. persicae on excised potato leaves, producing sexual morphs and conducting breeding experiments, have been described elsewhere (Blackman 1971, 1972). The resistant parent clone was "French R", originating from peach at Valergues, France in 1968, which has maintained a high level of resistance to organophosphates for several years in the absence of any selection pressure. This clone was chosen because it was holocyclic (produced both male and female sexual morphs), and because it seemed the most likely of those available to be homozygous for the resistance gene or

genes. Oviparae and males of this clone were crossed with three holocyclic susceptible clones, J, U and A<sub>2</sub>, from breeding lines which had been followed through several sexual generations without showing any indications of insecticide resistance.

Clones of the F<sub>1</sub> generation resulting from resistant X susceptible crosses were started from individual fundatrices and reared separately on excised potato leaves. To assess their resistance, use was made of the correlation between carboxylesterase activity, revealed electrophoretically, and organophosphate resistance (Beranek 1974b). Horizontal starch gel electrophoresis was employed and the technique was essentially that of Beranek (1974b), using 1-naphthyl acetate as substrate. All F<sub>1</sub> clones were maintained for 15-20 parthenogenetic generations during which their esterase activities were checked three times.

The resistance level of each F<sub>1</sub> clone was also estimated directly on at least one occasion by injecting various concentrations of dimethoate into the haemocoels of etherised aphids, using a fine glass needle. The dimethoate was initially dissolved in a drop of acetone and the solution then made up in insect saline. The range of concentrations used was from 0.0005% to 0.025%. Ten newly ecdysed adult apterae of each clone were injected at each concentration with a dose of about 0.02 µl, which was varied slightly according to the size of the aphid. Numbers dead after 24 hours were counted and compared with controls injected with insect saline only.

#### RESULTS

Seventeen F<sub>1</sub> clones were obtained from crosses made in autumn 1974. The matings between oviparae of French R and males of clone J were most successful and provided 12 of these F<sub>1</sub> clones. Electrophoresis of samples from each clone in the 2nd or 3rd parthenogenetic generation after the fundatrix indicated that all F<sub>1</sub> clones had a high level of activity of the carboxylesterase ("esterase 2" - Beranek 1974b) which is generally associated with organophosphate resistance. Esterase 2 activity was still high in all the F<sub>1</sub> clones on re-examination after 6-10, and after 16-21, parthenogenetic generations.

Bioassays by haemocoelic injection were done on samples taken from each F<sub>1</sub> clone between 10 and 16 generations after the fundatrix. It was not possible to estimate resistance levels accurately because of the wide range of variation (10-50%) in mortality of the saline-injected controls. No aphids survived injection with 0.025% dimethoate. Some French R survived 0.01% dimethoate and all lower concentrations, whereas the susceptible clone J was killed by all concentrations down to and including 0.0005%. All F<sub>1</sub> clones showed some evidence of resistance to dimethoate, and some of them appeared to have a similar level of resistance to French R. However, certain F<sub>1</sub> clones did seem to be less resistant than French R. More intensive bioassay work will be necessary to confirm relative resistance levels of parent and F<sub>1</sub> clones.

#### DISCUSSION

These preliminary results must be treated circumspectly, and any firm conclusions must await the results of F<sub>1</sub> matings and backcrosses made in autumn 1975. Nevertheless, there is good reason to believe on the basis of the generally high level of resistance found in all F<sub>1</sub> clones, that the parent clone French R is homozygous for a dominant or near-dominant major gene conferring resistance to organophosphates.



Until 1974 the problem of insecticide resistance in M. persicae in Britain was restricted to glasshouses, where high levels of organophosphate resistance have developed, coupled with resistance to DDT and other insecticides. At least one of these glasshouse populations has also recently developed cross-resistance to pirimicarb (P.H. Needham, pers. comm.). In the glasshouse environment, continuous parthenogenesis is possible and it seems likely that under the intensive selection pressure of repeated insecticide applications numerous resistance-conferring mutations have been incorporated successively into the same parthenogenetic lines to give high levels of resistance and cross-resistance. However, the basis for resistance to organophosphates may still be a single dominant gene. Resistant M. persicae in British glasshouses are not all of one clone, as there are at least three different genotypes with high esterase 2 activity (Beranek 1974b, Blackman 1975), but it does seem possible that there are only a small number of clones which can be characterised by their esterase patterns and other properties. A single chrysanthemum-adapted clone of M. persicae with a high level of cross-resistance to insecticides seems to be widespread in glasshouses in southern England, and is probably dispersed on chrysanthemum cuttings as suggested by Wyatt (1966). This clone produces an unusually high proportion of *alatae*, the immatures of which are reddish in colour.

There is one property which is common to all resistant M. persicae from glasshouses so far examined; they are all heterozygous for a chromosomal translocation (Blackman 1975). Eight samples of resistant M. persicae from British glasshouses, one sample from Czechoslovakia and one from Finland have now been examined, and all have a translocation between the first and third pairs of autosomes. A similar or identical translocation has been found in field populations of M. persicae from Japan and California, but no information is yet available on the degree of insecticide resistance in these populations. Certainly, organophosphate resistance and the translocation are not necessarily associated under field conditions; French R does not have the translocation, and neither did samples of resistant M. persicae from field populations on sugar beet in England in 1974. It remains to be seen, therefore, whether there is any direct link between resistance and the translocation, or whether they occur together in glasshouses simply because both are favoured by the glasshouse environment.

Changes within aphid clones in the level of insecticide resistance have been reported and discussed by many authors, most recently by Beranek (1974a), Boness & Unterstenhöfer (1974) and Hürková (1973). Many of the reported changes in properties within laboratory clones of aphids may be attributable to contamination, as it is clear that some workers have underestimated the degree of care and standards of hygiene necessary to achieve complete isolation of separate aphid stocks. However, changes in resistance levels within clones appear to be well substantiated, and are frequently explained as a manifestation of "endomeiosis", the genetic recombination which has been claimed to occur within parthenogenetic lines of aphids by Cognetti (1961). In the present work the stability of resistance in  $F_1$  clones, which are presumably initially heterozygous for the resistance factor, is evidence against any significant level of recombination involving the chromosome carrying the resistance gene. Such recombination, if it occurred, would be expected to result in loss of the resistance factor in at least some of the  $F_1$  clones. Endomeiosis does not in any case provide a satisfactory explanation of the intraclonal changes and variability observed by other workers. It seems more likely that some mechanism of gene activation and inactivation is implicated, so that in certain cases the expression of the resistance gene(s) can be modified directly or indirectly by factors in the environment.

Insecticide resistance is a far more complex situation genetically in field populations of aphids than in the glasshouse. Selection pressure for the development of resistance is not so intense as in the glasshouse, especially in a polyphagous aphid such as M. persicae where only part of the population of an area is

subjected to insecticide at any one time. Whether resistance develops to a significant level will depend on numerous factors of the aphid's ecology, about most of which we have inadequate information. The distances moved by migrating aphids, the extent to which host-specific races occur within the species, and the relative contributions of sexually and parthenogenetically overwintering aphids to the next year's populations, are especially important considerations. However, with some knowledge of the genetics of resistance of M. persicae we should be able to interpret the observed changes in resistance within field populations. In the long term, this may help towards a better understanding of some of the basic problems of aphid ecology, which in turn may enable us to predict, and perhaps overcome, some of the problems of insecticide resistance in M. persicae.

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THE ESTABLISHMENT OF ECONOMIC DAMAGE THRESHOLDS WITH PARTICULAR  
REFERENCE TO CEREAL AHBIDS

K.S. George

Plant Pathology Laboratory, MAFF, Hatching Green, Harpenden, Herts.

Summary An integrated control strategy for any crop includes the possibility of a level of insect attack which makes insecticidal spraying necessary. The level of aphid control on cereals plays an important role in the maximum production of grain.

This paper presents results of a co-operative experiment being done jointly in the UK and other north west European countries to establish the threshold in terms of cereal growth stage and aphid numbers per tiller at which spraying with an insecticide becomes economically justifiable.

Sommaire Pour toute culture, l'emploi de méthodes de lutte intégrée inclut la possibilité que le degré d'infestation de cette culture par les insectes rende nécessaire un traitement insecticide. Les insecticides employés contre les pucerons en cultures de céréales jouent un rôle important dans l'obtention d'un rendement maximum en grains.

Dans cet article nous donnons des résultats d'une expérimentation menée en coopération au Royaume Uni et dans d'autres pays de l'Europe du Nord-Ouest, en vue d'établir, en fonction du stade de croissance de la céréale et du nombre de pucerons par talle, le seuil au delà duquel un traitement insecticide devient économiquement rentable.

INTRODUCTION

At an earlier British Insecticide and Fungicide Conference, Way (1973) discussed methods of forecasting pest and disease attack on crops and emphasised the need to apply pesticides only when they are needed rather than on a routine, insurance basis. Strickland (1969) had earlier discussed pests of wheat and the costs of controlling them. The concept of economic thresholds in pest control is based in its simplest form on the necessity and the cost of spraying: the cost of buying and applying insecticide must be less than the value of the extra yield obtained by ridding the crop of the particular pest against which the treatment is applied. However, there are other factors on the input side of the equation which should be considered, but these apply mainly on a national rather than on an individual crop level and can seldom be heeded by the farmer who has an immediate pest problem to solve. Such factors as the cost of diagnosis, the risk of diminishing susceptibility to the insecticide, the side effects of the spray on the crop and other organisms, and the necessary back-up information required on the levels and effects of pesticide residues in the crop and the soil are recognised, but can seldom be taken into account, and most of them have not been adequately evaluated.

Most advice on the necessity of spraying to rid a crop of a particular pest is restricted to the immediate economics of the situation. Likewise, the farmer

has maximised his profit by exploiting the cropping system to the best of his ability and unless certain conditions apply, such as requirements for precautionary sprays on forward contracts, he accepts the need to spray only when he is convinced that the treatment is likely to be economically worthwhile. The establishment of economic thresholds is thus vital to decision-making by the farmer.

Adaptation and exploitation of normal farming practices within a cropping system are frequently necessary to minimise pest losses and maximise yield. Normally, cereals are grown to minimise the possibilities of overwhelming pest and/or disease attack and the need for chemical control. Recommended varieties are sown and, if these have inbuilt resistance to pests or diseases, they are used extensively in areas at risk. Cultural adaptations to minimise attack from certain pests are commonly practiced. Thus, sowing dates are exploited to the full to control pests such as wheat bulb fly and frit fly. Where the need for chemical control of pests is a certainty, seed treatments, if available, are used in preference to insecticidal sprays at a later growth stage of the crop.

Kennedy (1968), discussing the general case for integrated control of pests, pointed out that increasing agricultural productivity has resulted in altered ecosystems and decreased biological diversity on agricultural land. Within some crops, certain pest species have an overwhelming advantage because of modern farming techniques. One principle of integrated control is that as many and as diverse checks on the pests as possible be deployed to reduce this advantage to its lowest point. In this way the necessity for chemical control on larger acreages can be reduced to a minimum and the possible onset of insecticide resistance delayed.

Paradoxically, pests on intensively grown cereal crops are usually less important than those which occur in cereals grown in alternate husbandry rotations. For example, many of the pests of grassland are able to migrate to succeeding cereal crops but these pests become much less important under intensive cereal systems. However, certain pests assume a greater importance and it is with a group of pests in this category, cereal aphids, that this paper is concerned.

Work on cereal aphids has shown that considerable grain losses may be caused to spring barley crops by stem- and leaf-feeding aphids, mainly Metopolophium dirhodum (Wlk.) (George 1974, Lowe 1974, Wratten 1975). These are generally unnoticed by farmers who are more concerned with the easily visible aphid infestations, mainly Sitobion avenae (F.), in cereal ears.

At the earing stage, spraying the crop can be difficult because, even if done carefully, some plants will be damaged by the tractor and sprayer moving through the crop. The alternative is to spray from the air but this can be more expensive and it is often difficult to arrange at the time required. The farmer, therefore, is not anxious to spray unless the treatment is economic. Experimental work has been directed, therefore, towards establishing the frequency and timing of the sprays.

Much of this work has been done in South-east Essex where cereal aphids occur every year. Fortunately, Barley Yellow Dwarf Virus (BYDV), which is transmitted by cereal aphids, occurs only at a low level, or is absent in this area. Further work is needed before the effects of low levels of BYDV can be quantified and their contribution to grain yield loss assessed.

In 1972, three experiments were done (George 1974) and it was concluded that aphid burdens on ears of winter wheat before the grains start to fill have little effect on subsequent grain yield, but once filling begins a product of aphid numbers and time comes into operation and yield is decreased.

Similar experiments in 1973 were mainly inconclusive due to adverse weather conditions during ear emergence and grain ripening though this indicated the value of



one insecticidal spray against aphids on winter wheat ears at the beginning of flowering.

Other workers, notably Kolbe (1969 and 1970) in Germany and Reitzel (1967) in Denmark, have obtained similar results which suggest that aphid feeding at the bases of the filling grains has a detrimental effect on yield.

#### METHOD

At a meeting of the International Organization for Biological Control Working Party on Cereal Pests in December 1973 it was agreed that those participants interested in cereal aphids should take part as a co-operative project in 1974. This took the form of an experiment designed to establish the effectiveness against aphids of a single spray of insecticide applied at the beginning of flowering.

Each experiment consisted of a minimum of four replications of one treated and one untreated plot. The treatment was one spray of pirimicarb applied at growth stage 10.5.1. (Large 1954) i.e. the beginning of flowering. Pirimicarb was chosen because of its specificity against aphids. Plots were five metres square. Counts of aphids per ear and also per tiller were made just before spraying, and as often as possible thereafter until harvest and were based on 25 complete tillers per plot taken at ground level to eliminate selection bias. Forty-four experiments were completed, 18 of them in the Netherlands and 22 in the UK.

In the UK, experiments were based on five replications and, with the exception of one experiment, were done in the eastern and south-eastern parts of the country by members of the Agricultural Development and Advisory Service. All these experiments were on winter wheat and covered nine varieties. There were nine experiments on Maris Huntsman, four on Cappelle-Desprez, two each on Maris Nimrod and Maris Templar and single experiments on the varieties Atou, Bouquet, Chalk, Val and Maris Widgeon. The sprays were applied at the beginning of flowering which occurred mainly in the first half of June. In the Netherlands, 13 experiments were on variety Clement and two on Cyrano, and single experiments were done on Caribo, Lely and Manella.

As previous work had shown the possibility of yield loss occurring when a crop had a mean of five or more aphids per ear at flowering, with populations increasing, it was decided that this number should be taken as the base line or critical level for analysis of the experiments. In the U.K., aphid numbers rose to over five per ear in 11 experiments, and in the Netherlands, there were more than five aphids per ear on six experiments.

#### RESULTS

An analysis of the 22 UK sites taking out differences due to sites, showed that the overall mean yields were not significantly different (Table 1). However, the overall mean yield from the sprayed plots on those 11 sites with five or more aphids per head was significantly greater than that from the unsprayed plots (3.1 cwt per acre, 389 kg/ha). On the other 11 sites where there were less than five aphids per ear, there was no difference in yield between the treated and untreated plots. On the sites where most aphids occurred, the overall mean yield was much lower than on the remaining 11 sites, suggesting that these crops might have been under stress from some other factor before aphid infestation and build-up occurred. Wearing and Van Emden (1967) have shown that aphid reproduction is affected by water stress and its secondary effects in plants such as beans and Brussels sprouts. These results suggest that with cereals some stresses which may produce changes enhancing aphid reproduction act detrimentally on the plant itself.

Table 1

Winter wheat experiments, U.K., 1974. Single spray at growth stage 10.5.1.

Mean yield of 11 sites with >5 aphids per ear

Untreated	3562 kg/ha	(28.38 cwt/acre)
Treated	3951 kg/ha	(31.48 cwt/acre)
S.E.	± 108	± 0.86

Mean yield of 11 sites with <5 aphids per ear

Untreated	5981 kg/ha	(47.66 cwt/acre)
Treated	5877 kg/ha	(46.83 cwt/acre)
S.E.	± 108	± 0.86

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In the Netherlands 18 experiments were done but in only six of them were there more than five aphids per ear at flowering. In five of these six, the numbers of aphids at flowering were only 6, 9, 9, 9 and 18 and these diminished rapidly. In the other experiment the number rose from 9 to 13 in two weeks. There were no significant differences in yield between treated and untreated plots and differences in yield were slight (Table 2). The maximum was 9 per cent increase in one experiment, while the increase in all others fell within the range 95 - 106 per cent.

Table 2

Winter wheat experiments, Netherlands, 1974. Single spray at growth stage 10.5.1.

Mean yield of 6 sites with >5 aphids per ear

Untreated	5739 kg/ha	(45.73 cwt/acre)
Treated	5873 kg/ha	(46.80 cwt/acre)

Mean yield of 12 sites with <5 aphids per ear

Untreated	6021 kg/ha	(47.98 cwt/acre)
Treated	6078 kg/ha	(48.43 cwt/acre)

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Grain from each plot on the 22 UK sites was sieved into five fractions. Size group differences between treated and untreated plots were negligible on the 11 sites where aphid numbers were low [Untreated (U) 88.7 per cent, Treated (T) 88.6 per cent on the top two sieves] but more substantial on the other 11 sites (U 70.5, T 74.1).

The the Netherlands, 1.000 grain weights were calculated from treated and untreated plots at each of the 18 sites. Overall there was no difference in mean weights between treated and untreated plots (U 43.2, T 43.7). Unlike the UK results, and probably as a result of the very small aphid infestations, there were no differences in the means for the six sites with more than five aphids (U 42.2, T 42.7).

DISCUSSION

This co-operative work is continuing in 1975 to obtain sufficient data to define more fully the aphid population levels at which spraying is an economic proposition. At present, advice to farmers in the UK is that if mean populations on wheat ears at the beginning of flowering are five or higher, and further counts a day or two later



reveal that the population is increasing, then one spray is likely to be economically worthwhile. The same advice is given in the Netherlands. One advantage of spraying at the beginning of flowering is that further sprays are unlikely to be necessary as there is little aphid migration into the crop after this stage.

Table 3 illustrates the cost of spraying (1974 figures). The farmer can spray the field himself with his own tractor and spray machinery, or a ground contractor can be engaged to do the work, or spraying can be done from the air. Obviously, the first two methods are preferable if the outbreak is restricted to part of the field, as commonly happens. For example, an infestation often develops on one side of the field only and one or two passes with the sprayer are all that is required.

Table 3

Economics of single spray against cereal aphids  
Costs per acre in the U.K., 1974

	Farmer ££	Ground contractor ££	Air ££
Insecticide	1.40	1.40	1.40
Application	0.90	1.40	3.00
Wheeling losses, 3% of 2 tons/acre	3.80	3.80	-
Total	6.10	6.60	4.40
Cwt/acre at £63.38 per ton	1.9	2.1	1.4

The cost of the insecticide is about £1.40 per acre, and the cost of application is shown in Table 3. These are average figures obtained from my colleagues in ADAS. The wheeling losses figure derives from the work of Kolbe (1969), who suggested that spraying with a 15-metre boom through cereals at flowering results in a 2-4 per cent yield loss. The UK booms vary between 30ft (9m) and 60ft (18m) in length. I have taken 3 per cent yield loss as the mean and assumed a yield of two tons per acre (5021.4 kg/ha), which is slightly high but is a figure often given by farmers as the yield they expect to get. The yield which has to be recovered to offset the cost of spraying has been calculated from the price of wheat in 1974. Thus, a farmer whose crop is sprayed with a ground sprayer needs to recoup two cwt (251 kg/ha) per acre as a minimum. The amount is slightly less with aerial spraying. An increasingly common practice among West German farmers is to leave cereal rows unsown, or to form a lane of wheeling through the crop at an early growth stage, in positions corresponding to the track that must be taken by tractor and sprayer, (Bedford, 1974). Where this is done, those plants fringing the empty area tiller more vigorously and provide sufficient extra grain yield to compensate for the yield loss from the empty rows. This is an example of a cultural adaptation which can reduce the economic threshold for spraying against aphids. Where it is done, a yield increase of one cwt per acre (125 kg/ha) is sufficient to offset the cost of spraying.

Within the context of an integrated control system for cereals, spraying against aphids feeding on the grain from the beginning of flowering onwards poses few problems for within a few weeks of the spray being applied the crop is ripening and drying ready for harvest. Dean (1974) has indicated the effects of parasites and predators on the cereal aphid complex and suggests that their contribution to restricting build-up of aphids is small. The use of insecticides which adversely affect parasites and predators is unlikely, therefore, to increase the possibility of

subsequent aphid build-up within cereal crops though little is known about the overwintering and dynamics of parasite and predator populations from year to year. Dean and Wilding (1973), reporting on observations on Entomophthora spp. as controlling factors in cereal aphid populations show that the effects of these fungi can be important. Further work is needed to indicate whether or not sprays against cereal fungal diseases earlier in the growing season affect the build-up of aphid pathogenic fungi. There is evidence of this having happened on Myzus persicae (Sulzer) on potatoes (Nanne and Radcliffe 1971).

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NOTES



CROP RESISTANCE TO PESTS AS A COMPONENT OF INTEGRATED

CONTROL SYSTEMS

H. J. B. Lowe

Plant Breeding Institute, Maris Lane, Trumpington, Cambridge

Summary In large field plots of aphid-resistant and susceptible beet, numbers of *Myzus persicae* were always less on resistant plants. Incidence of virus yellows, which is mainly transmitted by *M. persicae*, was less on the resistant beet in two years out of four. Natural enemies appeared to exert significant control of the aphids on some occasions, especially on resistant beet. These results showed that even partial resistance in the crop can make real contributions to aphid control. If combined with other forms of resistance to beet yellowing viruses, inherited aphid-resistance should provide a basis for improved integrated control of virus yellows.

INTRODUCTION

The advantages of crop varieties with high degrees of resistance to pest attack have long been recognised. Successes, such as those with wheat resistance to Hessian fly or to wheat stem sawfly, or alfalfa resistant to aphids are well known and widely documented. Less is known of the advantages of lower degrees of resistance, which are insufficient to give commercially acceptable control under all normal conditions. Host-plant resistance at these moderate levels must be considered as an addition not as an alternative to conventional control procedures. Some experiments, for instance comparing susceptible with weevil-resistant alfalfa (Horber, 1972), have shown that fewer pesticide applications can give control on a moderately resistant variety. Work on rice (IRRI, 1973) has demonstrated variety x insecticide-treatment interactions that should be used in integrated pest control programmes, and varietal resistance characteristics must be considered when establishing threshold levels of pest population for spray treatment. Hills et al. (1969) found that growing the sugar beet variety USH9, resistant to virus yellows in California, together with chemical control of aphids gave yield increases over susceptible beet greater than either measure tested alone.

It is argued here, referring to work on sugar beet at the Plant Breeding Institute, that inherited resistance in crop plants is a most important factor in integrated control and that even apparently low levels of resistance may be the key to improved exploitation of natural control agents in integrated control systems.

## METHODS

The method of testing sugar beet for resistance to aphids in the glasshouse was described by Lowe (1974). The plants were arranged in replicated blocks, each containing one or two plants of each stock, with the plants in contact. The aphids, apterous adults of *Myzus persicae* from culture on *Brassica pekinensis* and *Aphis fabae* from *Vicia fabae*, were released on the plants, usually 5 and 3 per plant respectively. Numbers of aphids were counted on each plant after a few days.

In field experiments, sugar beet were grown under normal agricultural conditions of spacing, sowing date, herbicide treatments, etc., except that no insecticides were applied. In 1970 and 1971, small plots (up to 5 rows wide and 6 m long) of experimental sugar beet stocks were grown in replicated layouts. From 1972, experimental varieties were grown in large plots, approximately 27 x 27 m. These were intended to simulate a real crop situation without plot to plot movement of aphids, but were too big to allow full replication. Only one resistant and one susceptible variety were grown in each year, with only one or two plots of each variety. The same susceptible variety (VT 142) was grown every year, but 3 resistant experimental varieties were used. Except in 1974, field plots were artificially infested with *M. persicae* by scattering pieces of leaf cut from infested *B. pekinensis* from glasshouse cultures.

In 1975, additional large plots (one of each variety) were subjected to a normal spraying routine for aphid control, and another plot was treated with carbaryl to restrict the activity of the aphids' natural enemies. The carbaryl was applied to the soil between the rows at a rate of 25 g a.i./ha in 1000 l water; and pirimicarb at 140 g a.i./ha was used for the aphicidal sprays.

## RESULTS AND DISCUSSION

Under suitable conditions, normally prevailing in spring and autumn, clear and consistent separation of resistant and susceptible sugar-beet stocks was obtained in glasshouse tests (Table 1).

Table 1  
Average numbers of aphids on sugar beet stocks in glasshouse tests

	Commercial control	Susceptible VT 142)	Resistant (LMG)	Resistant (ERA 2)	S.E.
<i>M. persicae</i> , mean no./plant					
Test 1	26.7	61.4	13.2	25.4	5.9
Test 2	51.9	47.8	13.4	23.7	8.2
Test 3	44.0	42.7	7.4	25.2	6.4
Mean	44.2	50.6	11.3	24.7	-
<i>A. fabae</i> , mean no./plant					
Test 1	57.2	51.2	17.1	22.5	7.0
Test 2	56.7	50.4	22.2	35.1	5.6
Test 3	90.4	63.5	13.2	33.8	11.4
Mean	68.1	55.0	19.2	30.5	-



Over a range of beet stocks there was usually a continuous range of variation in the average numbers of aphids, and resistance to *M. persicae* and resistance to *A. fabae* were usually but not invariably associated. Average numbers of aphids in glasshouse experiments tended, however, to vary widely over the year, and it was difficult to assess from such results the possible value of the resistance observed for the sugar beet crop. Early field experiments in 1970 (Lowe 1972) and 1971 (Table 2) were made with small plots and the differences of aphid infestation amongst the beet stocks were comparable to and in agreement with glasshouse tests.

Table 2

Numbers of *M. persicae* per plant on sugar beet stocks in the glasshouse, average of 3 tests, and in the field, peak populations 1971

	Susceptible (VT 142)	Resistant (M. Viceroy)	Resistant (VT 104)	S.E.
Glasshouse tests	23.3	9.5	9.9	2.5
Field trial	62.3	34.2	37.4	6.0

Further observations were made on the large plots in order to assess the likely contribution of resistance to aphid control in fields of sugar beet. In the absence of any chemical treatments, the populations of *M. persicae* were consistently smaller on the plots of resistant beet varieties than on the susceptible plots (Table 3).

Table 3

Maximum numbers of *M. persicae* per plant on large field plots of aphid-resistant and susceptible beet

Source of aphid population	Year				
	1972	1973	1974	1975	
	Introduced	Introduced	Natural	Natural	Introduced
Date when max. nos observed	30 June	2 July	1 July	10 June	2 July
Resistant	2.6	7.8	11.7	0.9	30.4
Susceptible	11.6	16.5	26.0	1.2	41.2
P for difference	-	<0.002 >0.001	<0.001	≈0.3	≈0.05

The rather small numbers of aphids per plant were characteristic of *M. persicae* on beet in England, and natural populations are usually smaller, like that recorded in 1975. The plots were exposed to virus only from sources outside the experiment, and the incidence of virus yellows varied greatly from year to year. In 1973 and 1974, yellows spread rapidly throughout all plots in July and levels of infection did not differ between aphid-resistant and susceptible beet. In 1972 however virus incidence was lower and there was a clear difference between

susceptible beet plots with 33.5% of plants infected on average and the plot of the resistant variety (VT 99) with only 8.8% of plants infected on 4 September. VT 99 has resistance to the viruses as well as resistance to *M. persicae*, but it was not possible to apportion the effects of the different resistant characters in producing a lower incidence of yellows. The usual population peak of *M. persicae* was not observed on the plot of VT 99 in 1972, although peaks occurred on the susceptible plots, indicating a positive interaction between the effect of natural enemies and resistance to the aphids of the type proposed by van Emden & Wearing (1965), the resulting reduction in aphid numbers being important in decreasing virus incidence. There were many coccinellid larvae on these plots, and pitfall traps took on average 1.4 larvae per trap per day between 23 and 30 June 1972. It seemed that the coccinellids were able to exert control over the aphid population on the plot of resistant beet, but not on the susceptible plots.

In 1975, in addition to unsprayed plots, aphids were controlled on similar large plots by application of primicarb soon after populations of *M. persicae* exceeding one per four plants were detected. The plots of aphid-susceptible and resistant beet were first sprayed on 4 June and 9 June - respectively, a regime which permitted a resurgence of aphids on the susceptible beet but not on the resistant before the decline of the natural aphid population in mid June. Both plots were sprayed again on 20 June following artificial infestation with aphids on 17 June. On 11 July, the unsprayed plot of susceptible beet had significantly ( $P < 0.001$ ) more virus infected plants than the unsprayed resistant beet (Table 4), which did not differ significantly from either sprayed plot.

Table 4  
Incidence of virus yellows on aphid-resistant and susceptible  
sugar beet, 1975 (per cent of plants)

Beet Variety	Treatment			
	Unsprayed		Sprayed	
	Susceptible	Resistant	Susceptible	Resistant
11 July	21.2	12.5	11.3	10.3
28 July	86.6	55.6	41.9	19.3

This initial spread of virus was attributed to the natural aphid populations (Table 3). The increased incidence of virus yellows recorded on 28 July (Table 4) showed the effects of the introduced *M. persicae* populations and later immigrant aphids. All differences in virus incidence amongst the plots were significant (at  $P = 0.001$ ,  $\chi^2$  tests) with less virus infection of aphid-resistant than of susceptible beet both with and without spraying.

The initial natural infestation of *M. persicae* in 1975 was almost eliminated in mid June by predation, apparently mainly due to coccinellid larvae which were recorded on plants on 10 and 13 June. On the unsprayed plot of susceptible beet there were more coccinellid larvae than aphids on 13 June. A plot of resistant beet treated with carbaryl on 11 June had significantly more *M. persicae* ( $P = 0.001$ ) on 13 June than the neighbouring unsprayed plot of the same variety (1.15 and 0.36 aphids/plant respectively), and on 2 July had more (45.6 aphids/plant) than even the susceptible beet; 74 per cent of plants had yellows on 28 July. Pitfall traps, operating from 16 June indicated that the carbaryl treatment most affected small



carabids. Up to 27 June, only 0.06 Bembidion and Trechus spp. were taken per trap day compared with 0.76 per trap day on neighbouring unsprayed plots. Catches of Pterostichus spp. were not affected.

Sugar beet is a relatively poor host plant for M. persicae, and this aphid normally occurs in small populations which increase relatively slowly. In this situation, predators are most effective in controlling aphid numbers (Foster and van Emden, in press), and small differences in the plants, i.e. moderate resistance, are likely to make their most effective contribution to control, not only directly but also in combination with the effects of natural enemies, as here in 1972.

Clearly, neither the natural enemies nor the aphid-resistance of the varieties used in this work give reliable control of aphid numbers and hence virus yellows at the very low threshold aphid-populations necessary at present; but conversely they both may contribute significantly. Lowe (1972) and Russell (1972) argued that resistance to M. persicae will be most useful as one characteristic of "yellows resistant" sugar beet in combination with other forms of resistance to and tolerance of the yellowing viruses. The low incidence of virus on VT 99 in 1972 appeared to be an example of the benefit of such combined resistance. For integrated control, based in the current spray-warning system, varieties with the combined "yellows resistance" would have a higher threshold of aphid infestation, related to resistance to virus infection. The combination of resistance to aphids with a higher acceptable aphid infestation should allow more scope for control by natural enemies, and the yellows-resistant beet should need spraying substantially less often than at present. When spraying is needed, aphid-resistance in the crop may be expected to enhance aphicide efficiency in both short and long terms. In the short term, beneficial effects should resemble those observed by Hills et al. (1969) because slower aphid increase should allow the timing of spray applications to be more precise, and also resurgence of aphid populations under heavy aphid attack or difficult spraying conditions would be less. In the long term, increasing the significance of natural enemies and resistance of the plant in controlling beet yellows may be expected to reduce the relative selection pressure favouring insecticide-resistant aphid strains. In this way the continued effectiveness of particular chemicals can be supported by the use of pest-resistant crop varieties. Thus, even in a situation where pest populations must be controlled at a very low level, moderate levels of inherited resistance in the crop plants were observed to give real improvements; and it can be seen that increases in the pest and disease resistance in the crop, even if small, may give considerable benefits under a system of integrated control.

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APHID CONTROL BY A FUNGUS, VERTICILLIUM LECANII, WITHIN AN INTEGRATED  
PROGRAMME FOR CHRYSANTHEMUM PESTS AND DISEASES

R.A. Hall ✓

Glasshouse Crops Research Institute, Worthing Road, Littlehampton, Sussex, BN16 3PU

Summary Aqueous sprays of the spores of the entomopathogenic fungus, Verticillium lecanii, eliminated low populations of Brachycaudus helichrysi in the apical growth of chrysanthemums and established permanent control of Myzus persicae. Control of Macrosiphoniella sanborni was, however, only partial. Within an integrated programme, V. lecanii could, therefore, provide an alternative to parasitic control of M. persicae if fungicides for the control of phytopathogenic diseases were carefully selected.

Sommaire Les pulvérisations aqueuses des spores du champignon entomopathogénique Verticillium lecanii, ont éliminé les populations faibles de Brachycaudus helichrysi sur les croissances apicales des chrysanthèmes, et ont établi le contrôle permanent du Myzus persicae. Le contrôle de Macrosiphoniella sanborni ne fut cependant que partiel. Dans un programme intégré, le V. lecanii pourrait donc apporter un autre moyen de protection contre le M. persicae si les fongicides pour le contrôle des maladies phytopathogéniques étaient soigneusement choisis.

INTRODUCTION

The selection in recent years, of strains of glasshouse pests resistant to insecticides, has stimulated the investigation of alternative means of control. Several natural mortality factors have been examined with a view to their inclusion in integrated control programmes. The potential biological agents considered include entomogenous fungi which have often been tested for insect control in other circumstances. Many such attempts were unsuccessful since fungal epizootics are largely regulated by weather conditions. However, the glasshouse environment offers a unique opportunity to manipulate the critical physical factors, particularly relative humidity and temperature.

Natural infections of the fungus Verticillium lecanii have been found in dense populations of several glasshouse pests. For example, on cucumber the whitefly (Trialeurodes vaporariorum) and Aphis gossypii and on chrysanthemums the green peach aphid (Myzus persicae), the chrysanthemum aphid, (Macrosiphoniella sanborni) and the leaf curling plum aphid, (Brachycaudus helichrysi) have been infected. The effectiveness of the control produced by these natural infestations prompted an investigation of the potential of V. lecanii to prevent aphid infestations on chrysanthemums. Chrysanthemums were chosen since it was possible to elevate night-time humidity under the routinely used polythene black-outs.

## METHODS AND MATERIALS

### Production of infectious spores

Two types of spores are produced by *V. lecanii*; conidiospores on solid media but blastospores more abundantly and readily in liquid media. Since both are equally pathogenic in glasshouses (Hall, unpublished), it is more economic and expedient to culture blastospores. For the present experiments, blastospores were grown in agitated and aerated Sabouraud liquid medium. The culture was allowed to grow for four days at 20°C before the spores were harvested by centrifugation and washed four times in phosphate buffer (0.0003M). Viable spore concentrations were assessed by spreading replicate aliquots of 0.25 ml of suspension on to Sabouraud dextrose agar plates. Total spore counts were made in an improved Neubauer haemocytometer.

### Application of fungus to aphid infested plants

Experiments were performed in small glasshouses (2 x 3m), each containing a bed of approximately 30 chrysanthemum plants (cultivar "Deep Tuneful" which is very susceptible to aphids). Each house was infested with a single aphid species. When the aphids had increased to a density of 8-10 per plant, *V. lecanii* spores in buffer, containing 0.02% Triton X-100 as wetting agent, were sprayed on the plants to 'run-off'.

### Temperature and relative humidity

After spraying in the evening with fungal spores the beds were immediately covered with polythene blackouts. This ensured that the relative humidity was raised sufficiently for the spores to germinate and subsequently infect the aphids. The night-time temperatures were rigorously controlled and bed temperatures monitored by copper-constantan thermocouples. On evenings subsequent to the treatment, the beds were dampened by light overhead sprays of water to boost humidity prior to covering with blackouts. This was an important aid to the spread of infection from diseased to healthy aphids.

## RESULTS

### Myzus persicae

The green peach aphid initially infests the tips of young chrysanthemums but as the population increases it spreads rapidly to the undersides of leaves lower down the stem. *V. lecanii* has consistently controlled light infestations in small glasshouse experiments (Fig.1). Commercially adequate control was obtained by the 15th day after spraying at both 20°C and 15°C and virtual eradication achieved by the 24th day at 20°C and the 28th day at 15°C. Even aphids on the undersides of lower leaves were eliminated although it is certain that the fungal sprays did not cover these surfaces adequately. Secondary infection must therefore have spread from aphids higher up the plant.

Control has also been successful on plants with aphid infestations on the outside of tightly closed flower buds where there was less moisture than in the tips of unbudded plants.



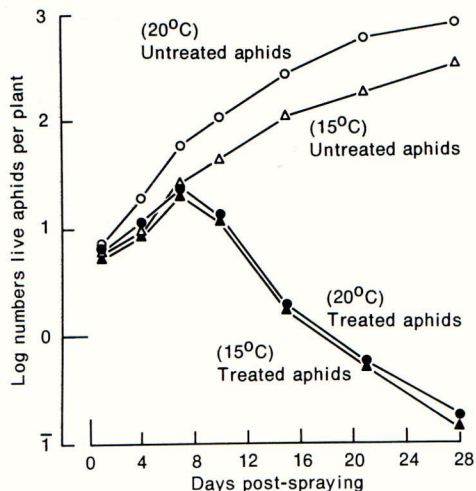


Fig.1 Control of Myzus persicae by V. lecanii at 15°C and 20°C on chrysanthemums sprayed with  $10^8$  blastospores/ml

#### Brachycaudus helichrysi

The leaf curling plum aphid aggregates exclusively in the vegetative tips of plants, until high population density induces it to spread. Once V. lecanii is established, rapid spread of the disease among aphids feeding deep within the tips is facilitated by trapped moisture and aphid crowding. On such unbudded plants, the pests can be eradicated completely in as short a time as 13 days (Fig.2).

However, in contrast to Myzus persicae, control was only partial on older plants where aphids were forced to feed in drier conditions on the outside of tightly closed flower buds (Fig.2).

#### Macrosiphoniella sanborni

Although excellent control of chrysanthemum aphid, a brown, unsightly aphid has been achieved in the laboratory, success in the glasshouse has often been unsatisfactory (Fig.3). Control of this pest by V. lecanii cannot therefore be guaranteed.

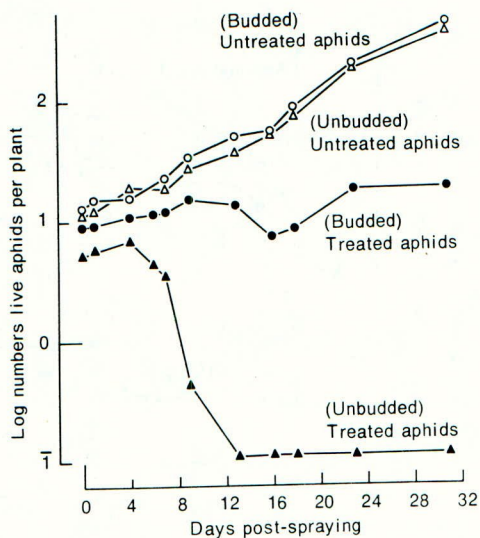


Fig. 2 Control of *Brachycaudus helichrysi* by *V. Lecanii* on budded and unbudded chrysanthemums sprayed with  $8 \times 10^7$  blastospores/ml

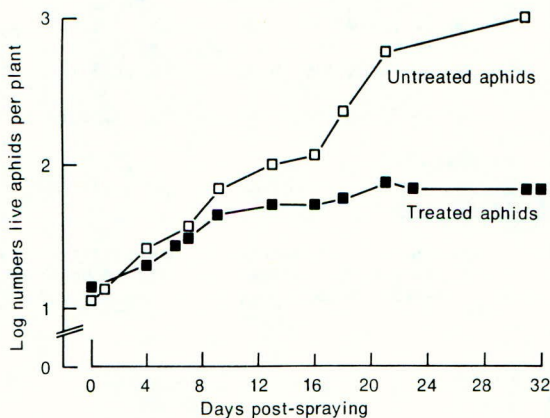


Fig. 3 Partial control of *Macrosiphoniella sanborni* by *V. lecanii* on chrysanthemums sprayed with  $8 \times 10^7$  blastospores



## DISCUSSION

The reasons for the differential control of these three aphid species are uncertain for their inherent susceptibility, as measured by laboratory bioassay, is the same (Hall, unpublished). Evidently, ecological or behavioural factors are involved. However, V. lecanii holds promise for controlling low-density populations of aphids, especially M. persicae, in commercial glasshouses for the duration of a crop and possibly longer in AYR houses. In seven trials in small glasshouses, M. persicae was virtually eliminated and B. helichrysi was as well controlled on young unbudded plants. On the otherhand, reliable control of M. sanborni seems unlikely at the present time. However, the fungus might be more effective in large commercial houses where the humidity is better maintained than in the small houses used in these trials. At present, there is little information on the formulation of fungal sprays. It may be possible to find additives to protect spores from premature desiccation thus permitting germination and infection at sub-optimal humidities thereby removing a major barrier to effective control. For this purpose water-retaining compounds such as carboxymethyl cellulose or sodium alginate might be used.

In the integrated programme proposed by Scopes and Biggerstaff (1973) for the control of pests and diseases on chrysanthemums, M. persicae was controlled by the parasite Aphidius matricariae. Because V. lecanii is as effective as Aphidius and easier to produce, it could replace the parasite in this programme.

Careful consideration of the whole pest and disease complex on chrysanthemums is, however, necessary before V. lecanii could be integrated within such a programme. Thus, Olmert and Kenneth (1974) showed that a wide range of insecticides adversely affected in vitro growth of their V. lecanii isolates. However, with chrysanthemum there may be no such difficulties. In their integrated control system Scopes and Biggerstaff envisaged that only dioxathion/pirimicarb sprays would be needed against leaf miner and minor aphids (B. helichrysi and M. sanborni). This mixture is applied only to the apical foliage and so should not affect epizootics of V. lecanii amongst Myzus persicae on the lower foliage - in the same way as predators and parasites are safeguarded (Scopes and Biggerstaff, 1973). Olmert and Kenneth (1974) also demonstrated that fungicides such as benomyl, thiabendazole, maneb and captan seriously affected in vitro growth of V. lecanii at one-tenth the recommended dosages. Thus, the injudicious use of fungicides against plant pathogens may adversely affect an entomogenous fungus (Jacques and Patterson, 1962). Fortunately, fungicides are not regularly applied to chrysanthemums since many diseases can be prevented by soil sterilization and general attention to crop hygiene. The most common diseases such as mould (Botrytis cinerea) and ray blight (Mycosphaerella ligulicola) occur mainly towards the end of the crop and attack flowers and buds. Since, at this stage, V. lecanii-induced aphid control should be complete, measures to control these diseases may be applied without affecting V. lecanii. Control of powdery mildew (Oidium chrysanthemi) may be required at any stage of crop growth and for this purpose a fungicide specific for powdery mildews such as dimethirimol should be employed since Wilding (1972) has shown it to be harmless to in vitro growth of Cephalosporium aphidicola (V. lecanii fide W. Gams 1971).

The pathogenicity of V. lecanii to parasites and predators already used for the biological control of other chrysanthemum pests has not yet been tested. However, it seems unlikely that they will be affected since the fungus has not attacked them when it occurred naturally in several crops where the predatory mite Phytoseiulus persimilis and the parasite Encarsia formosa were active.

Table 1

Incorporation of V. lecanii into an integrated control  
programme on chrysanthemums

<u>Insect pest or disease</u>	<u>Biological methods</u>	<u>Chemical sprays</u>
<u>Myzus persicae</u>	Spray with <u>V. lecanii</u> at initial detection of infestation	-
Red spider mite	Control by predators ( <u>Phytoseiulus persimilis</u> )	-
Leaf miner	-	Mist of dioxathion/pirimicarb over tops of plants at first sign of feeding
Leaf miner + <u>B. helichrysi</u> or <u>M. sanborni</u>	-	Dioxathion/pirimicarb spray over tops of plants
Caterpillars	<u>Bacillus thuringiensis</u>	-
Powdery mildew ( <u>Oidium chrysanthemi</u> )	-	dimethirimol
Grey Mould	-	captan or thiram at bud burst
Ray Blight	-	captan or mancozeb when buds show colour

Based upon the integrated system proposed by Scopes and Biggerstaff (1973), Table 1 shows how V. lecanii might be utilised. If a parasite was used against leaf miner B. helichrysi could be controlled by V. lecanii in the early vegetative stages of the crop. However, control of leaf miner by parasites is at present unreliable. Instead, insecticides can be misted over the apical foliage to control both leaf miner and minor aphid pests such as B. helichrysi and M. sanborni (Scopes and Biggerstaff, 1973).

On other glasshouse crops, pests such as Aphis gossypii and whitefly, Trialeurodes vaporariorum, are sensitive to V. lecanii. Although chrysanthemums are amenable to protection by entomopathogenic fungi because the existing black-out covers can be used to augment night-time humidity, it is possible that with slight modifications this fungus might be used on other crops.

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NOTES



POTENTIAL OF INTRA-CROP DIVERSITY FOR THE CONTROL

OF BRASSICA PESTS

M.S. O'Donnell and T.H. Coaker

Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX

Summary The effect of intra-crop diversity on insect pest populations on brassicas has been studied by undersowing Brussels sprout plants with clover. Cabbage aphid infestations were reduced by over 80% primarily due to interference with their immigration into the crop. Soil dwelling predatory arthropods were enhanced by the additional shelter provided by the clover and were responsible for the lower survival of cabbage caterpillars (30%) and cabbage root fly (60%), although ovipositing root flies may have been deterred from laying around sprout plants by the clover.

Competitive effects of the clover on the brassica crops were severe on light soil but were reduced by irrigation.

INTRODUCTION

It is generally recognised that insecticides alone do not provide permanent solutions to pest situations because of attendant problems such as insecticide resistance and the disturbance caused to natural controlling mechanisms. More recently the difficulties in maintaining attractive cost/benefit ratios from pesticide application, due to their increasing cost, adds to these problems. This has led to an intensive search for alternative methods of insect pest control not the least of which is the evolution of pest-management techniques that attempt to manipulate the agro-ecosystem to the detriment of the pest and so reduce insecticide use. Such an alternative approach to pest control has developed from a greater understanding of the ecological concepts affecting the abundance of insect species. It has been noticed by some ecologists (e.g. Elton, 1958), that there appears to be a greater stability of insect populations in more complex ecosystems than in more simple ones although this may not always be the case (Way, 1971). The evidence for a cause and effect interaction leading to pest outbreaks in present-day agriculture has, nevertheless, been often argued to be due to the intensive monoculture of annual crops (Dempster and Coaker, 1974). The possible importance of this association has, therefore led to studies on the diversity within agroecosystems either in the adjacent non-crop habitats or within the crop itself. The former approach is inconclusive and complex as both beneficial and detrimental influences prevail on the insect populations on adjacent crops (Lewis 1965, van Emden 1965, Pollard 1971). For some pests and crops, therefore, there may be a better opportunity to develop practical methods for reducing pest numbers and the damage they cause by increasing the diversity within the crop itself.

Earlier experiments of this type on brassicas showed that a weed cover under Brussels sprouts greatly reduced the incidence of cabbage aphid (Brevicoryne brassicae) on the crop. This was demonstrated to be principally due to the

reduced contrast between the crop and the soil background resulting in a lower immigration of the aphid and subsequently its numbers on the crop (Smith, 1969). Dempster (1969) showed, under similar experimental conditions, that the numbers of the cabbage caterpillar (Pieris rapae) were also reduced compared with crops grown on bare ground. In this instance enhanced predation of the young caterpillars by ground dwelling arthropods (Dempster, 1967), encouraged by the increased ground cover provided by the weeds, was the cause. These experiments also demonstrated that weed competition significantly reduced crop yield. For this reason and because weeds are unacceptable in good farming practice an alternative ground cover was selected that might minimise competition. A procumbent clover (cv. Kersey White) was used in field experiments in 1970 and 1971 (Dempster and Coaker, 1974). Observations on cabbage rootfly (Erioischia brassicae) were also included since it was possible that the increased ground cover would also increase the natural mortality of its eggs and larvae by beetle predators (Coaker and Williams, 1963).

Following the encouraging results from these experiments, further investigations have been made which are reported in this paper.

#### MATERIALS AND METHODS

Field experiments were carried out in 1973-1975 inclusive, on light, sandy soil at the Department of Applied Biology Field Station, Cambridge.

To approximate to a field situation, large square plots (1973 - 864m<sup>2</sup>, 1974 and 1975 - 625m<sup>2</sup>) were used for each treatment arranged in two randomised blocks.

The treatments were 0, 25, 50, 75 and 100% ground cover provided by strips of clover (cv. Kersey White) grown between rows of Brussels sprout plants. The clover was sown in February 1973 (22 kg/ha) for the 1973 experiment and in September 1973 (11 kg/ha) and August 1974 (22 kg/ha) for the 1974 and 1975 experiments, respectively. Autumn sowing was chosen for greater reliability in providing the required cover at the time of first generation cabbage root fly oviposition in late April/early May. Brussels sprout crops were used as, when planted early, their long growing season enabled each pest species to be studied fully. In 1973, cv. Cambridge No 5 (CN5) was used and in 1974 and 1975 cv. Early Half Tall (EHT), a variety with some resistance to cabbage aphid (Dodd, 1973). The crops were planted, at 1m spacing, in late April/early May for exposure to attack by the first generation cabbage root fly.

At least five guard rows surrounded the sampling area in each plot on which the populations of cabbage root fly, ground dwelling arthropods, cabbage aphid (immigrant alate and colonizers) and cabbage caterpillars were assessed (Coaker and Dempster, 1974).

Crop growth (fresh weight), and in 1975, clover production (fresh weight), cabbage rootfly root damage (Rolfe, 1969), yield and quality of the sprout buttons were measured (see Table 6).

In 1975, in consequence of the competition effects on the crop in previous experiments emphasised by the dry, light soil conditions, an irrigation experiment was carried out to assess the influence of three watering regimes on the growth of Brussels sprouts undersown with a 0, 50 and 100% clover cover. The watering treatments were rainfall only, rainfall plus irrigation for 6 weeks or 12 weeks following planting. Irrigation was applied to the latter two treatments to maintain a water balance of within 2.4 cm of field capacity.



## RESULTS

Cabbage root fly: In each generation and year, with the exception of the second generation in 1974 on the 25, 50 and 70% clover treatments, fewer eggs were found around the sprout plants growing in the clover treatments than around those growing in bare soil (Table 1). When the numbers of eggs were high as in 1971 and 1973, about 30% fewer eggs were found around plants in the 50 and 100% clover treatments. In these years the full benefit of the cover was not achieved as the spring sowing of clover did not reach the critical 60% soil coverage in the clover areas until after peak oviposition by the first generation flies. Below this level of cover there were no differences between the number of eggs found in each treatment. In 1974 and 1975, the autumn sown clover had exceeded this level at the time of transplanting the brassica crop and 60-70% fewer first generation eggs were found on these plots compared with those found around the sprout plants growing in bare soil. These results were, nevertheless, obtained when the overall numbers of eggs were smaller. Some of this difference may have been due to the lower preference for EHT by ovipositing flies since 25% eggs were laid on this variety than on CN5. The differences in egg numbers obtained from the various clover treatments are partially reflected in the amount of larval damage on the roots (RDI) of the sprout plants assessed after each generation attack. In none of the experiments were significant numbers of plants seen wilting and/or dying from cabbage rootfly damage.

Table 1

Cabbage root fly: mean number of eggs/plant  
(% difference from bare soil - 0)

% clover cover generation	0		25		50		75		100	
	1	2	1	2	1	2	1	2	1	2
1971	233		-	-	-	-	-	-	166 (-29)	
1973	385	318	-	-	241 (-37)	244 (-23)	-	-	260 (-32)	166 (-48)
1974	67	96	19 (-71)	107 (+11)	26 (-62)	117 (+21)	15 (-77)	141 (+46)	17 (-74)	58 (-39)
1975	78	-	23 (-71)	-	32 (-59)	-	-	-	-	-

Table 2

Cabbage root fly: mean root damage index (RDI) at harvest  
(% difference from bare soil - 0)

% clover cover	0	25	50	75	100
1970	45	-	-	-	35 (-22)
1971	77	-	-	-	56 (-27)
1973	54	-	18 (-48)	-	39 (-28)
1974	41	38	38	37	37

Predators: The commonest species trapped from May to September are shown in Table 3. Bembidion spp, important predators of cabbage rootfly eggs, were regularly trapped in larger numbers on the bare plots, but the other species, when present, were usually caught in greater numbers on the clover plots. In most cases more than twice as many were trapped including Harpalus rufipes an important predator of Pieris.



Table 3

Predators: Index of trapping in clover plots  
compared with bare plots

% clover cover	1970	1971	1973	
	100	100	50	100
species				
<u>Nebria brevicollis</u>	nt	nt	++	++
<u>Clivina fossor</u>	nt	nt	++	++
<u>Bembidion spp</u>	—	—	—	—
<u>Trechus spp</u>	nt	+	—	—
<u>Harpalus aeneus</u>	nt	nt	+	0
<u>H. rufipes</u>	++	nt	++	+
<u>Amara spp</u>	nt	nt	++	++
<u>Feronia spp</u>	++	++	++	+
<u>Agonum dosale</u>	nt	+	++	++

+, x 2 number trapped on bare plot  
 ++, x 2 " " " " " "  
 -, x 0.5 number trapped on bare plot  
 —, x 0.5 " " " " "  
 0, no difference  
 nt, not trapped

Table 4

Cabbage aphid: mean numbers /4 leaves/plant  
(% difference from bare soil - 0)

% clover cover	0	25	50	75	100
1971	11	—	—	—	1 (-96)
1973	7000	—	3000 (-57)	—	900 (-5)
1974	39	22 (-44)	24 (-38)	6 (-85)	5 (-87)

Cabbage aphid: As with weeds a similar interference in host-plant finding was obtained from the clover as yellow-water trap catches of immigrant alate were 74% less on the 100% clover cover plots than over bare plots. In consequence, in each experiment the presence of clover clearly reduced the aphid populations (Table 4). There was also a direct trend of fewer aphids with increase clover cover.

In 1974, the aphid resistant character of EHT was verified as the aphid population on this variety was 33% smaller than on CN5.

Plant growth: With the exception of the 1970 experiment when a 67% increase in total fresh weight of sprout plants growing in 100% clover cover was obtained compared with those growing on bare soil, growth in the other years was severely reduced by the clover. Apart from the 1975 experiment which suffered from severe dry soil conditions, plant growth was reduced relative to the amount of clover cover (Table 5).

Buttons: There was less difference in the yield of marketable buttons from the bare and clover plots in 1973 than in 1974 although in both years plant growth was seriously affected by the clover (Table 6). This was because there was a higher proportion of 'blown' buttons from the bare plots in 1973.

There was a greater proportion of buttons with fewer aphids in them from the

clover plots than the bare plots, even in 1973 when aphid numbers were exceptionally high (Table 4).

Table 5

Mean fresh weight (kg) of Brussels sprout plants at harvest  
(% difference from bare soil - 0)

<u>% clover cover</u>	<u>0</u>	<u>25</u>	<u>50</u>	<u>75</u>	<u>100</u>
1970	1.2	-	-	-	2.0 (+67)
1971	2.3	-	-	-	0.6 (-75)
1973	4.4	-	2.7 (-30)	-	2.3 (-48)
1974	4.8	3.0 (-38)	2.9 (-40)	2.1 (-56)	0.9 (-81)
1975*	0.7	0.2 (-74)	0.1 (-83)	-	-

\* July sample

Table 6

Mean fresh weight of buttons/plant and percentage  
in each quality category

<u>Quality category*</u>		<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>	
<u>% clover cover</u>	<u>wt(kg)</u>					
1973	0	0.4	2	44	46	8
	50	0.3	6	56	34	4
	100	0.3	4	47	44	5
1974	0	0.3	10	66	21	3
	25	0.1	30	64	6	0
	50	0.1	19	65	16	0
	75	0.1	25	66	8	1

\*Aphids/button; a - none, b - 1 - 5, c - 6 - 20, d 20

Irrigation experiment: The growth of the Brussels sprout plants on all the treatments receiving supplementary watering was improved (Table 7). Those plants growing in clover, however, did not benefit as well as plants growing in bare soil. This suggests that either the level of watering was insufficient to compensate for the competition from the clover or that there was an additional factor involved in the plots that affected crop growth.

Table 7

Mean fresh weight (kg/plant) of Brussels sprout plants  
from different watering treatments

<u>Watering treatments</u>	<u>Rainfall</u>	<u>Rainfall + 6 weeks watering</u>	<u>Rainfall + 12 weeks watering</u>
<u>% clover cover</u>			
0	0.8	0.9	2.1
50	0.1	0.2	0.8
100	0.01	0.1	0.3

See text for explanation of supplementary watering treatments

## DISCUSSION

As in earlier experiments (Dempster and Coaker, 1974) the numbers of aphids and rootflies were considerably reduced by the clover. Unfortunately caterpillar infestations were too low to reaffirm the lower survival (30%) in the presence of clover found earlier. The clover was grown under the crop to provide shelter for ground living predators and to reduce the contrast between the crop and bare soil. The larger numbers of predators in the more diverse clover plots were responsible for reducing caterpillar survival but with the aphid and cabbage rootfly any changes in survival cannot be separated from those of immigration. Laboratory experiments have shown that clover will substantially deter cabbage root fly from ovipositing around brassica plants even when the clover foliage is not in immediate contact with the plants. Consequently, even the narrow strips of clover may have affected oviposition but at the same time would favour some predators, e.g. Bembidion lampros that prefers open ground to shelter (Mitchell, 1963). The greatest reduction in cabbage root fly numbers occurred during the first generation, an advantageous effect since this attack occurs when the brassica plants are small and consequently suffer more severely from attack (Coaker and Finch, 1971).

The main difficulty to overcome is the competition between the clover and the crop. Narrower strips of clover do not affect crop growth as soon after planting as wider strips and full cover, but in dry soil conditions can be as detrimental to the final yield. This effect can be partly overcome by irrigation if the soil water availability becomes limiting to crop growth. As the 1971-5 experiments were done on very light soil maximum competitive effects were seen, heavier soil may be less detrimental. M.F.Ryan (pers. comm.) obtained a 57% increase in cauliflower yields interplanted with clover on peat soil in Ireland.

The optimum width of clover strips between the rows would also require determination for each crop/pest situation as full cover is more effective against aphid invasion than narrow strips although narrow strips are as effective against cabbage root fly. It is also likely that clover is not the best cover to use to increase the diversity within the brassica crop to reduce pest numbers.

It is important to apply a cost/benefit analysis to any pest control method and particularly so to any new innovations. Currently, to grow clover as narrow strips (25% cover at 11 kg/ha sowing rate) between brassica crops would cost one third of the cost of recommended insecticide treatments for brassica pests control. Irrigation treatments would add to the cost if clover was used but there would be a return from the clover as a forage crop and/or from its improvement to the soil.

The principle of intra-crop diversity described here for the control of brassica pests may with further development have potential as a practical method. Like other biological methods used for insect control, however, it would need to be integrated with insecticides if high quality produce was required, in this case with aphicides.

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NOTES

CONTROL OF THE LEAF-MINER LIRIOMYZA BRYONIAE WITHIN AN

INTEGRATED PROGRAMME FOR THE PESTS AND DISEASES

OF TOMATO

N.W. Hussey, D.L. Stacey & W.J. Parr ✓

Glasshouse Crops Research Institute, Littlehampton, Sussex BN16 3PU

Summary Control of this pest depends on the prevention of oviposition by adults emerging from overwintering pupae. Of seven pesticides tested for the control of Liriomyza bryoniae larvae 0.05% sprays of dioxathion and dimethoate granules in the compost (2 ppm) proved most effective. Limitations on the use of the predator Phytoseiulus and the parasite Encarsia in the presence of these materials are discussed.

INTRODUCTION

Widespread selection of resistant strains of the glasshouse red spider mite (Tetranychus urticae), and the glasshouse whitefly Trialeurodes vaporariorum, presents increasingly difficult problems in the chemical control of these pests on commercially grown tomatoes. Biological control, using predators (Phytoseiulus persimilis) and parasites (Encarsia formosa), has therefore been investigated as an alternative. Although practicable programmes for their use have been developed it is essential to integrate such techniques with the chemical control of other pests and diseases. To achieve such harmonization any pesticides used must be selective in action. Such selectivity may be achieved either chemically, by a narrow target spectrum, or ecologically, by isolating the pesticide from susceptible natural enemies either in space or time.

The tomato leaf-miner, Liriomyza bryoniae, is one pest for which such selective control must be developed. Although only a local problem in S. England it is a widespread and serious pest in the Channel Islands and Holland. Most of the larvae vacate the leaves to pupate in the soil and 1 cm below the surface. During the summer adults emerge within 18-20 days but pupae formed in early autumn will not emerge for 60 days even if kept at 15°C (Speyer & Parr, 1949). This extended pupal period, which is initiated when larvae develop under short days, encourages attacks on young seedlings where the winter soil sterilization has not been completely effective. As each female lays about 100 eggs, at the rate of ten per day, a few survivors can initiate an intensive attack on small plants. The eggs, laid within slits cut in the upper leaf surface, hatch within six days and the larvae feed for ten days within a constantly widening and meandering mine cut between the main veins of the leaf lamina. Young seedlings may be killed even by single larvae tunnelling within the cotyledons and first true leaves, but larger plants tolerate sizeable populations. Wolfenbarger (1966) reported that the yield of outdoor tomatoes was reduced when every leaflet was attacked by more than one larva of L. munda, but Lindquist (1974) showed that L. sativae had no effect on the yield of early trusses of greenhouse plants even when as many as 50 larvae were present in each leaf.



Although resistance has recently caused some concern (Wolfenbarger, 1958, and Hussey & Gurney, 1969), many insecticides applied as sprays have been found effective against tomato leaf-miners. Smith *et al* (1973) tested 30 insecticides against L. munda and found that the eggs and larvae are killed by azinphos-methyl, demeton-methyl, diazinon, dicrotophos, dimethoate, fenthion, lindane, parathion and phorate. De Brouwer and Offeren (1967) had earlier demonstrated the effectiveness of dichlorvos and mevinphos, while Wolfenbarger (1958) reported control of L. pusilla with aldrin, chlordane and toxaphene. Hoffman *et al* (1973) claim that omethoate is particularly effective against L. munda.

Salinas (1966) showed that carbaryl and endosulfan were effective, while Audemard and d'Aguilar (1969) added trichlorphon to the list though they claimed that bromophos and fenthion were phytotoxic to the cultivar Kordaat. The importance of achieving control of the eggs and larvae of the first spring generation in Holland was recognized by van den Linden (1968) who recommended applying malathion dust as soon as the mines appeared.

Although this considerable list of effective compounds suggests that leaf-miners are not difficult to kill most of the materials have a rather wide spectrum of activity and are not therefore easily incorporated within an integrated programme. Only Getzin (1960) has considered this aspect by attempting to preserve the parasite Derostenus varipes Crawford when controlling L. munda with dimethoate and dioxathion. These materials gave excellent control for up to nine days after application and allowed most parasite larvae to survive within the leaf-mines.

One of the most attractive possibilities for separating insecticides from natural enemies is the use of systemic compounds. Schread (1958, 1960), showed that both dimethoate and phorate controlled Phytophthora ilicis in holly, while Baronowski (1960) confirmed that phorate would kill L. pusilla in tomatoes. Lindquist and Bauerle (1972) showed that aldicarb and dimethoate prevented damage by L. munda in tomato seedlings when applied to the potting compost 48 h before transplanting seedlings.

The experiments reported here were designed to confirm the efficiency of some of the materials reported in the earlier literature and to evaluate the effect of the most promising on the natural enemies used in an integrated programme for tomatoes (Hussey, 1975).

## METHODS

### Contact Insecticides

Direct larval toxicity - Tomatoes, cv Minibelle, at the 6-7 leaf stage were exposed for three days to large numbers of L. bryoniae breeding on older plants in the same glasshouse. Before the test plants were moved to another, uninfested, glasshouse any remaining adults were killed by fumigation with dichlorvos. Each test chemical (Table 1) was applied 3 h before, or 3, 6 and 9 days after oviposition, using batches of six plants for each treatment and a similar number of controls. To confirm the claims of van den Linden (1968) one treatment tested malathion dust applied at 0.25 g/plant.

All sprays contained 0.01% wetter and were applied to run-off with a one pint pneumatic hand sprayer. In this way eggs and larvae of all ages were treated. No pupae were tested as they are known to be almost immune to pesticides. Mortality was assessed before the larvae emerged from the mines.

Residual Toxicity - Tomatoes were treated at 0.05 and 0.1% a.i. 1 h or 3, 6 and 9 days before exposure to ovipositing L. bryoniae for 3 days (Table 2) and were moved to an insect-free glasshouse for subsequent assessment.

#### Granular Insecticides

Toxicity to leaf-miner larvae - Pot tests were made by pricking-out tomatoes at the cotyledon stage into J.I. No.2 compost in which granules had been incorporated at rates of 2, 5 & 10 ppm. Six replicates of each treatment were kept for 8 weeks in a glasshouse within which large numbers of L. bryoniae were breeding.

Toxicity to spider mites, whiteflies and their natural enemies - Tomatoes (E1) at the 5-6 leaf stage were each infested with 30 female spider mites and, 13 days later, 4 predators were introduced onto each plant. Immediately before planting in 23 cm whalehide pots dimethoate granules were mixed into the top 5 cm of compost at rates of 10 and 40 ppm. Two replicates of four plants were used for each treatment and, with a comparable control series, were grown under the ADAS 'Blueprint' regime for tomatoes.

To assess the persistence of the treatments, predators were reintroduced every week until they became successfully established.

Adult whiteflies were released onto each plant 9 days before potting and Encarsia (50/plant) introduced 3 wk later.

In the light of the results obtained a second series of tomato seedlings (E2) were pricked out into compost containing lower rates of dimethoate (2, 5 & 10 ppm). Fourteen days later 200 adult whiteflies were caged over one plant from each treatment together with a control. After 5 days the surviving adults were killed by DDVP fumigation and the eggs counted. Assessments of the whitefly populations and of the % parasitism on the remaining plants were made 28 days after pricking-out.

## RESULTS

#### Contact Insecticides

Direct larval toxicity - Although malathion dust was completely ineffective, other materials (Table 1) killed the larvae if applied within 6 days of oviposition though azinphos-methyl and bromophos failed to kill older larvae. A freshly dried deposit of azinphos-methyl prevented oviposition while similar deposits of pirimiphos-ethyl and pirimiphos-methyl substantially reduced the number of eggs laid.

Malathion had no, and nicotine little, residual effect on leaf-miner larvae but dioxathion remained effective for 9 days (Table 2). Dimethoate was effective for 6 days but pirimiphos-ethyl and pirimiphos-methyl began to decrease in efficiency after 3 and 6 days respectively.



TABLE 1

Effect of insecticidal sprays (0.1% a.i.) on leaf-miner  
larvae of different age

Number and survival (%) after treatment at different intervals  
after infestation

Insecticide	Eggs	3-day larvae	6-day larvae	9-day larvae	Control
azinphos-methyl	0 (-)	233 (0)	254 (0)	147 (100)	160 (100)
bromophos	130 (5)	84 (0)	137 (0)	187 (100)	160 (100)
dimethoate	157 (0)	692 (0)	411 (0)	348 (0)	280 (100)
dioxathion	294 (0)	331 (0)	395 (0)	395 (0)	280 (100)
pirimiphos-ethyl	83 (0)	451 (0)	458 (0)	571 (0)	478 (100)
pirimiphos-methyl	67 (0)	435 (0)	415 (0)	576 (0)	478 (100)

TABLE 2

Residual effect of insecticidal sprays on survival of  
leaf-miner larvae

Number and survival (%) of larvae when eggs laid at different  
times after treatment

Insecticide (% a.i.)	1 h before	3 days	6 days	9 days	Control
dimethoate (0.1)	186 (0)	247 (0)	251 (0)	258 (100)	363 (100)
" (0.05)	197 (0)	265 (0)	282 (0)	355 (100)	
dioxathion (0.1)	27 (0)	75 (0)	62 (0)	44 (0)	37 (100)
" (0.05)	66 (0)	97 (0)	143 (0)	77 (0)	
nicotine (0.1)	65 (77)	199 (94)	238 (92)	243 (95)	275 (100)
" (0.05)	188 (94)	236 (95)	266 (97)	261 (97)	
pirimiphos-ethyl (0.1)	37 (0)	134 (0)	493 (45)	463 (79)	781 (100)
" " (0.05)	128 (0)	232 (17)	663 (46)	462 (84)	
pirimiphos-methyl (0.1)	21 (0)	122 (0)	231 (4)	257 (5)	1142 (100)
" " (0.05)	47 (0)	199 (13)	404 (20)	493 (33)	

## Granular Insecticides

Toxicity to leaf-miner larvae - At rates above 2 ppm dimethoate granules afforded complete protection for 6 wk and even after exposure to female flies for 8 wk only 11 mines developed on the 6 plants protected by 2 ppm, whereas all the control plants had been killed by the mining of hundreds of larvae.

Toxicity to spider mites, whiteflies and their natural enemies - Examination of leaflets from plants (E1) treated with dimethoate at 10 and 40 ppm revealed that, at the highest dosage, all active stages of red spider mite were killed while Phytoseiulus was killed at both rates. At 10 ppm the spider mite population therefore increased and in the absence of predatory control, 12 days after treatment the leaf-damage index (Parr & Hussey, 1969) was approximately the same as that on the untreated controls (Table 3).

Successive introductions revealed that Phytoseiulus could survive and increase 7 wk after treatment of the plants with 10 ppm, but not at 40 ppm dimethoate. In the second experiment (E2) predators successfully established 5 wk after treatment at 2 ppm, 6 wk at 5 ppm and 7 wk at 10 ppm Williams (1973).



Whitefly eggs were laid on treated plants (E1) though no development occurred on those treated at 40 ppm (Table 3). At 10 ppm the crawlers were killed but older scales developed normally.

TABLE 3

Effect of dimethoate granules on development of red spider mites and whiteflies

Concentration in compost (ppm)	Mites			Whitefly			
	Leaf-Damage index (days after treat- ment)			Crawlers		Scales	
	0	12	19	Alive	Dead	Alive	Dead
0	1.1	1.9	2.3	0	0	21	0
10	0.9	1.9	2.0	0	86	28	0
40	1.1	1.2	1.0	0	68	0	0

Following the release of Encarsia (30 days after treatment) black, parasitized scales developed on the plants treated at 10 ppm and these successfully matured to adult parasites.

The second experiment (E2), using lower dosages, showed that whitefly fecundity was markedly reduced at 10 ppm but less so at 5 and 2 ppm (Table 4). Subsequent scale development was affected even at the lowest rate. The development of Encarsia was similarly reduced at concentrations in excess of 2 ppm.

TABLE 4

Effect of dimethoate granules on development of whiteflies and Encarsia

Concentration in compost (ppm)	No.eggs/leaflet (after 5 days oviposition)	Whitefly		Encarsia	
		No.scales/ leaflet (after 28 days)	% survival to adults	%	
0	1334	251	96	72	
2	1078	176	69	71	
5	1180	36	61	50	
10	481	45	54	28	

Phytotoxicity

Damage from dimethoate granules proved to be unpredictable. No damage was observed in two trials in 1973, both treating 72 plants with 10 ppm, one sown in mid-summer the other in November. However, in 1974, 6 days after planting into compost containing 40 ppm dimethoate (raked into top 5 cm) necrotic spots (up to 5 mm diam.) appeared on the laminae and at the leaf edges. This symptom was accompanied by a pronounced curling of the leaves. At 10 ppm only the leaf-margins were affected. Five weeks later 45 cm of unaffected growth was present on plants grown in 10 ppm but only 20 cm in plants grown in 40 ppm.

Two weeks after pricking out seedlings into compost treated at 10 ppm all the cotyledons showed a slight tip-scorch and curling but at 5 ppm only 65% of the seedlings were slightly affected. No symptoms occurred when compost was treated at 2 ppm.

## DISCUSSION

Attacks by tomato leaf-miner are initiated by adults emerging from overwintering pupae surviving soil sterilization treatments. This conclusion is confirmed by the experience of Pennah (in litt.) in Guernsey who controlled the pest by covering the glasshouse soil with a polythene sheet after dusting the surface with BHC. The trapped vapour apparently killed the newly emerged adults so preventing their establishment in the new crop. Experience with this technique in the U.K. has not been so successful and, in any event, it is only practicable where tomatoes are grown in peat bales. Of the materials shown to be effective in the tests reported here pirimiphos-ethyl and pirimiphos-methyl are known to be lethal to parasites and predators. Dimethoate, while equally lethal, can be used as a systemic during the propagating phase by incorporating 2 ppm a.i. in the compost in the form of granules.

Artificial inoculation of the potted plants with red spider mite and subsequently with predators (Hussey et al., 1975), immediately before planting can then be accomplished without deleterious effects. Similarly, use of Encarsia soon after planting will be unaffected.

Such selective use of a broad spectrum pesticide is an example of its separation from natural enemies in time similar to that used in the case of parathion to control Tyroglyphus in an integrated programme for cucumber pests (Anon, 1972). This technique could well be exploited in other cases where parasites and predators are released at predictable times.

Infestations of leaf-miner occurring after planting can be prevented by sprays (0.05% a.i.) of dioxathion applied to the upper leaf surfaces as soon as adult feeding punctures are observed on the apical foliage. This technique separates another lethal pesticide from natural enemies on the abaxial surface of the lower foliage and is similar to that used successfully in our integrated programme on chrysanthemums (Scopes & Biggerstaff, 1973). Such methods isolate the pesticide from natural enemies in space and could be used similarly in other cases where pests occupy different niches on the plant.

While biologically effective, the use of dioxathion on tomatoes has yet to be officially cleared before the technique can be used commercially.

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