ESTERASE VARIATION IN THE BROWN PLANTHOPPER NILAPARVATA LUGENS AND ITS INVOLVEMENT IN RESISTANCE TO ORGANOPHOSPHORUS INSECTICIDES

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ABSTRACT

The susceptibility of eight populations of the brown planthopper Nilaparvata lugens was compared in the laboratory using five organophosphorus insecticides (malathion, diazinon, acephate, monocrotophos and carbophenothion). The Queensland strain was the most susceptible to the selected insecticides while the Japanese strain showed the most resistance, notably to malathion with a resistance factor of 409. The Philippine biotypes and carbofuran resistant strain showed resistance intermediate to that of the Japanese and susceptible strains. Total esterase activity of the hopper populations, measured using a colorimetric method, was highest in the most organophosphate resistant populations. Further investigation by polyacrylamide gel electrophoresis separated the esterases into eleven bands. Results from using selected inhibitors characterised the esterase bands as two arylesterases, one acetylcholinesterase and eight carboxylesterases. All resistant hoppers showed increased activity in one esterase, E10. The involvement of this esterase, a carboxylesterase, in resistance is discussed.

INTRODUCTION

Resistance to organophosphorus (OP) insecticides, notably malathion, has been linked to variations in esterase activity. The present study was conducted to determine if a correlation between insecticide resistance and esterase activity existed in the brown planthopper Nilaparvata lugens (BPH). Further characterisation of the esterases, by electrophoresis and the use of selected inhibitors, was undertaken to gain information on the types of esterase present in the BPH and to determine if a specific esterase was involved in resistance.

MATERIALS AND METHODS

Insect material

The origins of the BPH populations and rice cultivars used for culturing are given in Table 1. The biotypes from the International Rice Research Institute (IRRI), Philippines, are differentiated by their ability to colonise varieties of rice having different genes for conferring resistance to hopper attack. Therefore, rearing of the biotypes on suitable, susceptible rice varieties is essential for maintaining the correct biotype populations.

Bioassay

The large numbers of insects required during the bioassay necessitated the use of mixed populations of adult male and female hoppers along with a small percentage of 4th and 5th instar nymphs. The insecticides

Table 1

Origins of the BPH populations and their respective hostplants.

BPH population	Abbreviation used	Origin	Rice variety
Japanese strain	JS	Japan- ICI culture from Cardiff University	TN1
Queensland strain	QS	Queensland,Australia from Porton Down cultures	Blue Bonnet
Carbofuran resistant strain	CR	IRRI, Philippines IRRI glasshouse culture	TN1
Biotype 1	Bt1C	IRRI, Philippines from Cardiff University	TN1
2	Bt2C		Mudgo
3	Bt3C		ASD7
Biotype 1	Bt1P	IRRI, Philippines obtained directly from IRRI cultures.	TN1
2	Bt2P		Mudgo

malathion (25% e.c.), diazinon (60% e.c.), monocrotophos (60% e.c.), acephate (75% s.p.) and carbophenothion (40% e.c.) were evaluated using the infested plant activity test developed at ICI, Plant Protection Division, Jealott's Hill Research Station (Tranter 1983). Insecticides were applied to groups of approximately 20 insects using a Burkhard Potter Tower and percentage mortalities were assessed at 48 hours. After correction for control mortality the data were processed using logit analysis.

Esterase assay

The assay method was based on that of Devonshire (1975). Groups of male macropterous BPH were weighed and then homogenised in 20 mM phosphate buffer pH 7.0 using a PTFE-glass homogeniser. Aliquots of the uncentrifuged homogenates were incubated with 0.25 mM 1- or 2-naphthyl acetate as substrate in a total volume of 3.0 ml of phosphate buffer pH 7.0, at 25°C. After 30 minutes incubation 0.5 ml of a 0.3% Fast Blue B salt - 3.5% sodium lauryl sulphate solution was added. The optical density was measured 15 minutes later, at 605 nm for 1-naphthyl acetate and 555 nm for 2-naphthyl acetate.

Electrophoresis

Vertical slab acrylamide gel electrophoresis was performed using 6.5% polyacrylamide gels containing 0.25% (wt/vol) Triton X-100 with a continuous Tris borate EDTA running buffer system of pH 8.5. Male macropterous BPH were collected and bulk homogenates prepared in running buffer containing 10% sucrose, 0.5% Triton X-100 and 0.001% bromophenol blue. The concentration of homogenate depended on the stain used but typically was 50 µg insect material / µl homogenising solution. 5 µl of homogenate was layered in each gel pocket, 12 pockets per gel, and electrophoresis was carried out at 50 V constant voltage for 10 minutes followed by 150 V for 5 hours. The gels were then stained for esterase activity.

Staining and characterisation of esterases

To detect esterase activity 50 mg of 1- or 2-naphthyl acetate was dissolved in 1 ml of acetone and made up to 50 ml with a 0.2 M phosphate buffer pH 6.0 containing 50 mg of Fast Blue BB salt. Gels were stained in the solution for 60 minutes and then fixed in a mixture containing 5:5:1 methanol, water, acetic acid. By careful drying on cartridge paper each gel was kept as a permanent record. Acetylcholinesterase (AChE) activity was detected using the thiocholine method of Karnovsky and Roots (1964).

Several inhibitors were employed in the classification of esterases present in the BPH so that comparisons could be made with esterases found in other insects. Eserine, dichlorvos, carbofuran, carbophenothion and propoxur at concentrations of 10^{-4} M and 10^{-6} M were chosen as inhibitors. After 30 minutes incubation in a 0.2 M phosphate buffer solution pH 6.0 containing the inhibitor each gel was transferred to a fresh buffer solution containing the stain and inhibitor. Gels were then fixed and stored as above.

RESULTS AND DISCUSSION

The bioassay work confirmed that the QS was most susceptible to the five OP insecticides. Table 2 gives the resistance factors for the BPH populations when compared to the susceptible QS.

Table 2

Resistance factors (RF)* for the BPH populations

Insecticide	Bt1C	Bt1P	Bt2C	Bt2P	Bt3C	JS	CR
Malathion	55.7	29.0	47.3	98.4	60.4	409.7	39.6
Diazinon	9.6	11.1	9.6	16.3	10.5	26.3	10.4
Monocrotophos	15.6	25.8	23.9	37.9	28.8	59.3	17.5
Acephate	5.2	4.1	5.4	8.5	-	9.5	8.8
Carbophenothion	2.2	2.7	2.5	3.3	4.8	8.3	9.6

$$* \text{ RF} = \frac{LC_{50} \text{ of resistant strain}}{LC_{50} \text{ of susceptible strain}}$$

The JS was the most resistant strain and proved highly resistant to malathion with an RF of 409 (the JS LC_{50} was over 4000 ppm while that of the QS was only 9.81 ppm). The Phillipine biotypes were chosen for investigation because of reported variations in their resistance to insecticides (Heinrichs & Valencia 1978a). However, with the exception of Bt2P, which consistently showed higher resistance to malathion, results presented here would suggest that there is little difference between the biotype populations from Cardiff or the Philippines in their resistance to OPs. The CR strain in addition to its resistance to the carbamate carbofuran, was also reported to be resistant to monocrotophos and other OPs when compared with the original Philippine field strain (Heinrichs & Valencia 1978b). Significant resistance to OPs by the CR was not found in the present study although further work did confirm resistance to several carbamate and pyrethroid insecticides (Tranter 1983).

The esterases were investigated in the BPH because of the link between increased esterase activity and resistance to insecticides in insects such as Nephotettix cincticeps (Kojima et al. 1963) and Myzus persicae (Needham & Sawicki 1971).

Table 3

Total esterase activity of BPH populations using the substrates 1- and 2-naphthyl acetate.

BPH population	Total esterase activity 1-naphthyl acetate (a)		2-naphthyl acetate (b)		Ratio of b/a
Bt1C	1.23 ± 0.09	(6)	2.17 ± 0.21	(6)	1.8
Bt1P	1.10 ± 0.04	(6)	2.08 ± 0.08	(6)	1.9
Bt2C	1.23 ± 0.09	(5)	2.34 ± 0.24	(6)	1.9
Bt2P	1.68 ± 0.09	(7)	4.62 ± 0.42	(6)	2.7
Bt3C	1.07 ± 0.04	(5)	2.75 ± 0.12	(3)	2.5
CR	1.51 ± 0.07	(6)	2.85 ± 0.13	(6)	1.9
JS	3.88 ± 0.24	(7)	9.09 ± 0.34	(6)	2.3
QS	0.31 ± 0.01	(5)	0.34 ± 0.03	(5)	1.1

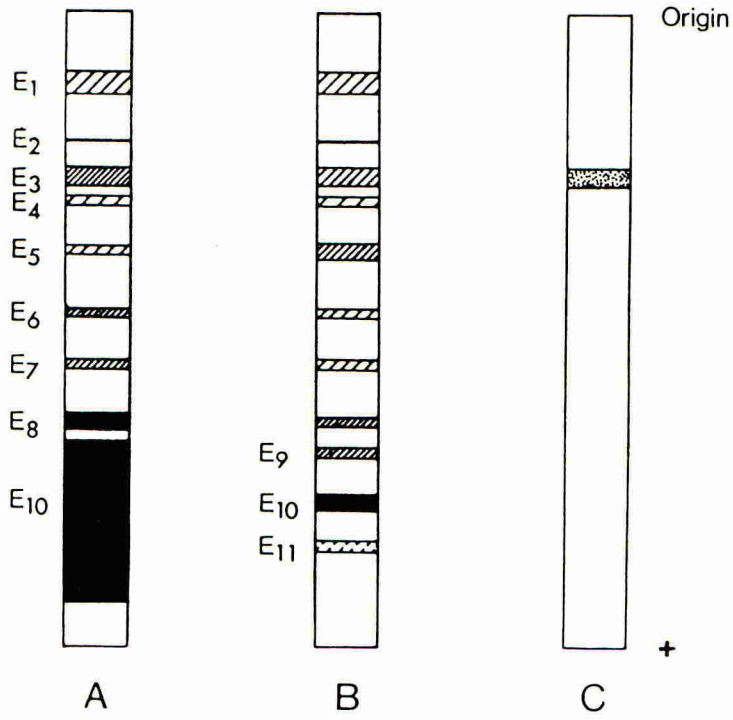
The results are expressed as μmol 1- or 2-naphthol/h/mg insect (mean ± S.E.) for the number of experiments in parentheses. Each experiment containing 10 to 15 insects.

Table 3 compares the total esterase activity of the BPH populations. Elevated levels of esterase activity were found in the resistant BPH, with the most resistant JS having a level of activity 10 times that of the susceptible QS and approximately 2-3 times that of the Philippine BPH. A comparison of the substrate specificity of the total esterase using 1- and 2-naphthyl acetate found a 1.8 to 2.7 times greater activity towards the 2-naphthyl ester in all of the populations with the exception of the QS, in which there appeared to be no preference between the two substrates. These results indicate that of the two naphthyl esters, the higher rate of hydrolysis by esterases from the the BPH occurs with the 2-naphthyl isomer. Esterases of resistant N.cincticeps also hydrolyse the 2-naphthyl ester in preference to the 1-naphthyl ester (Ozaki & Koike 1965). 1-naphthyl acetate was most actively hydrolysed in resistant M.persicae but there was no significant difference between the resistant and susceptible strains of aphid in their ability to hydrolyse the 2-naphthyl ester (Sudderuddin 1973).

To determine if increased activity was due to the increase in a single esterase, as in M.persicae (Devonshire 1975) or N.cincticeps (Ozaki et al. 1966) the esterases were examined using electrophoresis. Results of the electrophoretic separation are summarised in the zymogram (Figure 1). Triton X-100 was incorporated in both gels and homogenising buffer to obtain maximum solubilisation of esterases. Without Triton X-100 a high proportion of the esterase activity, especially AChE, remained in the gel pockets.

At least 11 esterases were distinguished in the QS using 1- or 2-naphthyl acetate as substrate. The resistant BPH showed a similar pattern

Figure 1. Zymograms of esterases of susceptible and resistant brown planthoppers after electrophoretic separation on polyacrylamide gels. A. esterases of the biotypes, CR or JS with 1-naphthyl acetate as substrate. B, esterases of the QS strain with 1-naphthyl acetate as substrate. C, acetylcholinesterase of all the BPH populations with acetylthiocholine iodide as substrate.



of esterases up to E8, with slight variations in the intensities of individual bands. However, the greatest difference between the BPH populations was seen in the region corresponding to E9-E11 in the QS zymogram. Shorter staining times and dilution of the homogenates indicated that this broad, highly active region was the result of one esterase which corresponded to E10 of the QS.

Using inhibitors the esterase E10 was classified as a carboxylesterase, as were esterases E4-E9 and E11. These esterases were all strongly inhibited by the OP dichlorvos but were only poorly inhibited by the carbamate compounds eserine and carbofuran. E1 and E2 were resistant to inhibition by carbamates and OPs and this suggested that both were aryl-esterases. E3 was classified as an AChE because this band was inhibited by both groups of insecticides and was also the only band to stain using the specific thiocholine method.

Although the results of electrophoresis are difficult to quantify accurately, the strongly staining E10 must be responsible for the majority

of the total esterase activity measured using the colorimetric method. The results of the biochemical investigation suggest that, as in other resistant insects, there is a correlation between increased esterase activity, specifically of one carboxylesterase E10, and increased resistance to malathion. However, the presence of increased carboxylesterase with increased resistance to malathion does not prove that the enzyme is the resistance mechanism involved. Therefore, further studies were conducted into the penetration and in vivo metabolism of malathion (Tranter 1983). No differences were observed between the BPH populations in the penetration of malathion. Results of the metabolism study demonstrated that the production of malathion metabolites, especially the carboxylesterase product malathion monoacid, was highest in the JS. This strongly suggests that the metabolism of malathion, by ester hydrolysis, is highest in the JS and supports the proposed link between malathion resistance and increased esterase activity.

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REFERENCES

Devonshire, A.L. (1975) Studies of the carboxylesterase of Myzus persicae resistant and susceptible to organophosphorus insecticides. Proceedings 8th British Insecticide and Fungicide Conference 1, 67-73.

Heinrichs, E.A.; Valencia, S.L. (1978a) Contact toxicity of insecticides to the three biotypes of brown planthopper. International Rice Research Newsletter 3(3), 19-20.

Heinrichs, E.A.; Valencia, S.L. (1978b) Resistance of the brown planthopper to carbofuran at IRRI. International Rice Research Newsletter 3(4), 18.

Karnovsky, M.J.; Roots, L. (1964) A "direct colouring" thiocholine method for cholinesterase. Journal of Histochemistry and Cytochemistry 12, 219-220.

Kojima, K.; Ishizuka, T.; Kitakata, S. (1963) Mechanisms of resistance to malathion in the green rice leafhopper Nephotettix cincticeps. Botyu-Kagaku 28, 17-25.

Needham, P.H.; Sawicki, R.M. (1971) Diagnosis of resistance to organophosphorus insecticides in Myzus persicae. Nature 230, 125-126.

Ozaki, K.; Koike, H. (1965) Naphthyl acetate esterase in the green rice leafhopper, Nephotettix cincticeps Uhler, with special reference to the resistant colony of the organophosphorus insecticide. Japanese Journal of Applied Entomology and Zoology 9, 53-59.

Ozaki, K.; Kurosu, Y.; Koike, H. (1966) The relationship between malathion resistance and esterase activity in the green rice leafhopper, Nephotettix cincticeps Uhler. SABCO Journal 2, 98-106.

Sudderuddin, K.I. (1973) An in vitro study of esterases, hydrolysing non-specific substrates, of an OP-resistant strain of the green peach aphid, Myzus persicae (Sulz.). Comparative Biochemistry and Physiology 44, 1067-1076.

Tranter, B.C. (1983) Elucidation of insecticide resistance mechanisms in the brown planthopper Nilaparvata lugens. PhD thesis, University of Reading.

THE RELATIONSHIP BETWEEN INSECTICIDE RESISTANCE AND CONTROL FAILURE

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ABSTRACT

The generally poor understanding of relationships between insecticide resistance and control failure reflects both the difficulties in using laboratory bioassay results to predict field control, and an ignorance of natural levels of tolerance shown by most pests. These problems can be overcome by measuring phenotypic levels of resistance (i.e. LD-values) and resistance gene frequencies of populations both well- and poorly-controlled by insecticides in the field. Recent work on pyrethroid resistance in house-flies (Musca domestica) on animal farms in south-east England has enabled the estimation of 'threshold' LD_{50} values for several pyrethroids above which control is likely to fail, and the approximate corresponding frequency of the major resistance gene kdr. These results are reviewed, and some problems of methodology and interpretation likely to be encountered for other insect pests are discussed.

INTRODUCTION

The reason(s) why insecticides fail to control insect pests are usually uncertain and often contentious. This is particularly so when the prime suspect is insecticide resistance, because the relationship between resistance and control failure is still very ill-defined. This arises from basic misunderstandings as to what constitutes resistance (Brown 1976, Ball 1981, Sawicki & Denholm 1984), and the inability to measure satisfactorily and simultaneously the three interdependent variables that define control failure through resistance, namely: frequency of a resistance (R) gene, phenotypic level of resistance measured by bioassays, and efficacy of control in the field.

In almost all cases of field resistance the nature and type of resistance genes are unknown, and criteria for defining control failure are imprecise; only absolute levels of tolerance can sometimes be measured objectively by laboratory bioassays. Recently, however, detailed work on the causes and effects of pyrethroid resistance in house-fly (Musca domestica) populations close to Rothamsted has provided sufficient data to establish the relationship between resistance and control failure for this species (Farnham et al. 1984). This paper reviews the major findings and highlights some of the problems of methodology and interpretation likely to be encountered for other pests.

EXPERIMENTAL APPROACH

To correlate levels of pyrethroid resistance with control failure, house-fly strains from 30 pig farms within 20 km of Harpenden, 10 from elsewhere in the U.K. and one from Canada were tested within two generations of receipt by topical application in the laboratory for tolerance to bioresmethrin, permethrin, deltamethrin and the natural pyrethrins with and without synergists. A discriminating dose of DDT + FDMC (2,2-bis-(4-chlorophenyl)-1,1,1 trifluoroethanol) determined the frequency of homozygotes for kdr (knockdown resistance), the major gene

conferring resistance to pyrethroids and cross resistance to DDT. Pyrethroid usage, if any, and control efficacy were recorded by direct observation and/or liaison with farmers. We considered that pyrethroids failed when, applied as sprays or residues, they barely reduced fly numbers or gave such transient control that populations recovered to or near their original level within 24-48h of treatment. By 1982, control failure was evident on 9 of the 23 farms where space-sprays of synergised non-persistent pyrethroids or residues of commercial formulations of permethrin had been used for house-fly control.

RELATIONSHIP BETWEEN RESISTANCE LEVELS AND CONTROL FAILURE

Most difficulties in interpreting resistance arise at the population level, when resistance can either denote a change in the response of a pest through selection, or control failure when the recommended dose no longer gives adequate control. Although these two are clearly interdependent, they are not always synonymous because an increase in tolerance need not cause loss of control (Sawicki & Denholm 1984). Standard laboratory bioassays can measure objectively differences in tolerance, but are much too far removed from reality to predict control efficacy with insecticides in the field. This is presumably why so few of the many papers describing bioassay results allude to the relationship between LC- or LD- values and field control (Ball 1981).

These problems of interpretation are compounded by the virtual ignorance of natural levels of tolerance shown by pest species in the field. Continued reliance on laboratory (S) strains for detecting and monitoring resistance disregards the fact that the overall response of natural populations alters continuously as pests come into contact with more, new or different insecticides. Since these S strains, defined as "populations never subjected to insecticidal pressure, in which resistant individuals are rare" (Busvine 1980), no longer represent the base line response of most pests, the value of arbitrary resistance factors, e.g. 10-fold (Brown 1976), as indicators of control failure at recommended doses is very limited. Resistance factors defining control failure vary very considerably depending on the pest and crop, the amount of overkill built into manufacturers' recommended doses, the insecticide used for diagnosis, the type of bioassay, etc. For Heliothis virescens in California, factors in excess of 50-fold for permethrin have apparently not impaired control with pyrethroids (Martinez-Carvillo & Reynolds 1983), whereas 6-fold resistance enabled Myzus persicae to survive recommended doses of demeton-S-methyl (Devonshire et al. 1975). Likewise, laboratory tests comparing Californian Tetranychus urticae populations well- and poorly-controlled with dicofol showed 5-fold resistance by a slide-dip bioassay but 544-fold resistance by a residual bioassay (Dennehy et al. 1983).

Some authors (Solomon et al. 1979, Micks et al. 1980, Sawicki & Denholm 1984), realising that resistance is a dynamic phenomenon, have advocated an alternative method to the use of S strains for obtaining base-line response data and detecting resistance. This is based on the frequency distribution of tolerances shown by field populations sampled recently and at random from nature, and shows not only the modal response of these populations but also the amount of variation about the mode. Using this approach, Solomon et al. (1979) found that tick populations differing 5 to 10-fold in LC- values were all 'susceptible' to recommended doses of organophosphorus insecticides in the field. However, since there

were no control problems, there was no indication of how far LC- values must lie outside this tolerance distribution before control failure is apparent. This was possible for house-flies by examining strains both well- and poorly-controlled by pyrethroids (Fig. 1).

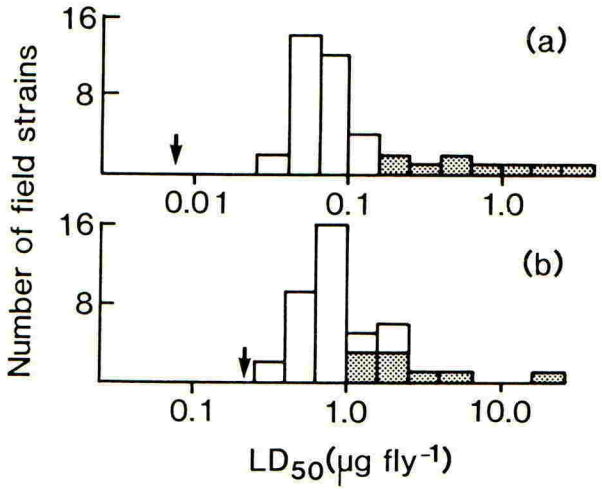

Fig. 1. Distribution of LD_{50}s of 41 field strains for permethrin (a) and unsynergised natural pyrethrins (b). Arrows indicate the response of the laboratory susceptible strain, shaded areas show strains from farms where pyrethroids failed.

The survey showed that LD_{50}s for permethrin (and bioresmethrin) of well-controlled strains followed an approximately log-normal distribution with a modal response 7-10x greater than the S strain (Fig. 1a). Poorly-controlled field strains had LD_{50}s up to 400x greater than the S strain and lay within an extended tail of the distribution, thus confirming resistance as the cause of control failure on these farms. However, the corresponding distribution of LD_{50}s for natural pyrethrins was much more compressed, and the resistance factors consequently much lower (Fig. 1b). These considerable differences in tolerance even between closely related chemicals enhance the difficulties in predicting when control failure will arise.

From these data we were able to estimate LD_{50}s for each compound corresponding to the control failure point (Table 1). These threshold LD_{50}s are obviously approximate, and represent the mid-points of 'grey areas' in which the outcome of control is indeterminate rather than absolute diagnostic values. Similar data are urgently needed for most pests, since they provide a realistic means of anticipating when control failure is likely to occur, and of confirming resistance as the cause when it has taken place.

TABLE 1

Approximate control failure threshold LD_{50}s for different pyrethroids, and resistance factors relative to both the susceptible (S) strain and the modal response of field populations.

Pyrethroid	LD_{50} (ug fly^{-1}) S strain	Modal response (MR)	Failure threshold (FT)	Resistance factor FT/S	FT/MR
Bioresmethrin	0.005	0.03	0.10	20	3.3
Permethrin	0.008	0.05	0.12	15	2.4
Deltamethrin	0.0004	0.003	0.012	30	4
Pyrethrins	0.28	0.75	1.10	4	1.5

RELATIONSHIP BETWEEN RESISTANCE GENE FREQUENCIES AND CONTROL FAILURE

Resistance has also been defined more broadly as "marking a genetic response to selection" (Crow 1957). This genetic response is expressed as an increase in the frequency of resistant (R) gene(s) in a treated population, which results in control failure when the proportion of individuals carrying the gene exceeds a threshold value. For a fuller understanding of the relationship between resistance and control failure, and to make results for different pests more comparable, it is highly desirable though seldom practicable to determine the R gene frequency at this control failure point.

Resistance factors based on the comparison of LD-values, which define the overall phenotypic response of a population, are very crude and indirect indicators of differences in R gene frequency. It is generally more informative and sometimes easier to determine the frequency of resistant individuals directly, e.g. by use of a discriminating dose or electrophoresis, than to calculate probit lines. For instance, electrophoresis of insecticide-resistant Myzus persicae establishes not only the frequency of resistant aphids but also the frequency of different variants for resistance (Sawicki et al. 1978). This technique is superior to toxicological bioassays, since the probit lines of susceptible and resistant variants overlap.

Even when resistant individuals can be identified, the conversion of such data to gene frequencies is difficult because the genotypic constitution of phenotypically-resistant individuals (i.e. those surviving a discriminating dose) depends on the effective dominance of an R gene at this dose. Unless all genotypes at an R locus have been isolated and characterised toxicologically, the effective dominance is unknown. Thus normally the R gene frequency corresponding to a discriminating dose response lies within a large range of possible values (Fig. 2).

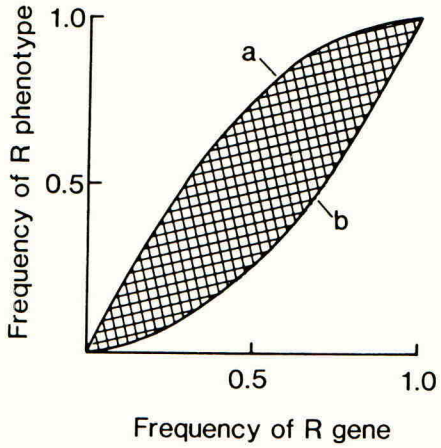

Fig. 2. Relationship between the frequency (p) of a resistance gene in a test strain and the proportion of insects responding to a discriminating dose. a - R gene fully dominant, b - R gene fully recessive; the value of p for genes of intermediate dominance lies between these two extremes. R genotypes are assumed to be in Hardy-Weinberg equilibrium at the time of testing, i.e. $R/R = p^2$, $R/S = 2pq$, $S/S = q^2$ ($q = p-1$).

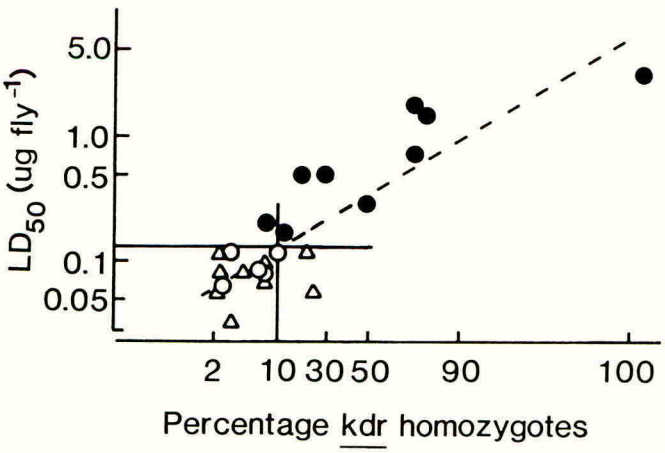

Fig. 3. Relationship between LD_{50}s for permethrin (log scale) and percentages of kdr homozygotes (probit scale). The fitted regression (dashed line) accounted for 73% of the observed variation. Solid lines show the approximate control failure threshold value of each variable. (○-pyrethroids used, control satisfactory; ●-pyrethroids used, control failure; △-no pyrethroids used).

In spite of these difficulties, R gene frequencies can give a more accurate impression of the genetic structure of field populations at the time of sampling than LD- values and discriminating dose results, which may be influenced by moving insects from the field to the laboratory. Populations undergoing selection with insecticides are seldom in Hardy-Weinberg equilibrium at an R locus; resistant genotypes being over-represented according to the intensity of selection. Equilibrium is restored under more benign laboratory conditions, normally after a single generation, but the resulting shift in genotype distribution can significantly reduce resistance levels even though the R gene frequency remains unchanged. Under prolonged laboratory culture, back pressure towards susceptibility may render field strains even less representative of parental populations by reducing the R gene frequency itself.

By examining the genetics of pyrethroid resistance in our field strains we were able to quantify the relationship between R gene frequencies and control failure. These studies showed that although pyrethroid resistance was caused by up to seven independent R genes, only two, kdr (knock-down resistance) and the esterase variant $E_{0.39}$, were frequent and of practical importance. 27 of the 41 strains tested had detectable levels of kdr, the major gene involved in pyrethroid resistance locally. There was a close relationship between the frequency of kdr homozygotes estimated by the DDT + FDMC test and LD_{50}s to permethrin (Fig. 3), confirming the importance of kdr in resistance to this compound. Variation about the regression line reflected in part the influence of other R genes such as the esterase $E_{0.39}$, which confers slight (2 to 3-fold) resistance to synthetic pyrethroids even in strains lacking kdr (Sawicki et al. 1984), but also no doubt the inadequacy of LD_{50}s or similar parameters for summarising the response of genetically-heterogeneous populations.

For the natural pyrethrins this relationship was much less clear-cut (Fig. 4), due to a greater clustering of points at the lower end of the tolerance scale, and a greater scattering at the higher end. The clustering reflected the relatively slight resistance conferred to natural pyrethrins by kdr alone or with $E_{0.39}$ compared to the synthetic compounds. The scattering was probably due to the strong selective intensification of kdr by other pyrethroid resistance factors present in local populations (R.M. Sawicki, unpublished data). Thus natural pyrethrins are relatively poor diagnosers of pyrethroid resistance in house-flies caused by kdr.

These graphs demonstrated that control failed when c. 10% (more realistically 7-13%) or more flies in a field strain were homozygous for kdr (i.e. phenotypically-resistant individuals). Since kdr is fully recessive under laboratory conditions, this corresponds to an estimated threshold gene frequency of 0.25-0.40 (Fig. 2). The magnitude of these figures illustrates how far recessive genes can be selected before control failure becomes apparent, and together with the slight (1.5 to 4-fold) difference between the modal response of field strains and control failure threshold LD_{50}s (Table 1), explains how over-frequent or continuous use of pyrethroids against house-flies can lead rapidly to control failure, sometimes after a single application (Denholm et al. 1983). Had kdr been fully dominant and of similar expressivity, the corresponding threshold gene frequency would be 0.03-0.06, i.e. 5-8 times less.

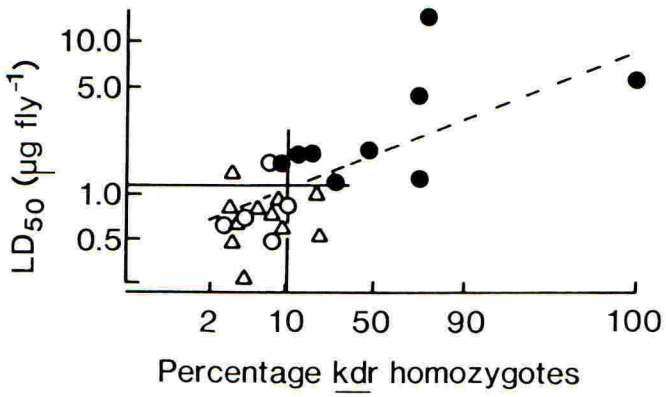

Fig. 4. As Fig. 3., but with results for natural pyrethrins. The fitted regression accounted for 51% of the observed variation.

CONCLUSIONS

The quantification of the relationship between R gene frequencies, resistance levels and control failure has many important applications in the study of insecticide resistance. The basic approach of correlating bioassay data with control efficacy should be accessible for most insect pests and its advantages over other methods of detecting, monitoring and predicting the practical importance of resistance are evident from the example provided. The genetic component of the relationship will be less accessible until the nature and properties of R genes are understood for a larger number of organisms. However, a fuller understanding of how all three variables interact is needed to resolve the factors causing control failure through resistance, and to assist multidisciplinary research into how this might be avoided.

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REFERENCES

Ball, H.J. (1981) Insecticide resistance - a practical assessment. Bulletin of the Entomological Society of America 27, 261-262.

Brown, A.W.A. (1976) How have entomologists dealt with resistance? Proceedings of the American Phytopathological Society 3, 67-74.

Busvine, J.R. (1980) Recommended methods for measurement of resistance to pesticides. FAO Plant Production and Protection Paper 21, pp.132.

Crow, J.F. (1957) Genetics of insect resistance to chemicals. Annual Review of Entomology 2, 227-247.

Denholm, I.; Farnham, A.W.; O'Dell, K.; Sawicki, R.M. (1983) Factors affecting resistance to insecticides in house-flies, Musca domestica L. (Diptera: Muscidae). I. Long-term control with bioresmethrin of flies with strong pyrethroid-resistance potential. Bulletin of Entomological Research 73, 481-489.

Dennehy, T.J.; Granett, J.; Leigh, T.F. (1983) Relevance of slide-dip and residual bioassay comparisons to detection of resistance in spider mites. Journal of Economic Entomology 76, 1225-1230.

Devonshire, A.L.; Needham, P.H.; Rice, A.D.; Sawicki, R.M.; (1975) Monitoring for resistance to organophosphorus insecticides in Myzus persicae from sugar beet. Proceedings 8th British Insecticide and Fungicide Conference 1, 21-24.

Farnham, A.W.; O'Dell, K.; Denholm, I.; Sawicki, R.M. (1984) Factors affecting resistance to insecticides in house-flies Musca domestica L. (Diptera: Muscidae). III. Relationship between the level of resistance to pyrethroids, control failure in the field, and the frequency of gene kdr. Bulletin of Entomological Research 74 (in press).

Martinez-Carvillo, J.L.; Reynolds, H.J. (1983) Dosage-mortality studies with pyrethroids and other insecticides on the tobacco budworm (Lepidoptera: Noctuidae) from the Imperial Valley, California. Journal of Economic Entomology 76, 983-986.

Micks, D.W.; Moon, W.B.; McNeill, J.C. (1980) Malathion tolerance vs. resistance in Culex quinquefasciatus. Mosquito News 40, 520-523.

Sawicki, R.M.; Denholm, I. (1984) Adaptation of insects to insecticides. In: Origins and development of adaptation (Ciba Foundation Symposium 102), London: Pitman Books, pp. 152-166.

Sawicki, R.M.; Devonshire, A.L.; Farnham, A.W.; O'Dell, K.; Moores, G.D.; Denholm, I. (1984) Factors affecting resistance to insecticides in house-flies, Musca domestica L. (Diptera: Muscidae). II. Close linkage on autosome 2 between an esterase and resistance to trichlorphon and pyrethroids. Bulletin of Entomological Research 74, 197-206.

Sawicki, R.M.; Devonshire, A.L.; Rice, A.D.; Moores, G.D.; Petzing, S.M.; Cameron, A. (1978) The detection and distribution of organophosphorus and carbamate insecticide-resistant Myzus persicae (Sulz.) in Britain in 1976. Pesticide Science 9, 189-201.

Solomon, K.R.; Heyne, H.; Van Kleef, J. (1979) The use of a population approach in the survey of resistance to pesticides in ticks in southern Africa. In: Recent Advances in Acarology Volume 2 Rodriguez, J.A. (Ed), New York: Academic Press, pp.65-69.

A FURTHER EXAMINATION OF INSECTICIDE RESISTANCE IN HOUSEFLIES (MUSCA DOMESTICA) IN THE UNITED KINGDOM

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ABSTRACT

A second survey of insecticide resistance of UK houseflies was carried out between March 1981 and June 1982. 39 strains were tested against trichlorphon, dimethoate, pyrethrins + PB, permethrin and methomyl. 14 strains were also tested against dichlorvos. The level of response to trichlorphon and pyrethrins + PB was similar to that previously reported in 1981. Very high resistance to permethrin was found in 90% of strains. The generally lower levels of resistance to dimethoate and methomyl suggest their potential usefulness as housefly control agents.

INTRODUCTION

The survey for resistance in houseflies (Musca domestica) previously reported by Chapman and Lloyd, (1981), showed widespread resistance to DDT, δ-HCH, trichlorphon, tetrachlorovinphos, bendiocarb and pyrethrins synergised by piperonyl butoxide (PB). It was decided to continue to monitor for insecticidal resistance using a slightly different range of insecticides. Resistance to trichlorphon and pyrethrins + PB was still monitored since these compounds were in widespread use and this also provided a link with the previous screening programme. Dimethoate was included although not used for fly control in animal units in the United Kingdom. Permethrin resistance was examined since it was felt that its resistance status should be qualified even though its use for fly control has been discouraged in the UK. Resistance to methomyl was monitored because at the time of the screening programme methomyl incorporated into fly bait, had recently been introduced for use in UK farms. Dichlorvos was tested against 14 strains.

MATERIALS AND METHODS

The test methods were the same as described in Chapman and Lloyd 1981. The discriminating doses (DD) were obtained from computed line data to give a knockdown of 99.99% of susceptible flies 48 h after treatment (table 1). A multiple of 8x DD was used for the organophosphorus insecticides and 2x DD for the carbamate and pyrethroid insecticides.

Houseflies were obtained from sites in 18 counties of England (36 strains), the Grampion region in Scotland (2 strains), County Down, Northern Ireland (1 strain). Information on insecticide usage at each site was also collected where possible.

The insecticide solutions were prepared from technical samples containing more than 90% a.i. except for pyrethrins which were prepared from a decolourised extract containing 24.1% total pyrethrins. The synergist piperonyl butoxide was added to the pyrethrins in a 10:1 ratio respectively.

RESULTS

The dose response data for susceptible flies and the discriminating doses (DD) derived from computed KD99.99 values are given in table 1.

TABLE 1

Dose-response data for susceptible houseflies, 48 hours after topical treatment at 20°C

Toxicant	µg/♀ fly KD50	(95% limits)	KD99.99	(S.E.)	Chi^2	DF
Trichlorphon	0.21	(0.20, 0.22)	0.62	7.8 (0.7)	29.0	23
Dimethoate	0.0082	(0.0077, 0.0086)	0.025	7.5 (0.7)	25.4	23
Dichlorvos	0.0067	(0.0063, 0.007)	0.025	6.5 (0.6)	18.1	19
Pyrethrins + PB	0.025	(0.023, 0.027)	0.11	5.8 (0.6)	8.7	15
Permethrin	0.0059	(0.0055, 0.0061)	0.017	7.8 (0.7)	27.8	23
Methomyl	0.068	(0.063, 0.073)	0.73	4.2 (0.3)	25.1	23

The 48 hour KD responses of housefly strains to trichlorphon, dimethoate and dichlorvos is shown in table 2 and the responses to pyrethrins + PB, permethrin and methomyl in table 3.

The quality of the information on insecticide usage was very variable because reliable records were not available at some sites, hence this aspect of the survey must be treated with caution. All the units visited where information could be obtained, had used organophosphorous insecticides at some time. The most common being trichlorphon (in the form of a bait), tetrachlorvinphos, fenitrothion, iodofenphos and dichlorvos. Six farms reported use of carbamates. The use of pyrethrins and permethrin is indicated in table 3.

Eighty-seven percent of strains exhibited resistance to trichlorphon in the 'high' to 'very high' categories while 87% showed only 'moderate' resistance to dimethoate. No strain was fully susceptible to either insecticide. Two strains were susceptible to dichlorvos and 3 showed 'high', to 'very high' resistance.

At the only site where flies were susceptible to synergised pyrethrins, this insecticide had been used 'when necessary' over the last 4-5 years and up to 3 successive days per week in summer. Fifteen strains showed 'high' to 'very high' resistance to pyrethrins. At 12 of these sites permethrin or 'regularly' used pyrethrins or non-persistant pyrethroids had been used. 'Regular' use varied between 2x per day and 2x per week over an extended period. Pyrethrins or pyrethroids had not been used on two sites and usage data could not be obtained from one site. At 5 sites where permethrin had reportedly been used only 'moderate' resistance to pyrethrins was observed although it is not known what dosage or treatment regime was used at these sites.

'High' to 'very high' resistance to permethrin was found in 90% of strains. Of these 35 strains, 17 reported use of permethrin or 'regular' use of pyrethrins. No records were available on 3 sites and at 7 sites pyrethrins or pyrethroids had not reportedly been used.

TABLE 2

48 hour response to topically applied discriminating doses (DD), of housefly strains collected from farms in the United Kingdom.

STRAIN	TRICHLORPHON % KD at			DIMETHOATE % KD at			DICHLORVOS % KD at		
	DDx1	DDx8	R.cat[b]	DDx1	DDx8	R cat.	DDx1	DDx8	R cat.
AA1 (AA)[a]	8	93	+	29	99	+	-	-	
AB1 (AB)[a]	2	3	+++	20	96	+	2	96	+
AC (V)[a]	0	19	+++	20	100	+	-	-	
AD	5	5	+++	7	89	+	-	-	
AE	6	10	+++	23	100	+	-	-	
AF (W)[a]	0	60	++	59	100	+	-	-	
AG	5	16	+++	25	99	+	-	-	
AH	2	40	++	36	100	+	-	-	
AI	4	22	+++	39	100	+	-	-	
AJ (I)[a]	1	10	+++	8	98	+	-	-	
AK	0	26	++	26	100	+	-	-	
AL	4	30	++	76	100	+	-	-	
AM	2	2	+++	3	40	++	-	-	
AN	19	74	++	15	98	+	18	100	+
AO	6	68	++	66	100	+	-	-	
AP	3	23	+++	10	100	+	-	-	
AQ	0	3	+++	2	66	++	-	-	
AR	18	83	+	66	100	+	-	-	
AS	1	15	+++	2	94	+	-	-	
AT	4	14	+++	5	69	++	-	-	
AU	0	17	+++	22	95	+	-	-	
AV	1	55	++	42	100	+	12	100	+
AW	5	20	+++	12	100	+	-	-	
AX	12	74	++	74	100	+	-	-	
AY	0	7	+++	22	97	+	-	-	
AZ	7	41	++	33	92	+	-	-	
BA	5	44	++	35	99	+	10	93	+
BB	0	9	+++	43	97	+	-	-	
BC	1	16	+++	43	100	+	3	80	+
BD	5	47	++	39	98	+	3	83	+
BE	9	99	+	75	100	+	59	100	+
BF	3	41	++	69	100	+	-	-	
BG	19	96	+	7	100	+	100	100	0
BH	6	68	++	62	100	+	100	100	0
BI	0	1	+++	20	96	+	6	73	++
BJ	21	95	+	65	100	+	26	100	+
BK	3	14	+++	17	95	+	53	94	+
BL	5	17	+++	10	68	++	5	43	++
BM	0	2	+++	2	70	++	0	7	+++

a Indicates a further sample from a site tested in the previous monitoring programme. The letters in brackets refer to the strain designation used in a previous paper (Chapman & Lloyd 1981).

b Resistance category:

0 = Susceptible (100% KD at DD x1)
\+ = Moderately resistant (> 75% KD at upper dose)
++ = High resistance (25-75% KD at upper dose)
+++ = Very high resistance (< 25% at upper dose)

TABLE 3

48 hour response to topically applied discriminating doses (DD), of housefly strains collected from farms in the United Kingdom.

STRAIN	PYRETHRINS % KD at DDx1	PYRETHRINS % KD at DDx2	PYRETHRINS R cat.[b]		PERMETHRIN % KD at DDx1	PERMETHRIN % KD at DDx2	PERMETHRIN R cat.		METHOMYL % KD at DDx1	METHOMYL % KD at DDx2	METHOMYL R cat.
AA1 (AA)[a]	49	86	+	c	8	21	+++		79	97	+
AB1 (AB)[a]	63	91	+	c	9	22	+++		30	71	++
AC (V)[a]	0	34	++	c	0	2	+++	d	81	100	+
AD	6	9	+++	cc	3	10	+++		57	93	+
AE	100	100	0	cc	31	85	+		97	100	+
AF (W)[a]	31	92	+		4	4	+++	d	100	100	0
AG	5	19	+++	c	1	6	+++	d	93	100	+
AH	94	100	+	c	10	44	++		100	100	0
AI	31	96	+		8	10	+++	d	86	100	+
AJ (I)[a]	7	47	++		3	4	+++	d	79	97	+
AK	91	100	+	c	21	64	++		96	100	+
AL	87	100	+		12	67	++		99	100	+
AM	10	42	++	cc	3	4	+++		7	81	+
AN	95	100	+		5	24	+++		91	100	+
AO	15	59	++	c	1	4	+++	d	93	100	+
AP	8	51	++		1	1	+++		94	100	+
AQ	0	27	++	c	0	0	+++	d	56	87	+
AR	48	85	+		7	21	+++		88	100	+
AS	65	97	+		7	21	+++		70	100	+
AT	18	69	++	cc	3	4	+++		53	93	+
AU	83	90	+		1	13	+++	d	83	99	+
AV	32	87	+	c	0	0	+++	d	91	100	+
AW	17	85	+	c	3	4	+++	d	71	100	+
AX	98	100	+	c	46	82	+		96	100	+
AY	70	89	+	e	11	25	+++	e	70	100	+
AZ	9	62	++	e	2	3	+++	e	91	100	+
BA	46	82	+	c	4	14	+++		85	100	+
BB	60	84	+		5	19	+++		99	100	+
BC	4	42	++		8	10	+++	d	89	100	+
BD	86	91	+	c	40	78	+		99	100	+
BE	97	100	+	c	25	66	++		86	100	+
BF	92	100	+		25	84	+		98	100	+
BG	10	49	++	cc	1	1	+++		85	100	+
BH	97	100	+	e	8	18	++	e	100	100	0
BI	24	56	++		5	9	+++		91	100	+
BJ	96	100	+	c	4	22	+++		92	100	+
BK	9	34	++	cc	1	2	+++		92	100	+
BL	82	100	+	c	22	50	++		23	86	+
BM	2	54	++		0	0	+++	d	78	100	+

a As Table 2
b Resistance category as Table 2
c Indicates farms where synergised pyrethrins or non-persistent pyrethroids have been used
cc Synergised pyrethrins or non-persistent pyrethroids 'regularly' used
d Permethrin used as a residual treatment
e No insecticide usage data available

There were 3 strains which were susceptible to methomyl and only 1 strain where the resistance was in the 'high' category. In only 9 instances was there less than 100% response at twice the discriminating dose.

DISCUSSION

The similarity of responses to trichlorphon and synergised pyrethrins between this screening programme and the previous one indicates an overall similarity in insecticidal resistance to these compounds throughout the entire period 1979-1982.

In spite of widespread trichlorphon resistance this insecticide may still be of value as a bait in certain sites and its use should be continued where it is still effective.

The generally lower levels of resistance to dimethoate and dichlorvos probably reflect their lack of use in the UK for fly control. In Denmark, resistance to dimethoate appeared after 3 years use as a residual spray although it was a further 5 years before control failures with this product appeared (Keiding 1975). In Belgium and West Germany, dimethoate is in current use and only low levels (x 14) of dimethoate resistance have as yet been recorded (De Deken and Geerts 1983, Kunast and Messner 1979). In Czechoslovakia and the German Democratic Republic dimethoate use started in 1976, and after 3-4 years serious resistance appeared in certain areas while remaining low in others. The degree of resistance generally reflected the intensity of dimethoate use in these countries (Rupes *et al.* 1982). From the UK results and the experience in other European countries dimethoate would appear to have potential for useful fly control in UK animal units for between 4 and 8 years if used in moderation. However, use of dimethoate would have to be carefully monitored to avoid development of high levels of resistance which could lead to serious cross-resistance to other compounds (Keiding, 1975). If incorporated into a bait formulation rather than used as a residual spray it is possible that its effective life could be extended.

The relatively low resistance to the oxime carbamate methomyl is of great interest because of its successful use in a bait and particularly in view of the high resistance to other carbamates as seen in the UK (Chapman and LLoyd 1981) and in Europe (DeDeken and Geerts 1983, Keiding 1975-82).

The very high levels of resistance to permethrin found in the UK are typical of that found in many European countries (DeDeken and Geerts 1983, Kunast 1979, Keiding 1975-82). 'High' to 'very high' resistance to synergised pyrethrins, likely to cause control failure, in the UK appears to be the result of either excessive use of pyrethrins or non-persistent pyrethroids or to permethrin residual treatments. The information on insecticide usage (table 3) gives strong circumstantial evidence for this view and is further supported by data from laboratory and field trials involving intensive use of non-persistent pyrethroid space sprays and permethrin residual treatments in the UK (Anon. 1981, Wildey and Webb, Personal communication) and other parts of Europe (DeDeken and Geerts 1983, Kunast 1979, Keiding 1976). Permethrin has now been withdrawn for use against houseflies in intensive animal units in the UK and this use is not permitted in many other European countries. If the use of persistent

pyrethroids and regular and frequent use of pyrethrins and non-persistent pyrethroids can be discouraged a reversion in pyrethrin resistance may occur as demonstrated by Denholm *et al.* 1983 so that even sites which are currently experiencing failure of pyrethrins may again successfully use these insecticides for fly control.

Continued monitoring for housefly resistance particularly to newer compounds, together with detailed genetic and biochemical examination of the interaction of the insecticides and resistance mechanisms is essential if successful control of houseflies in intensive animal units is to be maintained in the future.

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REFERENCES

Anon, 1981 Practical trials of fly control techniques MAFF/ADAS Reference Book 251 (81), Storage Pests 1981, London HMSO, 51-52.

Chapman, P.A. & Lloyd, C.J. (1981) The spread of resistance among houseflies from farms in the United Kingdom. Proceedings 1981 British Crop Protection Conference - Pests and Diseases 2, 625-631.

DeDeken, R. and Geerts, S. (1983) The present status of insecticide resistance in the housefly (Musca domestica) in Belgium. Vlaams Diergeneeskundig Tijdschrift 52, 439-449.

Denholm, I.; Farnham, A.W.; O'Dell, K. and Sawicki, R.M. (1983) Factors affecting resistance to insecticides in houseflies,(Musca domestica)L. (Diptera : Muscidae). I. Long-term control with bioresmethrin of flies with strong pyrethorid-resistance potential. Bulletin of Entomological Research 73, 481-489.

Keiding, J. (1975) Problems of housefly (Musca domestica) control due to multiresistance to insecticides. Journal of Hygiene, Epidemiology Microbiology and Immunology 19, 340-355.

Keiding, J. (1976) Development of Resistance to Pyrethroids in field populations of Danish Houseflies. Pesticide Science 7 283-291.

Keiding, J. (1975-1982) Insecticide resistance in Musca domestica. Statens Skadedyrlaboratorium Årsberetning 1975, 1976, 1977, 1978, 1979, 1980, 1981, 1982.

Kunast, C. (1979) The development of permethrin resistance in the housefly (Musca domestica L.) in South Germany. Zeitschrift fur Angewandte Zoologie 66, 385-390.

Kunast, C. and Messner, K. (1979) Studies on the present status of insecticide resistance in the housefly (Musca domestica L.) in Southern Germany. Anzeiger Schällingskunde, Pflangen-und Umweltschutz 52 163-167. Zoologie 66, 385-390).

Rupes, V.; Muller, P.; Ledvinka, J. and Pinterova, J. (1982) The development of resistance to dimethoate in Musca domestica in Czechloslovakia and the German Democratic Republic. Acta Entomologica Bohemoslovaca 79, 412-421.

INSECTICIDE RESISTANCE IN ENCARSIA FORMOSA

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ABSTRACT

Stocks of Encarsia formosa, parasitoid of the greenhouse whitefly, from Britain, Holland and New Zealand were compared in terms of their susceptibilities to gamma-HCH and permethrin. Pupae within host scales were sprayed with a range of insecticide concentrations and mortality recorded as those failing to emerge and reach the 48 h adult stage. For both chemicals the British stock was more susceptible than either of the others which showed similar susceptibility levels. When treated with gamma-HCH all stocks exhibited a discontinuous mortality-concentration response with little change in mortality at low concentrations but a steep change at higher concentrations. Repeated exposure of the British stock to a gamma-HCH concentration initially causing 70% pupal mortality resulted in an increase in resistance which after nine generations was similar to that of the other two stocks. The differences in susceptibilities of the stocks and the significance of the selection results for possible breeding of pesticide resistant Encarsia for use in greenhouse integrated control programmes are discussed.

INTRODUCTION

Many growers of greenhouse crops use biological control of major pests such as whitefly and spider mites but other pests and diseases, which cannot be controlled biologically, require the use of chemicals, many of which are harmful to natural enemies. Careful timing of applications or localised applications such as soil drenches or polybutene formulations may help but when foliar applications are necessary biological control is threatened. One solution would be to use natural enemies which are resistant to commonly used chemicals.

Pesticide resistance has been reported for only fifteen natural enemy species compared with over 400 pest species (Croft & Brown 1975; Croft & Strickler 1983; Georghiou & Mellon 1983). This may be due to poor detoxifying mechanisms in natural enemies or the need for resistance to develop first in prey to ensure survival of an adequate food supply, or may simply reflect a limited search for resistant natural enemies in the field. Insecticide resistance has been selected for in the laboratory in a few species, mostly phytoseiid mites (Hoy 1982; Croft 1983), but resistance has not been reported in Encarsia formosa, parasitoid of the greenhouse whitefly.

In this study the susceptibilities of three different Encarsia stocks to the insecticides gamma-HCH and permethrin were tested with a view to detecting any existing resistance and the most susceptible stock was repeatedly exposed to gamma-HCH in an attempt to select for resistance.

MATERIALS AND METHODS

Encarsia

In 1982 Encarsia stocks were obtained from commercial suppliers in Britain (Bunting & Sons, Essex) and Holland (Koppert B.V., Berkel en Rodenrijs), both their stocks having originated in the 1960s from cultures kept at the Glasshouse Crops Research Institute, Littlehampton, England, and from New Zealand (Department of Scientific and Industrial Research, Auckland). The latter were introduced from England around 1930 but declined in the 1940s when synthetic insecticides were used in greenhouses and cultures were established in 1970 from individuals captured in the wild. Three cultures of these stocks, referred to as BS, KS and NZ respectively, were kept at 25 ± 0.5°C, 60% RH and a 16h light cycle using greenhouse whitefly (Trialeurodes vaporariorum) reared on dwarf French beans (Phaseolus vulgaris as hosts.

Insecticide treatments

Two persistent, broad-spectrum insecticides were used: gamma-HCH, an organochlorine and permethrin, a synthetic pyrethroid. Dilutions of commercial formulations were made in distilled water and 1 ml applied by Potter tower to replicated batches of 50-200 black whitefly scales parasitised 9-10 days previously. After the insecticide had dried scales were removed from leaves with a fine paint-brush and stored at 25°C to record Encarsia emergence. This prevented contact of Encarsia with insecticide other than that applied directly to scales. Mortality was recorded as unemerged adults plus adults dying within 48 h of emergence. Results were corrected for control mortality using Abbott's formula.

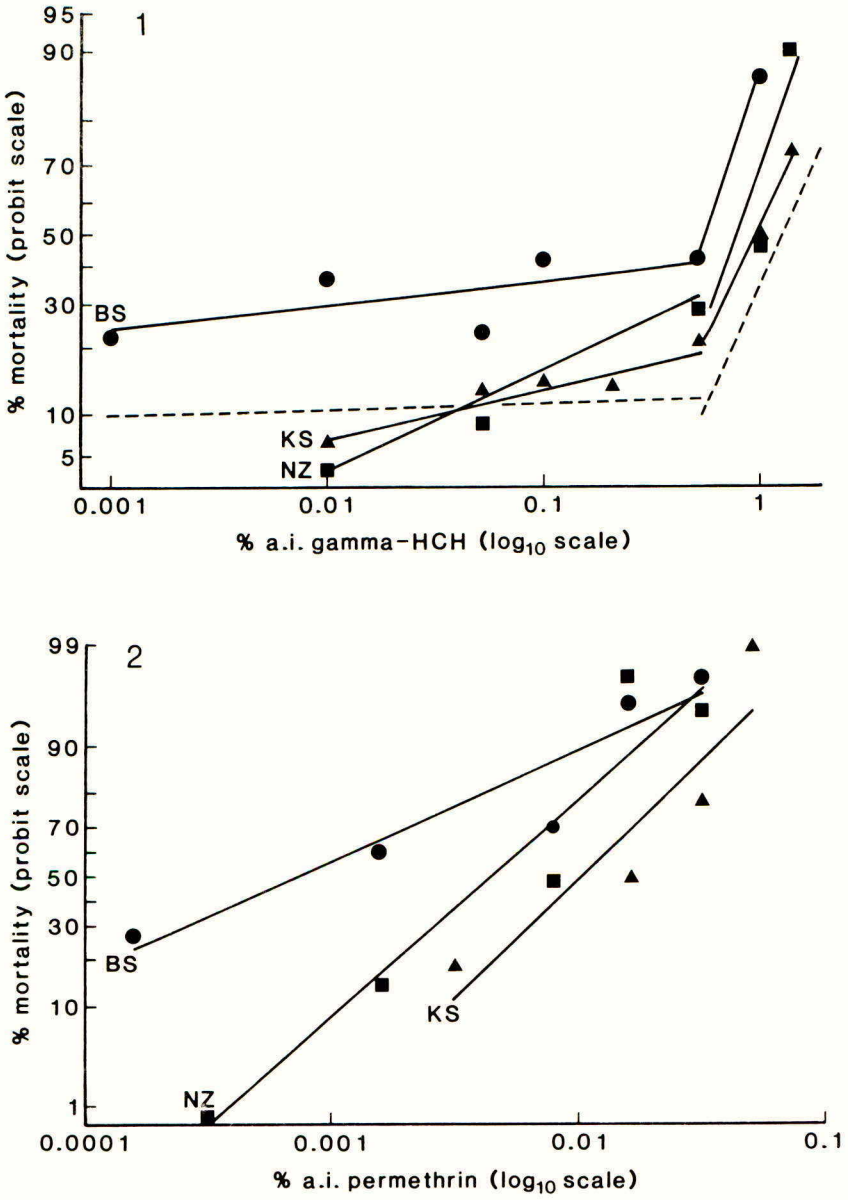
Resistance selection of British Encarsia involved spraying up to 400 scales as above with 1% a.i. gamma-HCH, which initially caused 70% pupal mortality, and allowing the survivors to parasitise whitefly scales for 48h. Selection was repeated for nine consecutive generations and then discontinued for a further three. The susceptibility to gamma-HCH of each generation was tested by applying a range of concentrations and constructing a mortality response curve.

RESULTS

Insecticide susceptibility

British(BS) Encarsia were more susceptible to gamma-HCH than both Dutch(KS) and New Zealand(NZ) which showed similar susceptibilities. In all three there was a small increase in mortality at concentrations below about 0.5% a.i. but a large increase above 0.5% a.i. Therefore separate probit-$\log_{10}$ regression lines were fitted above and below this concentration (Fig. 1). Analysis of the intercept values showed that the position of the shallow BS line is significantly different from those for KS and NZ but that there are no significant differences between the positions of the three steeper lines, although the BS line is above the other two. The BS LC25 was x538 and x249 lower than for KS and NZ respectictively but the LC50 was less than x2 those for KS and NZ.

When treated with permethrin all three stocks exhibited a continuous increase in mortality with concentration at a rate similar to that of the steep responses to gamma-HCH (Fig. 2). Although BS appeared more susceptible than KS and NZ the only significant difference was between BS and KS.



Figs 1 & 2. Initial susceptibility of British(BS), Dutch(KS) and New Zealand(NZ) Encarsia to (1) gamma-HCH and (2) permethrin applied to pupae, and susceptibility of BS Encarsia to gamma-HCH after selection with 1% a.i. for nine consecutive generations (- - -) (data points omitted for clarity). Mortality recorded as individuals dying before the 48 h adult stage.

Resistance selection

Selection of BS Encarsia with 1% a.i. gamma-HCH for nine consecutive generations increased resistance x2.5 at the selecting concentration, most of the increase occurring during the first three generations. The discontinuous shape of the mortality line remained after the final selection when the line was significantly different from that of the unselected stock but not significantly different from the unselected KS and NZ lines (Fig. 1). After selection the LC25 had increased x857, the LC50 x2 and the LC90 x6. After a further three generations without selection there was no change in the mortality-concentration line. There were no significant differences between parasitisation rates of selected and unselected individuals.

DISCUSSION

Pupae of all three Encarsia stocks were less susceptible to both gamma-HCH and permethrin than were adults tested by Ledieu(1979). In the case of KS pupae the LC50 for gamma-HCH was x36 and for permethrin x26 those for adults. These differences are probably due to protection of the Encarsia pupa by its host scale but may also result from use of different insecticide application methods and Encarsia strains.

The discontinuous mortality curves obtained for all stocks with gamma-HCH but not permethrin suggest a response related to penetration and/or mode of action of gamma-HCH. Below about 0.5% a.i. the host scale may absorb the chemical so that fumigant action is the only cause of mortality which varies little with concentration. Above 0.5% a.i. penetration of the host scale may occur to make direct contact with the Encarsia pupa thus causing a steep increase in mortality with concentration. The sharp increase in mortality in all stocks at the same concentration supports this hypothesis.

The significantly higher susceptibility of BS Encarsia to both chemicals may be due to breeding for many generations in a pesticide free environment providing no resistance selection pressure. This is in contrast to the resistant NZ stock which was established from a wild population almost certainly exposed to pesticides, which had arisen in the 1940s from survivors of glasshouse populations destroyed by pesticides. The slightly more resistant KS stock may have included individuals from populations which had been exposed to chemicals used to control secondary pests or adult whitefly (Koppert 1979). Hassan (1982) found Phytoseiulus persimilis from the commercial supplier Koppert which were resistant to the fungicide pyrazophos, and Schulten & van de Klashorst (1974) suggested that resistant P. persimilis may have arisen after accidental exposure to pesticides during the 1960s.

Resistance of BS Encarsia to gamma-HCH approached the KS resistance level within three generations and thereafter increased slowly and appeared to reach a plateau. Similarly rapid but small resistance increases occurred in the parasitoid Macrocentrus ancylivorus selected with DDT (Robertson 1957) and in the phytoseiid mite Amblyseius fallacis selected with azinphosmethyl (Strickler & Croft 1982). Rapid resistance development in Encarsia could indicate the existence of a resistance gene which quickly increases in the population because of the thelytokous parthenogenetic reproduction. Resistance levels possibly could have been increased further had higher concentrations of insecticide been used in successive generations to maintain the same high mortality level. Maintenance of

parasitisation rates in selected individuals is reassuring. However, selection for higher levels of resistance may cause reduced control performance and this would require careful testing before using resistant parasitoids.

The gamma-HCH resistance mechanism in BS Encarsia was not investigated but it may involve mixed-function oxidases as in many organochlorine resistant insects, which are believed to be fully active only during feeding stages (Wilkinson 1983). If this is the case then resistance selection using pupae may be limited and high resistance would be more rapidly achieved by treating a feeding stage such as the adult.

Finally, it is worth considering whether the thelytokous parthenogenetic reproduction of Encarsia limits possibilities for generating high resistance levels because of limited genetic plasticity. Traditionally sexual reproduction is stated to confer more genetic variability than parthenogensis, whereas thelytokous parthenogenetic organisms in which meiosis may not take place, and variation is dependent on mutation, may be considered at an evolutionary dead-end. However, thelytokous parthenogenesis has the advantages that gene loss is minimal, new genetic combinations may become fixed and beneficial mutations are passed to all progeny, all of which will speed resistance establishment as demonstrated by insecticide resistant thelytokous whitefly (Wardlow et al. 1976). Therefore species such as E. formosa should not be prematurely dismissed as candidates suitable for selective breeding.

This study has shown that Encarsia stocks differ in their susceptibilities to gamma-HCH and permethrin even though they may have fairly recent common origins. It has also shown that resistance to gamma-HCH can be increased by selective breeding and it is suggested that higher levels of resistance may be achieved by increasing the selection pressure and possibly by treating a different developmental stage. As long as it is desirable to use Encarsia to control whitefly and necessary to use chemicals against other pests, the possibility of breeding pesticide resistant Encarsia, capable of giving effective control in the presence of chemicals, warrants further investigation.

ACKNOWLEDGEMENTS

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REFERENCES

Croft, B.A. (1983) Status and management of pyrethroid resistance in the predatory mite Amblyseius fallacis (Acarina: Phytoseiidae). Great Lakes Entomologist 16, 17-32.

Croft, B.A.; Brown, A.W.A. (1975) Responses of arthropod natural enemies to insecticides. Annual Review of Entomology 20, 285-335.

Croft, B.A.; Strickler, K. (1983) Natural enemy resistance to pesticides: Documentation, characterization, theory and application. In: Pest Resistance to Pesticides G.P. Georghiou and T. Saito (Eds), New York: Plenum, pp. 669-702.

Georghiou, G.P.; Mellon, R.B. (1983) Pesticide resistance in time and space. In: Pest Resistance to Pesticides G.P. Georghiou and T. Saito (Eds), New York: Plenum, pp. 1-46.

Hassan, S.A. (1982) Relative tolerance of three different strains of the predatory mite Phytoseiulus persimilis A.-H. (Acari, Phytoseiidae) to 11 pesticides used on glasshouse crops. Zeitschrift für angewandte Entomologie 93, 55-63.

Hoy, M.A. (1982) Aerial dispersal and field efficacy of a genetically improved strain of the spider mite predator Metaseiulus occidentalis. Entomologia experimentalis et applicata 32, 205-212

Koppert, P.C. (1979) Biological control in glasshouses in the Netherlands. Proceedings of the International Symposium of IOBC/WPRS on Integrated Control in Agriculture and Forestry p. 484.

Ledieu, M.S. (1970) Laboratory and glasshouse screening of pesticides for adverse effects on the parasite Encarsia formosa Gahan. Pesticide Science 10, 123-132

Robertson, J.G. (1957) Changes in resistance to DDT in Macrocentrus ancylivorus Rohw. (Hymenoptera: Braconidae). Canadian Journal of Zoology 35, 629-633.

Schulten, G.G.M.; van de Klashorst, G. (1974) Genetics of resistance to parathion and demeton-S-methyl in Phytoseiulus persimilis A.-H. (Acari: Phytoseiidae). Proceedings of the 4th International Congress of Acarology pp. 519-524

Strickler, K.; Croft, B.A. (1982) Selection for permethrin resistance in the predatory mite Amblyseius fallacis. Entomologia experimentalis et applicata 31, 339-345.

Wardlow, L.R.; Ludlam, A.B.; Bradley, L.F. (1976) Pesticide resistance in glasshouse whitefly (Trialeurodes vaporariorum West.). Pesticide Science 7, 320-324.

Wilkinson, C.F. (1983) Role of mixed-function oxidases in insecticide resistance. In: Pest Resistance to Pesticides G.P. Georghiou and T. Saito (Eds), New York: Plenum, pp. 175-206.

EFFICACY OF ALDICARB AGAINST SUSCEPTIBLE AND RESISTANT MYZUS PERSICAE

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ABSTRACT

An insecticide-susceptible and two insecticide-resistant clones of Myzus persicae were caged at intervals over a nine-week period on potted Chinese cabbage or sugar beet plants grown in compost alone, or in compost containing aldicarb at the highest field-equivalent rate. Treatment reduced aphid survival, settling and ability to transmit virus; the numbers which survived closely reflected resistance status but the numbers of survivors settled, and their ability to transmit, did not, suggesting a repellent effect of the treatment. Results are discussed in the context of aphid and virus control in sugar beet.

INTRODUCTION

Aldicarb is routinely applied at planting by many sugar-beet growers to protect against seedling pests and, especially since the epidemic years of the mid 1970s, aphid-borne virus diseases. The widespread use of aldicarb intended, at least in part, as an insurance treatment against beet yellows viruses, continues despite doubts as to its value and effectiveness (Heijbroek et al. 1979, Heathcote 1983, Jepson & Green 1983). Resistance to carbamate insecticides in the vector Myzus persicae is well-documented (Sawicki et al. 1978, Sawicki & Rice 1978, Devonshire & Moores 1982) but the extent to which it further restricts the usefulness of aldicarb has been little investigated (see Heathcote 1983). We therefore report laboratory experiments which tested the ability of aldicarb to kill susceptible and resistant M. persicae and to control their transmission of beet yellows virus (BYV).

MATERIALS AND METHODS

Aldicarb was tested against three aphid clones; clone US1L was carbamate-susceptible, clone 405D slightly carbamate-resistant and clone T1V moderately carbamate-resistant, and represented respectively S, R1 and R2 UK field variants (see Sawicki & Rice 1978). Young adult apterae were raised for testing as described previously (Sawicki et al. 1980) in mass culture either on Chinese cabbage (Brassica pekinensis) or sugar beet, depending on which of these plants was to be used in tests. Beet plants used in tests had been infected as seedlings with the semi-persistent BYV, using M. persicae as vector.

Aldicarb was applied, once only, as 10% a.i. granules ('Temik 10G', Union Carbide) mixed, in an amount approximately equivalent to the highest recommended field rate of 51g per 100m row, with the compost (Fison's 'Eff') in which test plants were grown. Young seedlings (4-6 leaf stage), transferred into 5-inch-diameter plastic pots containing either compost alone or the compost/aldicarb mixture and standing in saucers, were watered sparingly to minimise loss of chemical.

The efficacy of aldicarb was tested 3, 4, 5, 7 and 9 weeks after planting the seedlings in treated compost. In each test, a single, similar leaf of each of 9 untreated and 9 aldicarb-treated plants was

infested with aphids of each clone. Groups of 10 aphids were confined to the undersides of leaves in small clip-on cages for access periods of 24h or 4 days on Chinese cabbage, and 4h or 24h on beet. (Longer access to beet gave excessive control mortality). Surviving adults were counted after each access period, but surviving progeny only after 4-day access to Chinese cabbage. Beet tests included counting of survivors settled after 24h access, and also assessed ability to acquire BYV during 4h and 24h access; they were too short to provide data on nymph survival. Ability to acquire BYV was determined by transferring survivors at the ends of access periods, in threes (to give adequate transmission), to untreated beet seedlings and counting plants which subsequently developed symptoms. Aphids remaining on test plants were removed, and plants grown-on in the glasshouse for repeat testing.

RESULTS

Tests with Chinese cabbage

No aphids survived 4-day access to Chinese cabbage 3 or 4 weeks after aldicarb application, but thereafter progressively more aphids survived (fig. 1a), more surviving 24h access (fig.1b) than corresponding 4-day access. Protection against R2 aphids was of shortest duration, followed by R1 and S aphids. More R2 nymphs were alive after 4-day access to treated plants than R1 or S nymphs, especially after 5 and 7 weeks (fig. 1c). Mortality on untreated plants was slight.

Tests with sugar beet

Over the five tests, most R2 and about half of S and R1 aphids survived 24h access to treated beet (fig. 2a). Most aphids, irrespective of clone, survived 4h tests though, after 3 and 4 weeks in particular, survival was R2>R1>S (fig. 2b). All aphids survived on untreated beet. BYV-transmission data from 4h tests reveal only a marginal overall decrease due to the treatment (fig. 3b) but transmission during 24h tests was considerably depressed (fig. 3a). There was no consistent difference in transmission amongst clones. In 24h access periods proportionately fewer surviving aphids settled on treated than on untreated plants (fig. 4).

DISCUSSION

Aldicarb killed fewer resistant than susceptible aphids. Numbers surviving reflected resistance status and rose over the 9-week period. Kill was greater in longer tests and on the preferred host, Chinese cabbage, presumably because more-sustained feeding resulted in more ingestion of poison. Less-consistent feeding may also have accounted for the greater variability in results of beet tests. The larger number of nymphs apparently produced on Chinese cabbage by more-resistant aphids probably reflected enhanced nymphal and parental survival rather than increased larviposition, though this is induced by some other insecticides (Rice, unpublished results).

It is recognised that aldicarb does not act fast enough to control BYV-introduction into a crop, but rather restricts secondary spread by reducing the numbers of aphids moving within it (Heathcote 1983, Jepson & Green 1983). In our tests, aldicarb reduced aphid numbers differentially, dependent on resistance status, and would presumably have concomitant differential effect on restriction of virus spread. However, frequency of transmission was also reduced amongst survivors, to an extent which did not clearly correspond to resistance status. In fact, after 24h access,

Figure 1 Aphid survival on untreated and aldicarb-treated Chinese cabbage

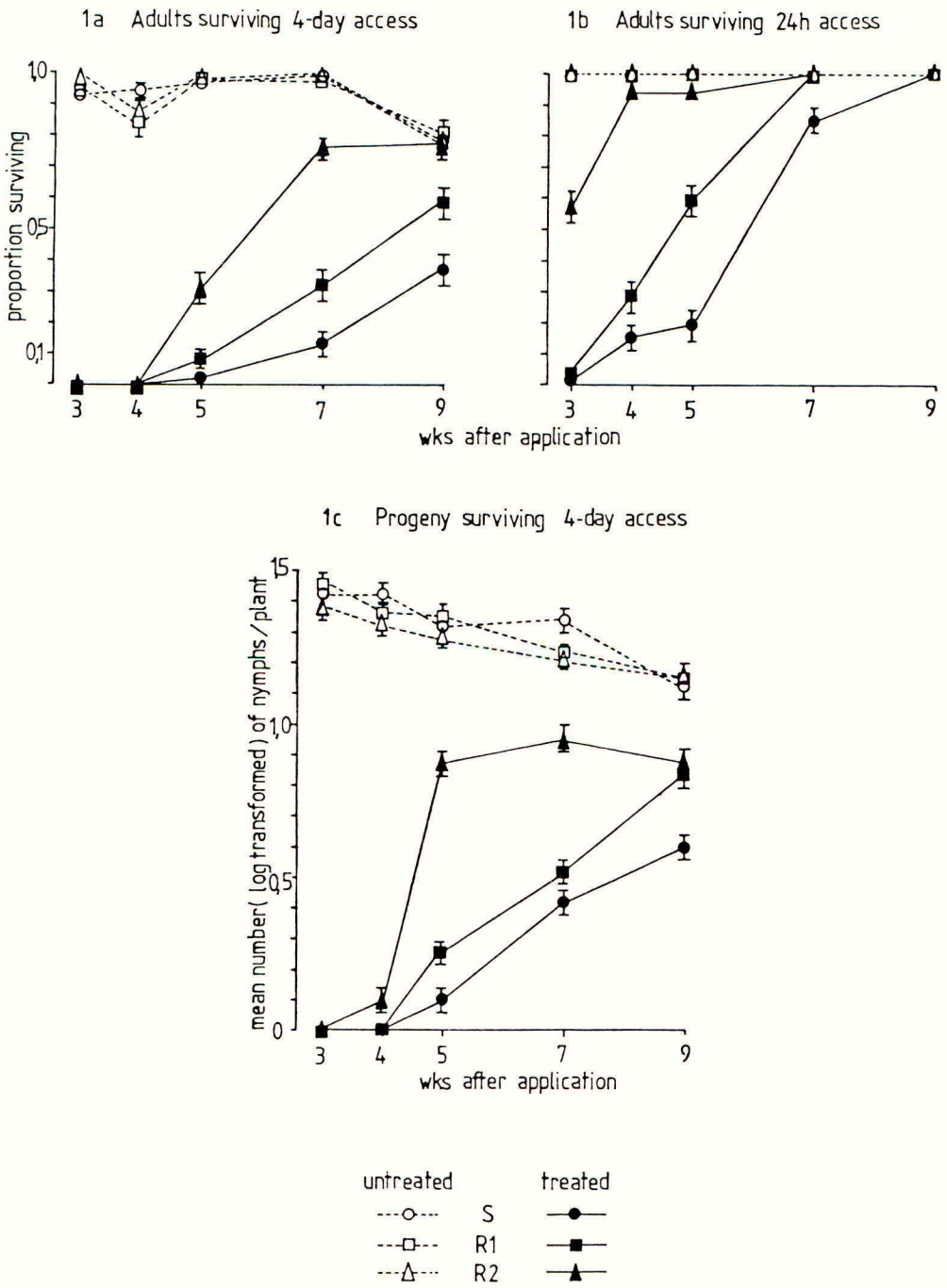


Figure 2 Aphid survival on untreated and aldicarb-treated sugar beet

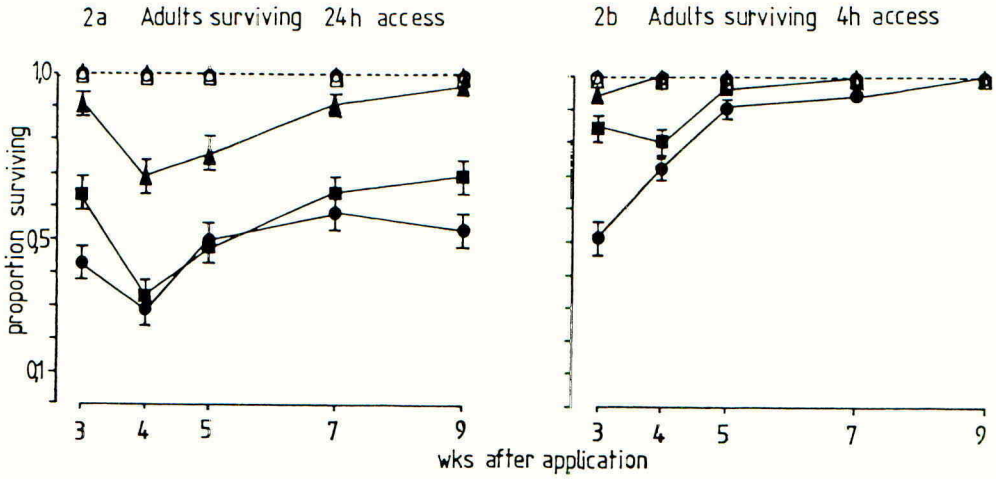


Figure 3 BYV-acquisition from untreated and aldicarb-treated sugar beet

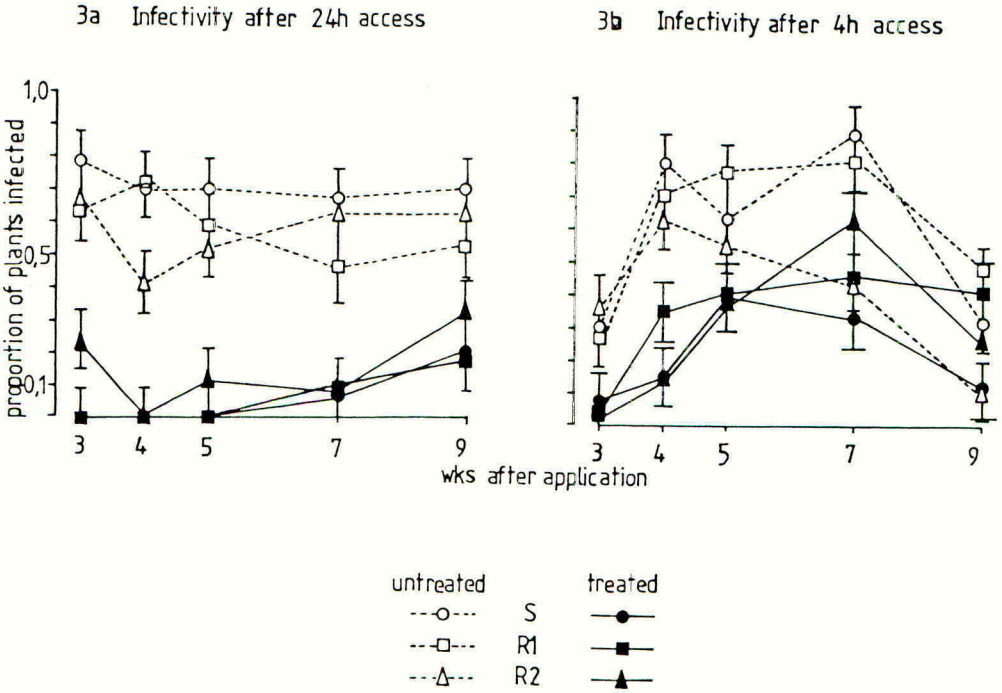
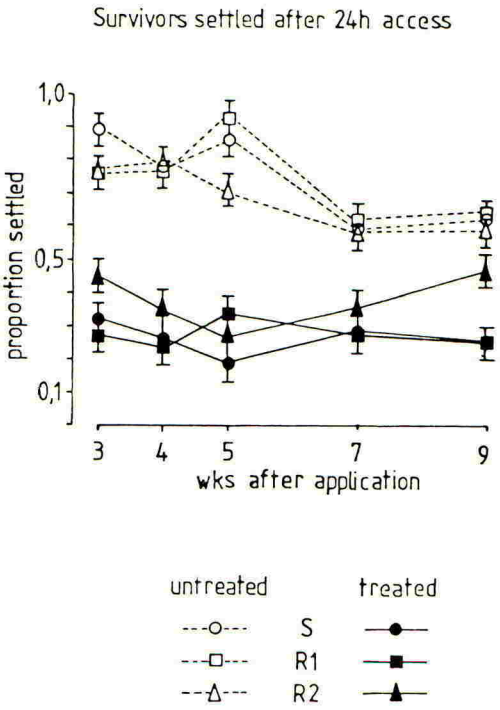


Figure 4 Aphid settling on untreated and aldicarb-treated sugar beet



BYV-transmission by all three clones was similarly depressed over most of the 9-week period. This could have been due to delayed toxicity resulting in death of survivors before they had infected seedlings, or, since transmission was not differentially reduced in susceptible and resistant clones, to decreased acquisition resulting from poor settling on test plants. Such an unsettling effect may be responsible for more-even distribution of aphids amongst aldicarb-treated plants (Heijbroek et al. 1979) and increased non-persistent virus transmission in aldicarb-treated sweet corn (Ferro et al. 1980). Aldicarb induces aphids to secrete alarm pheromone (Heathcote 1983) which causes restlessness of populations on insecticide-treated beet (Rice et al. 1983) but may have other unknown repellent effects on the individual. The better survival and ability of resistant aphids to colonise treated plants seems likely to detract from the ability of aldicarb to prevent BYV-spread for long into the season, and so casts further doubt on its usefulness as an insurance treatment (see Introduction), though worthwhile control of the less-readily transmitted beet mild yellowing virus might still be achieved. However the position is not clear, since the ability of aldicarb to restrict transmission by aphids which survived limited access to treated plants was not deleteriously affected by resistance. Sub-lethal, behaviour-modifying properties of aldicarb may therefore prove to be of considerable importance in determining its effectiveness in virus control.

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REFERENCES

Devonshire, A.L.; Moores, G.D. (1982) A carboxylesterase with broad substrate specificity causes organophorphorus, carbamate and pyrethroid resistance in peach-potato aphids (Myzus persicae). Pesticide Biochemistry and Physiology 18, 235-246.

Ferro, D.N.; Mackenzie, J.D.; Margolies, D.C. (1980) Effect of a mineral oil and a systemic insecticide on field spread of aphid-borne maize dwarf mosaic virus in sweet corn. Journal of Economic Entomology 73 (5), 730-735.

Heathcote, G.D. (1983) Aphicidal persistence of aldicarb and possibilities for forecasting the need for its use before sowing surgar-beet crops. Aspects of Applied Biology 2, 1983. Pests, diseases, weeds and weed beet in sugar beet, 19-27.

Heijbroek, W.; Jorritsma, J.; Hazen, P.J.; Kerstens, M.; Withagen, L. (1979) The application of aldicarb granules in sugar beet growing practice and its profitability. Institut voor Rationele Suikerproductie, Mededeling 4, 1-12.

Jepson, P.C.; Green, R.E. (1983) Prospects for improving control strategies for sugar-beet pests in England. In: Advances in Applied Biology, 7, T.H. Coaker (Ed.), Academic Press, 175-250.

Rice, A.D.; Gibson, R.W.; Stribley, M.F. (1983) Alarm pheromone secretion by insecticide-susceptible and -resistant Myzus persicae treated with demeton-S-methyl; aphid dispersal and transfer of plant viruses. Annals of Applied Biology 103, 375-381.

Sawicki, R.M.; Devonshire, A.L.; Payne, R.W.; Petzing, S.M. (1980) Stability of insecticide resistance in the peach-potato aphid, Myzus persicae (Sulz.). Pesticide Science 11, 33-42.

Sawicki, R.M.; Devonshire, A.L.; Rice, A.D.; Moores, G.D.; Petzing, S.M.; Cameron, A. (1978) The detection and distribution of organophosphorus and carbamate insecticide-resistant Myzus persicae (Sulz.) in Britain in 1976. Pesticide Science 9, 189-201.

Sawicki, R.M.; Rice, A.D. (1978) Response of susceptible and resistant peach-potato aphids Myzus persicae (Sulz.) to insecticides in leaf-dip bioassays. Pesticide Science 9, 513-516.

POTENTIAL USE OF DELTAMETHRIN AS A REPELLENT AND ANTIFEEDANT IN CONTROLLING PLUTELLA XYLOSTELLA

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ABSTRACT

Third instar larvae of a susceptible (S) strain and a deltamethrin-resistant (R) strain of Plutella xylostella were shown to move away from leaves treated with various concentrations of deltamethrin (e.c.) in water. The number of larvae that left the leaves increased with time and deltamethrin concentrations. The repellent effect on both S and R strains was very similar, but the mortality was higher in the S larvae. Tissue of leaves treated with the pyrethroid was consumed more than that of untreated leaves by both strains. The higher the dosage, the greater was the feeding inhibition. The potential of deltamethrin as a repellent and antifeedant against the resistant insect is discussed.

INTRODUCTION

The photostable synthetic pyrethroids have recently gained considerable importance as agricultural insecticides owing to their high insecticidal activity, low mammalian toxicity and stability in the field (Elliott 1977). They are particularly effective against lepidopterous pests (Elliott et al. 1978, Ruscoe 1979). In addition to having a direct lethal action, pyrethroids may possess other properties such as repellency and feeding inhibition, which are an added advantage in crop protection. Repellent effects have been reported for deltamethrin on spider mites (Aerts 1978) and on aphids (Rice et al. 1983), while sublethal doses of permethrin and cypermethrin have an antifeeding effect on lepidopterous larvae (Ruscoe 1977, Tan 1981).

This paper examines the potential use of the repellent and antifeeding action of deltamethrin to control the larvae of the diamond-back moth, Plutella xylostella L., a serious cruciferous pest, which has developed resistance to a wide range of insecticides (Sudderuddin & Kok 1978).

MATERIALS AND METHODS

Two strains of P. xylostella, a susceptible (S) strain obtained from a culture in Kuala Lumpur, Malaysia, and a deltamethrin-resistant (R) strain collected from vegetable farms in Singapore, were reared in the insectaries (Ho et al. 1983).

Brassica chinensis var. chinensis was grown in the greenhouse. The experiments were carried out at 26 ± 2°C and uncontrolled humidity (r.h. about 80%). Various concentrations of deltamethrin in distilled water were prepared from Decis (1.25% a.i. deltamethrin, e.c.). Leaves of B. chinensis of approximately the same size (weighing 3-5 g) were immersed in the appropriate deltamethrin concentrations for 30 seconds. The water was then allowed to evaporate from the leaves, and 20 third instar larvae were placed on each leaf in a plastic bowl. Three replicates were carried out for each concentration. Observations were made 2 hours and 24 hours later. The number of larvae that had wandered off each leaf was recorded and mortality was noted after 24 hours. The amount of leaf tissue consumed by the larvae was assessed by measuring the difference between the area of the leaf before and after the experiment.

RESULTS

Table 1 shows that deltamethrin at all dosages tested exhibited repellent effects on both S and R strains. For the S strain deltamethrin concentrations of as low as 10^{-5}% caused the larvae to wander off the leaves 2 hours after treatment. As time progressed more larvae left the leaves. In contrast, the larvae in the controls were all feeding on the leaves even 24 hours later. The R strain larvae behaved similarly, wandering off the treated leaves but stayed on the untreated ones. In general, the repellent action increased with dosage and time.

TABLE 1

Repellent effect of deltamethrin on larvae of P. xylostella

Strain	Deltamethrin concentration (% a.i.)	% larvae leaving leaves (Mean ± S.E.) 2 h	24 h	24 h % mortality (Mean ± S.E.)
S	0	0	0	0
	10^{-5}	7.5 ± 2.8	10.0 ± 3.9	1.2 ± 1.1
	10^{-4}	5.0 ± 5.0	20.0 ± 2.9	15.0 ± 9.2
	10^{-3}	6.0 ± 3.8	25.0 ± 2.9	42.5 ± 16.2
R	0	0	0	0
	10^{-4}	5.0 ± 2.9	16.7 ± 5.4	0
	10^{-3}	6.7 ± 3.6	28.3 ± 4.9	1.7 ± 1.4
	10^{-2}	5.0 ± 0.0	33.3 ± 5.9	8.7 ± 3.8
	10^{-1}	37.5 ± 1.8	37.5 ± 5.3	18.5 ± 1.8

A comparison of the mortality of the two strains confirmed that the R larvae were more resistant than the S larvae. Therefore different concentrations of the pyrethroid were used for the two strains, and a direct comparison of the dosage-response between the two strains is not possible. However, at 10^{-4} % and 10^{-3}%, deltamethrin appeared to repel the larvae of both strains to the same degree, there being no significant difference ($p > 0.005$)in repellency between the two strains at each of the two concentrations. When leaves were treated with a relatively high dosage, 10^{-1}%, of deltamethrin, a large proportion of the R larvae moved away within 2 hours, but no further movement occurred after this period. The larvae that remained on the leaves were inactive and fed very little. Leaf area measurements showed that the larvae fed less on the treated leaf tissue than they did on the controls (Fig. 1). The amount of feeding decreased with increasing dosages. A high level of feeding inhibition (c. 89%) was achieved at 10^{-3}% and 10^{-1}% deltamethrin for the S and R strains respectively.

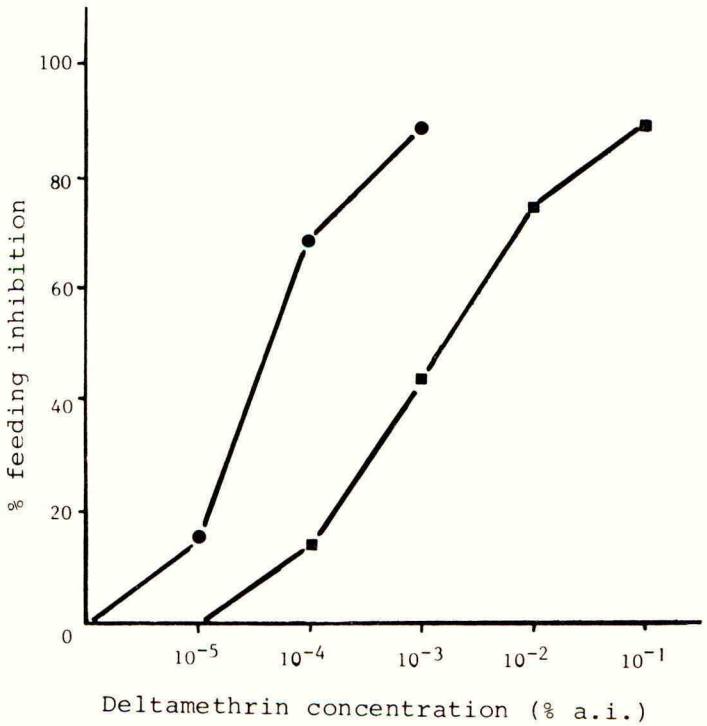

Fig. 1. Antifeeding action of deltamethrin on P. xylostella larvae (● S strain, ■ R strain).

DISCUSSION

The results of this investigation demonstrated that deltamethrin has repellent as well as antifeeding properties. These two properties are likely to be related to the action of pyrethroids on the insect's nervous system. Pyrethrins have been shown to cause excitation followed by block of nerve conduction in arthropods (Lowenstein 1942, Welsh & Gordon 1947, Camougis and Davis 1971). Synthetic pyrethroids also act on arthropod nerve tissues (Negherbon 1959, Narahashi 1962a, 1962b, 1971). Hyperexcitation, being an early symptom of pyrethroid poisoning in insects (Wouters & van den Bercken 1978), probably caused the larvae to move away from the irritant at lower dosages. However, at higher dosages of the insecticide, the blocking action led to paralysis of the larvae. Feeding inhibition is probably due to a direct action of the pyrethroid on the sensory nervous system (Ruscoe 1977), causing the mouthparts to be paralysed.

In an earlier study, high resistance to deltamethrin was detected in the fourth instar larvae of the R strain of P. xylostella (Ho et al. 1983). In view of this, even a large dosage of 10^{-1}% deltamethrin only killed 18% of the larvae (Table 1). However, almost 90% feeding inhibition was achieved by the same dosage. This would mean a considerable reduction in the yield loss of Brassica crops.

The full potential of deltamethrin as an agricultural chemical would not be realised if its properties other than a direct lethal action on insects were not exploited. The repellent and antifeeding action may prove valuable in controlling resistant strains of P. xylostella. Even though the larvae may survive the insecticide application (as they often do), they could be prevented from feeding and would eventually die of starvation. However, further work needs to be carried out to establish the effectiveness of deltamethrin as a repellent and antifeedant in the field.

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REFERENCES

Aerts, J. (1978). Repellent-werking van decamethrine tegen kasspint bij tomaten. Mededelingen van de Facultelt Landbouwwetenschappen, Rijksuniversitelt Gent 43, 655-660.

Camougis, G; Davis, W.M. (1971). A comparative study of the

neuropharmacological basis of action of pyrethrins. Pyrethrum Post 11, 7-14.

Elliott, M. (1977). Synthetic pyrethroids. In:Synthetic pyrethroids. ACS Symposium Series No.42, American Chemical Society, Washington, D.C., 229 PP.

Elliott, M.; Janes, N.F.; Potter, C. (1978). The future of pyrethroids in insect control. Annual Review of Entomology 23, 243-269.

Ho, S.H.; Lee, B.H.; See, D. (1983). Toxicity of deltamethrin and cypermethrin to the larvae of the diamond-back moth, Plutella xylostella L. Toxicology Letters 19, 127-131.

Lowenstein, O. (1942). A method of physiological assay of pyrethrum extracts. Nature,London 150, 760-762.

Narahashi, T. (1962a). Effectof the insecticide allethrin on membrane potentials of cockroach giant axons. Journal of Cellular and Comparative Physiology 59, 61-65.

Narahashi, T. (1962b). Nature of the negative after-potential increased by the insecticide allethrin in cokroach giant axons. Journal of Cellular and Comparative Physiology 59, 67-76.

Narahashi, T. (1971). Mode of action of pyrethroids. Bulletin of the World Health Organisation 44, 337-345.

Negherbon, W.O. (1959). Handbook of toxicology, Volume III: Insecticides. W.B. Saunders Co., London, 854pp.

Rice, A.D.; Gibson, R.W.; Stribley, M.F. (1983). Effects of deltamethrin on walking, flight and potato virus Y-transmission by pyrethroid-resistant Myzus persicae. Annals of Applied Biology 102, 229-236.

Ruscoe, C.N.E. (1977). The new NRDC pyrethroids as agricultural insecticides. Pesticide Science 8, 236-242.

Ruscoe, C.N.E. (1979). The impact of the photostable pyrethroids as agricultural insecticides. Proceedings 1977 British Crop Protection Conference - Pests and Diseases 3, 803-814.

Sudderuddin, K.I. ; Kok, P.F. (1978). Insecticide resistance in Plutella xylostella collected from the Cameron Highlands of Malaysia. FAO Plant Protection Bulletin 26, 53-57.

Tan, K.H. (1981). Antifeeding effect of cypermethrin and permethrinat sublethal levels against Pieris brassicae larvae. Pesticide Science 12, 619-626.

Welsh, J.H.; Gordon, H.T. (1947). The mode of action of certain insecticides on the arthropod nerve axon. Journal of

Cellular and Comparative Physiology 30, 147-171.

Wouters, W.; Van den Bercken, J. (1978). Action of pyrethroids. General Pharmacology 9, 387-398.

A SIMPLE TEST KIT FOR FIELD EVALUATION OF THE SUSCEPTIBILITY OF INSECT PESTS TO INSECTICIDES

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ABSTRACT

A dip test kit has been developed for field monitoring of the susceptibility of various insect pests to a range of commercial insecticides in areas where laboratory facilities are not readily available. The test method which is simple yet robust gives results 24 hours after treating field collected insects and can be adapted to generate either LC_{50} values for local populations or to test individual insects with a discriminating dose.

INTRODUCTION

Discussions on the susceptibility of field populations of insect pests to insecticide treatment almost invariably founder on the paucity of hard data. This lack totally undermines further discussions on optimal strategies for maintaining susceptibility, since debate is forced back upon a reiteration of first principles based upon pragmatism rather than upon experimental evidence. While LD_{50} determinations may be carried out on laboratory colonies of field strains which have been pressurised with insecticides, the relationship between the results of such studies and commercial insecticide use has yet to be established. An additional disadvantage is that such detailed work can clearly only be carried out where extensive laboratory facilities are available together with highly skilled staff. However, even when both are available, experience has shown that the establishment and maintenance of colonies of field strains of important pests such as Heliothis, Spodoptera and Plutella can be time consuming and costly. Even more importantly, comparisons between "field strains" and long established laboratory "susceptible" strains can be positively misleading, since they lead to the generation of spurious, or at best artificially inflated, resistance factors.

MATERIALS AND METHODS

In an effort to overcome such deficiencies and to facilitate generation of reliable field data a simple test kit was developed and evaluated against a series of prerequisites. Firstly, results had to be obtainable within a day of treating test insects. Secondly that the procedure should use simple, locally available equipment and not depend upon complicated techniques such as topical application. Thirdly that the test could be carried out by relatively unskilled staff where laboratory facilities were either minimal or nonexistent. Finally that all parts of the kit that came into contact with insecticides (especially pyrethroids) should be disposable to eliminate the very real problem of accidental contamination. A check list of the contents of the field test kit is as follows:

Petri dishes (large - 900 mm and small - 50 mm) for holding test larvae
Filter papers for same (large and small)
Vials for testing larvae
Nylon mesh to strain larvae from test solutions
Wide glass tube to support nylon mesh
Scissors
Paint brushes (fine and coarse) to handle larvae
Plastic trays to sort larvae
Autopipettes (two, 5-50 ul, 50-200 ul)
Graduated glass pipettes (1 ml and 10 ml)
Plastic bags (large and small) to collect and dispose of larvae
Elastic bands (large and small)
Plastic vials for preserving larvae
Sandwich boxes with cotton wool to pack vials
Insect fixer/preservative
Hand lens
Self adhesive labels
Tissues

In practice, the dip test technique has proved applicable to a range of insects such as beetles, caterpillars and aphids provided that care is taken to avoid reducing the vigour of any test insects through stress and that strict precautions are followed to avoid chemical contamination of non-disposable items.

Insects for testing can be collected in the field and readily transported or even held overnight in polythene bags containing appropriate foliage. They are then sorted into groups of ten selected on the basis of uniformity of size for any given test and placed in 50 mm plastic Petri dishes. These also contain filter papers on which details of the insecticide and concentration to be used can be recorded. It should be borne in mind that field collected larvae are often far more aggressive than laboratory strains so that lower densities of, say, five larvae per Petri dish may be preferable in order to avoid complications caused by fighting or cannibalism during the holding period.

Test solutions are made up over a concentration range of about 1000x using serial dilutions of 1/3rd, i.e. 0.01%, 0.003%, 0.001% ..... 0.00001% in volumes of 10 ml. Autopipettes with disposable tips have proved the most effective method of measuring small volumes while the disposable tips eliminate contamination problems. The glass vials used for dipping are labelled beforehand with the respective concentrations to be used. These are then used strictly according to the labels and subsequently washed in acetone. To reduce contamination, vials are only reused for the same toxicant at the same or a higher concentration in subsequent tests.

Groups of insects are transferred into glass vials containing the test solutions which are gently shaken for 30 seconds to ensure thorough wetting before draining through the Nylon mesh. The mesh is then laid on tissues to absorb surplus solution from test insects before their transfer to the labelled holding dishes which are then securely fastened with elastic bands. For any given insecticide, lowest concentrations are used first so that the same Nylon mesh can be used for the entire series before

being discarded. Treated insects, which are not fed during the 24 hour holding period, can be readily transported provided that extremes of temperature are avoided, although uniform holding conditions are preferred.

Assessments of mortality, which are usually made after 24 hours, can cause problems for inexperienced operators unless the criteria used to distinguish between dead and living larvae are rigorously and consistently applied. The inability of larvae to respond to a stimulus from a sharpened pencil has proved successful especially when the behaviour of untreated control insects is referred to for comparison.

As an aid to obtaining definitive data from field tests it is essential that samples of all insects should be retained for identification. These should be treated with fixative before being preserved in 70% ethanol. Polyethylene scintillation vials are particularly useful in this respect being both shatter proof and solvent resistant. Full data (i.e. date, location, crop) should be recorded at the time of collection in soft pencil on good quality paper and included with the insects in the vial.

RESULTS

When devising the test kit several important sources of variation were anticipated and investigated experimentally. These included the stability of dilutions of commercial formulations at concentrations far below those normally employed and their ability to wet test insects together with the effect of variation in dipping time on mortality.

The dilution problem was investigated by preparing solutions in three different ways:

A : serial dilution of commercial EC formulations with tap water.

B : serial dilution of commercial EC formulation with water containing an EC blank (i.e. no insecticide) to ensure constant levels of wetter and other adjuvants at each dilution

C : serial dilution of commercial EC with EC blank before dilution with tap water.

Results obtained with fenvalerate are given in Table 1.

These results indicated that mortality could indeed be affected by the method used to prepare solutions. However, it was noted that the simplest and most convenient approach, i.e. serial dilution of commercial formulations with water (A) consistently gave the lowest LC_{50} values and this method has consequently been adopted. To date no problems have arisen with a wide range of commercially available insecticide formulations including EC's, WP's and suspension concentrates (SC's) which have been used in this manner.

A similar approach was used to investigate the effect of varying immersion time. The results given in Table 2 show that this has surprisingly little influence on the final LC_{50} value so that the recommended time of 30 seconds was chosen largely on the basis of convenience.

TABLE 1

Dose/mortality relationships with fenvalerate against Plutella xylostella using solutions made up according to three different procedures

	% Mortality* of larvae after treatment		
Concentration, % a.i.	A	B	C
1×10^{-3}	100.0	87.5	90.0
3×10^{-4}	97.5	57.5	85.0
1×10^{-4}	92.5	10.0	40.0
3×10^{-5}	65.0	5.0	12.5
1×10^{-5}	22.5	0.0	5.0
3×10^{-6}	0.0	0.0	2.5
1×10^{-6}	5.0	0.0	0.0
3×10^{-7}	2.5	0.0	0.0
LC_{50}	2×10^{-5}	3×10^{-4}	1×10^{-4}

*Each mortality assessment is the mean for four separate results using 10 third instar larvae per concentration

TABLE 2

Effect of larval dipping time on the dose/mortality* relationships of fenvalerate against Plutella

	% mortality* of larvae after the following dipping times (secs):				
Concentration, % a.i.	10	20	30	45	60
3×10^{-4}	78	88	90	75	70
1×10^{-4}	65	68	68	50	48
3×10^{-5}	13	30	30	18	25
1×10^{-5}	7	2	5	15	2
3×10^{-6}	5	0	0	5	2
Average LC_{50} % a.i.	8×10^{-5}	8×10^{-5}	7×10^{-5}	7×10^{-5}	1×10^{-4}

*Each mortality assessment is the mean for four separate results using 10 third instar larvae per concentration

Some results obtained with other economically important caterpillar species and also a far more delicate insect, the black bean aphid (Aphis fabae), are given in Table 3.

TABLE 3

LC_{50} determination with fenvalerate against a range of species using the test kit

Concentration, % a.i.	% Mortality* with different species			
	Trichoplusia ni	Spodoptera littoralis	Heliothis virescens	Aphis fabae
1×10^{-2}	-	100	100	100
3×10^{-3}	-	100	95	100
1×10^{-3}	100	100	90	100
3×10^{-4}	100	40	77	90
1×10^{-4}	95	10	33	70
3×10^{-5}	100	0	10	50
1×10^{-5}	73	0	0	20
3×10^{-6}	37	0	-	20
1×10^{-6}	40	0	-	20
3×10^{-7}	10	0	-	0
1×10^{-7}	20	-	-	-
3×10^{-8}	10	-	-	-
LC_{50} % a.i.	1.5×10^{-6}	2.5×10^{-4}	2×10^{-4}	2×10^{-5}

*Each mortality assessment is the mean of 3 separate results using 10 third instar larvae or adult aphids per concentration

The outcome with this aphid was perhaps not surprising in view of the successful use of this technique with Myzus persicae by Sawicki et al (1978) but does indicate that this technique is by no means restricted to robust insects such as caterpillars and beetles.

The result with Trichoplusia ni is very similar to that obtained using a field strain in El Salvador in 1978 when a commercial aerial application of fenvalerate (at 0.03% active ingredient in 26.5 l/ha) had failed to give acceptable control and resistance was alleged. Further investigation showed that the grower had doubled the recommended plant population per hectare so that conventional sprays were not penetrating the dense canopy sufficiently to control Trichoplusia although control of Heliothis was maintained. On neighbouring fields with conventional plant spacings control of both pests remained satisfactory (J.P. Fisher pers. comm.).

DISCUSSION

Extensive field evaluation has shown that the test kit offers a relatively robust method of generating field data on the susceptibility of pest insect populations. Results can be used to obtain a rapid estimate of the LC_{50} value for a local population using probit paper or a more detailed analysis can be carried out, using a computer programme, to provide LC_{50} values together with confidence limits and estimates of the slope of the probit line (a measure of the spread of tolerances of the population) and also Chi-square values (an estimate of the fit of the data to the probit line). These latter statistics enable weighting to be placed on various data sets when considering seasonal or geographical changes in susceptibility.

An alternative approach to LC_{50} determinations is the use of dose mortality relationships to fix a discriminating dose (based upon the lowest concentration that consistently causes complete mortality) which can be used to test for changes in susceptibility in a given crop in a given locality as successive insecticide applications are made. Where a dip test has been evaluated in parallel with time consuming biochemical methods it has proved almost as effective at distinguishing between resistant and susceptible aphids (Sawicki *et al.*, *loc. cit.*) and offers the advantage of use in areas where biochemical methods would be out of the question.

REFERENCE

Sawicki, R.M., Devonshire, A.L., Rice, A.D., Moores, G.D., Petzing, S.M. and Cameron, D. The detection and distribution of organophosphorus and carbamate insecticide-resistant *Myzus persicae* (Sulz.) in Britain in 1976. *Pestic. Sci.* 1978, *9*, 189-201.

QUANTITATIVE EVALUATION OF STRATEGIES TO DELAY FUNGICIDE RESISTANCE

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ABSTRACT

In the last fifteen years significant changes in the fungicide sensitivity of plant pathogen populations have led to crop damage and the discontinuation or substantial modification in the use of important fungicides. Circumstantial evidence suggests that the use of fungicides alternately or in mixtures can delay the appearance of resistance. Several mathematical models, devised to describe the events that lead to resistance outbreaks, also predict that alternation or mixtures increase the time necessary for resistance outbreaks to occur. These models can be used to provide quantitative estimates of the delay. Direct experimental evidence on the effects of alternation and mixtures is relatively thin; in the majority of cases it has not provided evidence of any delay. A possible explanation for the discrepancy between circumstantial evidence and theory on the one hand and direct experimental evidence on the other lies in the low sensitivity of experimental techniques that have been used. It is suggested that further research work involving inherently slow pathogens with a low starting frequency of resistant strains would have a better chance to resolve the issue. Experimental designs that would minimise cross contamination should be used and results should be assessed when overall disease severity is still low.

INTRODUCTION

In the last fifteen years substantial changes in the population of several major plant pathogens in terms of their sensitivity to agricultural fungicides have been observed. These changes have led frequently to significant crop damage, and the discontinuation or substantial modification in the use of important products.

There seems to be general agreement that resistance problems are caused by subpopulations of resistant mutants that exist at some low but finite frequency before the exposure of the total population to a new fungicide (Dekker 1976, Georgopoulos 1977). Once the population is exposed to the action of the new fungicide, selection in favour of these resistant mutants starts to act so that their frequency may increase until eventually disease control may break down and the use of the new fungicide has to be abandoned or significantly modified.

It has been suggested (Edgington et al 1980) that the use of fungicides alternately or in mixtures can delay the appearance of resistance problems, and the circumstantial evidence that supports this claim has been relatively recently summarised (Delp 1980). Support to the use of these strategies has also been provided by the development of a number of mathematical models that have been devised in an attempt to describe the events that lead to resistance outbreaks. However, it has been recently suggested (Ogawa 1983) that mixtures should not be used to prevent or delay the development of resistance in Monilinia, and the overall

direct experimental evidence either in favour or against fungicide mixtures or alternation is very limited.

This paper will review the limited available experimental evidence, summarise the predictions of the mathematical models as to the effect of mixtures and alternation on the speed of resistance buildup, and attempt to define additional research that could improve our understanding of these phenomena.

REVIEW OF EXPERIMENTAL EVIDENCE

Available experimental evidence either in favour or against the predictions of the theoretical models is indeed very scarce. It can be meaningfully examined by pathogen and crop.

Cercospora beticola on sugar beets

Dovas et al (1976) attempted to quantify the effect of mixing and alternating benomyl and triphenyl tin hydroxide on the speed of buildup of benomyl resistance. They carried their study in the field starting with an artificial inoculation with mixed inoculum consisting of one resistant and one sensitive strain in a ratio 1:9. Both alternation and mixture provided better disease control than benomyl alone, with the mixture outperforming the alternation. The data clearly suggest a delaying effect of 40-60% in resistance buildup provided by the alternation. The mixture which was included in part only of the experiment failed to provide a clearcut delay. Finally, the occurence of resistant isolates in plots initially inoculated with the sensitive strain suggest movement of resistant inoculum in spite of the precautions taken.

Venturia inaequalis on apple

McGee and Zuck (1981) tested the effect of mixing and alternating benomyl and captan on the speed of benomyl resistance buildup. They carried their study in the glasshouse starting with an artificial inoculation using inoculum consisting of several resistant and sensitive strains in a ratio 1:9. They subjected this inoculum to five consecutive passages on apple seedlings preventively treated with the fungicides. Their data suggest that the mixture caused faster resistance buildup than benomyl alone and the alternation caused no measurable delay. Even more surprisingly benomyl alone caused a marginal only increase in the proportion of the resistant subpopulation.

Monilinia fructicola on nectarines

Sonoda et al (1983) compared the effect of benomyl alone and mixed with captan to the speed of resistance buildup to benomyl. They carried their study on a natural population at the end of the previous season. Under those conditions benomyl and benomyl plus captan provided equal and very good disease control in spite of the fact that at the end of the trial the observed frequency of resistant strains was 87% and 86% respectively. Captan alone totally failed to control the disease in this experiment. Substantial increase in the proportion of resistant strains observed in both the captan and the untreated plots suggest cross contamination.

Botrytis cinerea on grapes

Locher et al (1983) tested over several years in the field mixtures of vinclozolin and myclozolin with a number of protectant fungicides on naturally occurring populations of strains resistant and sensitive to the

dicarboximides. Among all the protectants tested in mixtures only chlorothalonil provided improved disease control and none of the mixtures affected the proportion of the resistant strains that was generally high.

Botrytis cinerea on strawberries

Hunter and Brent (1983) studied the effect of mixing and alternating procymidone and dichlofluanid. Their experiment was carried on tunnel grown strawberries infected by a naturally occurring mixed population with 23% resistant strains at the start of the experiment. Procymidone alone as well as in mixture and in alternation with dichlofluanid provided satisfactory disease control in spite of the fact that at the end of the experiment the proportion of resistant strains had increased to above 85%. There was no evidence to suggest that any delay in resistance buildup was provided by the mixture or the alternation.

Phytophthora infestans on potatoes

Staub and Sozzi (1983) studied the effect of mixing metalaxyl with mancozeb to the speed of resistance buildup to metalaxyl. They carried their study by creating an artificial epidemic in growth chambers started with mixed artificial inoculum with a ratio of resistant to sensitive sporangia $1x10^{-4}$. Their data clearly indicate that the mixture with mancozeb delayed resistance buildup. Graphically measured the proportion of resistant strains in the growth chamber treated with the mixture had reached at the end of the experiment, i.e. after 61 days, the same level as the one reached after 39 days in the metalaxyl treated growth chamber.

THE MODELS AND THEIR PREDICTIONS

The various models developed over the last few years have been recently summarised by Skylakakis (1982b). Since then, one more dynamic model has been presented by Levy et al (1983), in which the effects of the pathogen's apparent infection rate, fungicide weathering, coverage and degree of additivity as well as competition for the host's susceptible sites have first been expressed in a set of differential equations and then quantitatively evaluated through computer simulation by numeric integration of these equations.

The speed of resistance buildup

All dynamic models agree that fast (high apparent infection rate) pathogens will build resistance faster than slow ones. Quantitative evaluations of this effect of the pathogen's apparent infection rate have been summarised in Table 1.

Overall there is good agreement between two models starting from different premises. As already predicted (Skylakakis 1980, 1982b) the model (Levy et al 1983) allowing for the effects of competition between the two subpopulations provides estimates for slower resistance buildup. It is because this competition will occur earlier for fast pathogens that the greater discrepancy between the estimates of the two models is observed at the higher infection rate in Table 1.

There is a second area of agreement among all models (Delp 1980, Kable and Jeffery 1980, Skylakakis 1981, 1982b, Levy et al 1983). The higher the efficacy of the fungicide at risk, either inherently or because of its pattern of use, and the greater the degree of resistance to it, the faster the resistance buildup.

TABLE 1

The effect of apparent infection rate on the speed of resistance buildup measured by standard selection time

Apparent infection rate per day	Standard selection time days	
	Skylakakis 1982a	Levy et al 1983
0.4	3.9 - 5.8	8.6 - 11.6
0.2	6.4 - 11.5	10.3 - 12.7
0.05	22.7 - 25.6	32.3

Standard selection time (Skylakakis 1981) is the time necessary for the proportion of the resistant population to increase by e (2.7...) times.
Calculations after Levy et al approximated assuming linear change of ln(b/a) with time.

Delaying strategies and their effects

There is general agreement (Skylakakis 1982b, Levy et al 1983) that alternation and mixtures delay resistance. There are only two exceptions to this general rule; Kable and Jeffery (1980) predict that mixtures will provide no delay if fungicide coverage is complete, and Levy et al (1983) predict that a mixture may even increase the speed of resistance buildup if the second fungicide has a high degree of additive action against the sensitive subpopulation. Leaving aside the exceptions, a range of estimates for the delay caused by mixtures and alternation has been provided in Table 2.

The relative delay, expressed as percent of time increase for the resistant subpopulation to reach a given frequency under a defined fungicide regime, can be measured by the percent increase in standard selection time and ranges between 55% and 122% for mixtures, 35% and 48% for alternations. It has to be observed that the delay associated with alternations is related to the proportion of the total epidemic covered by applications of the fungicide at risk, and the delay will increase as this proportion decreases.

All models accept that in the case of alternation no increase in the proportion of resistant subpopulation takes place in the periods during which the disease is controlled by the second fungicide. It then follows that the efficacy of this second fungicide has no influence on the delay caused by alternation as long as that disease severity is kept at a level that makes competition between the two subpopulations insignificant. Should competition become a factor, then a less efficacious second fungicide will cause increased delay by allowing disease to develop faster and enhancing the delaying effect of competition.

In the case of mixtures increased efficacy of the second fungicide will also increase the delay at low disease severity. At higher levels of disease severity this effect can be neutralised or even reversed by the adverse influence of the second fungicide's efficacy to the delay provided by competition.

TABLE 2

The delaying effect of mixtures and alternations on the speed of resistance buildup measured by standard selection time

Apparent infection rate per day	Standard selection time days					
	after Skylakakis 1981			after Levy et al 1983		
	Systemic	Mixture	Altern.	Systemic	Mixture	Altern.
0.4	3.9	7.1	5.6	8.6	14.3	11.8
0.2	7.9	14.3	11.3	10.4	16.4	15.4
0.05	25.6	56.9	36.6	32.3	50.0	43.5

For calculations after Skylakakis 1981 the following assumptions have been used: Latent periods 5, 10 and 20 days corresponding to infection rates of 0.4, 0.2 and 0.05. Efficacy of alternative fungicide R/R' = 7. Alternation programme 70% of time systemic 30% of time protectant.
For calculations after Levy et al 1983 the following assumptions have been used: Initial proportion of resistant subpopulation 5×10^{-12}. Alternation programme 70% systemic, 30% protectant. Protectant fungicide has no additive effect in mixture with systemic. Calculation approximated assuming linear change of ln(b/a) with time.

DISCUSSION

Out of the six reports summarised in this paper, where mixtures have been tested, only one (Staub and Sozzi 1983) provides evidence for delay to resistance buildup provided by mixtures, one (Dovas et al 1976) is not conclusive as a mixture was used only in one part of the experiment and in that part the initial proportion of the resistant subpopulation fluctuated widely, three did not provide any evidence of delay, and in one (McGee and Zuck 1981) resistance developed faster in the presence of the mixture than in the presence of the fungicide at risk alone.

Out of the three reports, where alternations were tested, only one (Dovas et al 1976) provides evidence on the delay they cause, while the other two fail to support it.

Since all the models uniformly predict that some delay should occur yet in most experiments none is observed, there are two possible conclusions: either the models misrepresent reality or the techniques used in most cases were not sensitive enough to register the delay. The fact that in some experiments no delay is observed even if alternation is used, when, not only models, but also common sense would predict a delay, tends to suggest that, at least in some cases, it is the experimental techniques that are at fault.

There seem to be three main sources of reduction in sensitivity of these experimental techniques.

First the speed of the resistance buildup may make the delay difficult

to observe, especially when the starting proportion of the sensitive subpopulation is relatively high. This is well illustrated in Table 3.

TABLE 3

The effect of standard selection time on the percent of a fit resistant subpopulation during the course of an epidemic

Standard selection time days	% of resistant subpopulation Epidemic duration days		
	0	30	60
5	0.1	29.0	99.4
	5.0	95.5	≈100.0
7.5	0.1	5.2	75.1
	5.0	73.8	99.3
10	0.1	2.0	29.0
	5.0	50.9	95.5
15	0.1	0.7	5.2
	5.0	28.0	73.8

Although there is no published evidence of the observed variation when sampling is carried out to define the proportion of the resistant subpopulation, it is fair to assume that with a starting occurence of 5% for the resistant subpopulation no sampling at 60 days of epidemic could differentiate standard selection times varying between 5 and 10, while reasonable differentiation should be achieved at the same sampling time and range of standard selection times with a starting occurence of 0.1%. Interestinly enough the single experiment that clearly supports the delaying effect of mixtures (Staub and Sozzi 1983) had a starting occurence of $1x10^{-4}$, while most others had a starting occurence above 5%.

Cross contamination among plots would also tend to dilute the delaying effect of alternation and mixture. Obviously, the extent of this dilution will depend on the test organism's mobility and plot size. Indirect proof of cross contamination may be provided by those experiments where the resistant subpopulation increases even in plots not treated by the fungicide at risk. Again, interestingly enough, all the positive evidence in favour of mixture or alternation delay has come from experiments where cross contamination had been either totally eliminated (Staub and Sozzi 1983) or somewhat reduced (Dovas et al 1976).

Finally, alternations or mixtures tend to control the disease better than the systemic alone when a significant proportion of the population is resistant, and plots treated with these treatments tend to have significantly lower disease severity than plots treated with the fungicide at risk alone. In such cases the delaying effect of competition in plots of high disease severity may balance the delaying effect of the mixture or alternation in the plots of lower disease severity.

In conclusion, experimental evidence which supports and quantifies the delaying effect of mixtures and alternations is extremely thin.

The only two existing results, however, of 40%-60% delay through alternation provided by Dovas et al (1976), and 56% delay by a mixture provided by Staub and Sozzi (1983) are in good agreement with the predictions of the models. It can be suggested that further experiments would have a better chance to resolve the issue, if

- they start with a low level of resistant subpopulation, not above 0.1%,
- relatively slow (apparent infection rate below 0.2) pathogens are used as test organisms,
- cross contamination is avoided by large plots and/or less mobile pathogens,
- assessments are carried out at low disease severity (below 5%) to remove the effects of competition.

REFERENCES

Dekker, J. (1976) Acquired resistance to fungicides. Annual Review of Phytopathology 14, 405-428.

Delp, C.J. (1980) Coping with resistance to plant disease. Plant Disease 64, 652-657.

Dovas, C.; Skylakakis, G.; Georgopoulos, S.G. (1976) The adaptability of benomyl-resistant population of Cercospora beticola in northern Greece. Phytopathology 66, 1452-1456.

Edgington, L.V.; Martin, R.A.; Bruin, G.C.; Parsons, I.M. (1980) Systemic fungicides: A perspective after 10 years. Plant Disease 64, 19-23.

Georgopoulos, S.G. (1977) Development of fungal resistance to fungicides. In: Antifungal Compounds M.R. Siegel and H.D. Sisler (Eds.), New York, Marcel Dekker, Vol 2, pp 439-495.

Hunter, T.; Brent, K.J. (1983) Effects of different spray regimes on dicarboximide resistance in Botrytis cinerea on strawberries. Proceedings 10th International Congress of Plant Protection, 2, 631.

Kable, P.F.; Jeffery, H. (1980) Selection for tolerance in organisms exposed to sprays of biocide mixtures: A theoretical model. Phytopathology 70, 8-12.

Levy, Y.; Levi, R.; Cohen, Y. (1983) Buildup of a pathogen subpopulation resistant to a systemic fungicide under various control strategies: A flexible simulation model. Phytopathology 73, 1475-1480.

Locher, F.; Lorenz, K.J.; Beetz, K.J. (1983) Influence of vinclozolin mixtures on the development of resistance and on disease control in Botrytis cinerea Pers. of grapes. Proceedings 10th International Congress of Plant Protection, 2, 627-628.

McGee, D.C.; Zuck, M.G. (1981) Competition between benomyl-resistant and sensitive strains of Venturia inaequalis on apple seedlings. Phytopathology 71, 529-532.

Ogawa, J.M. (1983) Strategies for testing and management of fungicides for control of Monilinia in stone fruit crops. Proceedings 10th International Congress of Plant Protection, 2, 616-622.

Skylakakis, G. (1980) Estimating parasitic fitness of plant pathogenic fungi: A theoretical contribution. Phytopathology 70, 696-698.

Skylakakis, G. (1981) Effects of alternating and mixing pesticides on the buildup of fungal resistance. Phytopathology 71, 1119-1121.

Skylakakis, G. (1982a) Epidemiological factors affecting the rate of selection of biocide resistant plant pathogenic fungi. Phytopathology 72, 271-273.

Skylakakis, G. (1982b) The development and use of models describing outbreaks of resistance to fungicides. Crop Protection 1, 249-262.

Sonoda, R.M.; Ogawa, J.M.; Manji, B.T. (1983) Factors affecting control of blossom blight in a peach orchard with low-level benomyl-resistant Monilinia fructicola. Plant Disease 67, 681-684

Staub, T.; Sozzi, D. (1983) Recent practical experiences with fungicide resistance. Proceedings 10th International Congress of Plant Protection, 2, 591-598.

NEGATIVELY CORRELATED CROSS-RESISTANCE AND SYNERGISM AS STRATEGIES IN COPING WITH FUNGICIDE RESISTANCE

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ABSTRACT

Strategies to cope with fungicide resistance may include the use of chemicals to which fungicide-resistant populations display negatively correlated cross-resistance or of synergists which alleviate or reverse the level of resistance. A survey of such chemicals is given. So far, these chemicals have not found intentional application in practice but in some cases results of laboratory studies are promising, especially in the field of negatively correlated cross-resistance to benzimidazoles. Chemicals with pronounced synergistic activity *in vivo* against fungicide-resistant isolates are rare. Compounds which *in vitro* enhance accumulation by fungi or ergosterol biosynthesis inhibitors (EBIs) may possibly be a key for the detection of chemicals which synergize EBI-toxicity to both sensitive and EBI-resistant populations. This type of synergism might already be of importance in existing mixtures of conventional fungicides and EBIs. The application of negatively correlated cross-resistance and of synergism in practice can probably also suffer from development of resistance. It is, therefore, concluded that the chemicals described certainly will not overcome the resistance problem in the near future but can play a modest role in resistance management, if integrated with other strategies.

INTRODUCTION

Fungicide resistance in crop protection has become a serious problem. There is a rapid increase in the number of recorded cases of fungicide resistance (Delp 1980). The emergence of resistance is determined by a variety of genetical, biological, and operational factors which together determine the degree of selection pressure exerted in a given ecological situation (Georghiou 1983). Genetical and biological factors are inherent qualities of the population and are generally beyond man's control. Therefore, countermeasures should be mainly based on operational factors. These can be changed to any extent necessary and feasible, depending on the risks implied by the genetical and biological factors (Georghiou 1983). The operational factor can be related to the chemical and to its application. The relevance of the latter subject in fungicide management will be dealt with during this conference by Skylakakis. With respect to the chemical the nature of its mechanism of action is of prime importance. Development of resistance to conventional fungicides with a multisite action is rare. Virtually all recorded cases of fungicide resistance relate to fungicides with a site-specific action. However, further search for conventional fungicides does not seem attractive since they lack systemicity and eradicative action. Therefore, there is urgent need for systemics which do not evoke so readily the development of resistance. This may apply for fungicides which inhibit ergosterol biosynthesis (EBIs) (Fuchs & Drandarevski 1976). Strategies to cope with fungicide resistance may also include the use of chemicals to which resistant mutants display negatively correlated cross-resistance or of synergists which alleviate or reverse the level of resistance. The importance of these chemicals for resistance management will be evaluated in this paper.

CONCEPTS OF NEGATIVELY CORRELATED CROSS-RESISTANCE AND SYNERGISM

According to Georgopoulos (1977) cross-resistance means resistance to two or more toxicants mediated by the same genetic factor. The term should clearly be distinguished from multiple resistance. In this instance different genes for resistance independently account for resistance to different chemicals. Genetic analysis is the only decisive way to discriminate between the two possibilities. Mutants selected for resistance to one fungicide are usually also resistant to related fungicides of the same chemical group or with a similar mechanism of action: positively correlated cross-resistance. Sometimes, a mutation may give resistance to unrelated toxicants. This may f.i. be the case when resistance is not due to decreased affinity of the target site for the toxicant but to altered metabolization or decreased accumulation at the target site. Similarly, in rare cases a mutation towards resistance to a particular chemical may lead to increased or collateral sensitivity to other chemicals with a related or non-related mechanism of action. This phenomenon is defined as negatively correlated cross-resistance. In this paper the terms positively and negatively correlated cross-resistance will be loosely applied and also be used in apparent cases of cross-resistance without decisive genetic data. The term 'reciprocal cross-resistance' is used when selection for resistance to one toxicant results in positively- or negatively-correlated cross-resistance to another toxicant, and vice versa.

Mixtures of chemicals may result in additive, antagonistic or synergistic fungitoxic action. In these instances the toxicity of the mixture is equal to, or lower or higher than the sum of the toxicity of the individual components, respectively. The activity of synergists can f.i. be based on the potential to enhance fungicide accumulation at the target site or on the inhibition of activity of detoxication enzymes like mixed-function oxidases and hydrolases.

ACYLALANINES

Analogue synthesis of acylaniline herbicides, which may also possess weak fungicidal activity, has led to the discovery of the chemically related acylalanine fungicides (Hubele *et al.* 1983). The mode of action of these fungicides is based on specific interference with RNA polymerase activity (Davidse *et al.* 1983). Isolates of several Oömycetes with cross-resistance to different acylalanines could easily be selected. Isolates of Phytophthora cactorum lacked cross-resistance to chloroacetanilide herbicides (Leroux & Gredt 1981). Other results obtained with the same fungus point to positively correlated cross-resistance to metolachlor and negatively correlated cross-resistance to propachlor (Art & Buchenauer 1981). Metalaxyl-resistant isolates of Phytophthora megasperma f. sp. medicaginis displayed positively correlated cross-resistance to both these herbicides but lacked cross-resistance to others (Davidse *et al.* 1984). The divers cross-resistance patterns may be due to a second mechanism of action of these herbicides.

Metalaxyl-resistant isolates of Phytophthora infestans and Plasmopara viticola showed cross-resistance to oxadixyl (SAN 371 F), an oxazolidinone fungicide. However, the level of cross-resistance to oxadixyl was relatively low. In greenhouse tests it could be decreased to an insignificant level by application of a mixture with mancozeb. This is ascribed to the synergistic effect of the mixture. Synergism was also found upon control of the wild-type isolates (Gisi *et al.* 1983).

BENZIMIDAZOLES

It is generally accepted that the primary mode of action of benzimidazole fungicides is based on interference with microtubule assembly because of binding to fungal tubulin (Davidse & Flach 1977). Resistance to benzimidazoles and benzimidazole-generating fungicides is a widespread problem in agriculture. In a particular fungus several loci and allelic mutations in the same locus may play a role (Van Tuyl 1977). The mutations give rise to biosynthesis of tubulin with a decreased binding affinity to benzimidazole fungicides (Davidse & Flach 1977). Resistance to one of them usually involves cross-resistance to others. However, about 1% of the benomyl-resistant strains of Aspergillus nidulans and Aspergillus niger were as sensitive to thiabendazole as the wild-type. In addition, a significant proportion (2 - 20%) of thiabendazole-resistant strains of five different fungal species showed negatively correlated cross-resistance to benomyl (Van Tuyl *et al.* 1974, Van Tuyl 1977). The negatively correlated cross-resistance to carbendazim in A. nidulans was due to allelic mutations in the same benA locus (Van Tuyl 1977). Binding affinity between carbendazim and tubulin of these strains appeared to be increased (Davidse & Flach 1977).

The first recorded case of negatively correlated cross-resistance in benomyl-resistant field isolates has been described for zineb in Verticillium malthousei (Lambert & Wuest 1975). Equivalent reduction in spore germination and germ tube growth of four resistant isolates occurred at zineb concentrations half those required for four sensitive strains.

Benzimidazole-sensitive and resistant isolates of Botrytis cinerea are mostly insensitive to the Oömycete fungicide cymoxanil. Some isolates from the Bordeaux vine-yard area were extremely sensitive to the fungicide. No causal explanation for the phenomenon is available (Leroux & Gredt 1981).

Negatively correlated cross-resistance to benzimidazoles has also been described for N-phenyl carbamate herbicides like barban, chlorbufam, chlorpropham, and propham (Leroux & Gredt 1979, 1983). It was observed in a part of laboratory and field isolates of B. cinerea and Penicillium expansum. A carbendazim-resistant isolate of A. nidulans did not show the phenomenon. N-phenyl carbamates are known to interfere with mitosis but at a site differing from the one of benzimidazoles. The biochemical basis of this negatively correlated cross-resistance is, therefore, not clear.

Recently, methyl N-(3,5-dichlorophenyl)carbamate (MDPC), a chemical structurally related to the herbicides mentioned above, has been presented (Kato *et al.* 1983, 1984). In pot tests, MDPC controlled gray mold of cucumber, Cercospora leaf spot of sugarbeet, scab of Japanese pear and powdery mildew of cucumber when caused by benzimidazole-resistant strains but not upon inoculation with wild-type strains. The toxic effect was ascribed to interference with mitosis (Suzuki *et al.* 1984). The negatively correlated cross-resistance seemed to be a universal phenomenon since 100 benzimidazole-resistant isolates of B. cinerea selected at random from various locations were all more sensitive to the chemical than wild-type isolates (Kato *et al.* 1983). However, *in vitro* studies with Venturia nashicola revealed that of this fungus only isolates with a high degree of resistance to carbendazim showed increased sensitivity to MDPC. Isolates with an intermediate or low degree of resistance to carbendazim displayed a wild-type sensitivity to the chemical (Ishii *et al.* 1984). Since the latter isolates play a major role in the failure of scab control by carbendazim in Japanese pear orchards, practical use of MDPC in this instance is not likely.

Similar studies should be carried out with other pathogens in order to elucidate the importance of MDPC in the control of diseases incited by benzimidazole-resistant pathogens.

Another recent case of negatively correlated cross-resistance to benzimidazoles has been reported for diphenylamine (DPA) (Rosenberger & Meyer 1983, 1984). DPA is a chemical used in practice in postharvest dip or drench treatments to prevent storage scald, a physiological disorder caused by oxidation of α-farnesene. It is combined with a benzimidazole treatment for control of decay caused by P. expansum. *In vitro* experiments established that increasing levels of benomyl resistance were associated in most isolates with increasing levels of DPA sensitivity. The authors suggest that the interaction described may represent the first commercial application (albeit totally accidentally) of negatively correlated cross-resistance. Three of the 18 benomyl-resistant isolates tested were simultaneously resistant to DPA. Inclusion of such isolates as inoculum in storage trials reduced the effectiveness of DPA-benzimidazole mixtures. Therefore, the practical usefulness of such mixtures cannot be assessed until more is known about the relative incidence of the various isolates in apple storages. A physiological explanation of the phenomenon described is not yet known, but of importance as a starting point for further research.

CARBOXAMIDES

Extensive investigations of the structure-activity relationships of large groups of oxathiin and thiophene carboxamides have been carried out with wild-type and carboxin-resistant mutants of A. nidulans and Ustilago maydis (White *et al.* 1978, White & Thorn 1980). Resistance in these mutants to carboxamides is due to the presence of a succinate dehydrogenase complex (SDC) with a lower sensitivity to the fungicide. Mutants with varying levels of resistance c.q. degrees of sensitivity of SDC do exist. Positively correlated cross-resistance to various carboxamides such as to 3'-methylcarboxin is the most common response. However, for any given mutation affecting carboxin sensitivity of the SDC, also a specific structural group of oxathiin or thiophene carboxamides (or even a specific chemical) may be found which will alleviate or reverse the effect of the mutation in terms of inhibition of SDC activity. For instance, altered SDC from mutants of U. maydis with a moderate degree of resistance to carboxin showed an increased sensitivity to 4'-phenylcarboxin and some 4'-substituted analogues of 3-methylthiophene-2-carboxanilides. SDC of mutants with a high resistance level to carboxin showed an extremely low level of cross-resistance to chemicals like f.i. 3'-decylcarboxin, 3-methylthiophene-2-carboxanilide and the 3'-phenoxy analogue of the latter chemical. In addition, the sensitivity level of SDC may vary significantly; f.i. in the case of 3'-decylcarboxin the concentration needed for 50% inhibition of SDC activity of a highly carboxin-resistant mutant is 638x as low as for carboxin. Such observations are of importance for the development of structural analogues as practical fungicides for the control of carboxin-resistant pathogens. The authors hope that with the use of such analogues the pathogen population may be forced to return to its original-sensitive state. The strategy, however, has several limitations since it cannot be excluded that a given analogue may also select for mutants with positively correlated cross-resistance. Theoretically, this may be anticipated by monitoring the genotype composition of a pathogen population and use of a suitable mixtures of different analogues. A second limitation is that analogues inhibitory towards SDC activity of carboxin-resistant mutants of a particular pathogen are not necessarily effective against the SDC of other resistant pathogens. Finally, *in vitro* sensitivity of SDC and *in vivo* control of the corresponding pathogen do not always correlate positively. Probably, it will

take tremendous efforts to overcome these limitations. Practical interest to commercialize any of these analogues seems, therefore, to be nil. A significant practical testcase would be a study on the control of oxycarboxin-resistant populations of Puccinia horiana in chrysanthemum glasshouses.

DICARBOXIMIDES

The term dicarboximides has been loosely applied to fungicides like iprodione, procymidone, and vinclozolin. Fungal variants with cross-resistance to dicarboximides usually also display decreased sensitivity to aromatic hydrocarbon fungicides (cf. Beever & Byrde 1982). The majority of dicarboximide-resistant isolates of various fungal species showed an increased osmotic sensitivity on agar media amended with salts or sugars (Leroux *et al.* 1981, Beever 1983). No obvious explanation for this pleiotropic effect is available. It has been suggested that the phenomenon may explain why no highly dicarboximide-resistant strains of B. cinerea were collected from grape berries (Leroux & Gredt 1982).

ERGOSTEROL BIOSYNTHESIS INHIBITORS (EBIs)

Extensive studies on cross-resistance to EBIs have been carried out with various fungi. The literature up to 1980 primarily relates to *in vitro* studies with laboratory mutants and EBIs classified as piperazines, pyrimidines, imidazoles and triazoles, which inhibit C-14 demethylation (DMIs). The studies showed that positively correlated cross-resistance to DMIs is the rule, although the level of cross-resistance to a particular fungicide may differ significantly and can occasionally be nil (cf. De Waard & Fuchs 1982). These results have been confirmed and extended in *in vivo* studies with various pathogens like Erysiphe graminis f. sp. hordei (Hollomon 1982, Buchenauer 1984), Penicillium italicum (De Waard *et al.* 1982), Sphaerotheca fuliginea (Schepers 1983) and E. graminis f. sp. tritici (Buchenauer 1984, De Waard *et al.* 1984).

EBIs classified as morpholines probably interfere with ergosterol biosynthesis by inhibition of Δ^{14}-reductase (RIs) (Kerkenaar 1983, Kerkenaar *et al.* 1984). Cross-resistance patterns between DMIs and RIs cannot be generalized. *In vitro* studies with U. maydis showed that mutants selected on DMI-amended agar showed cross-resistance to tridemorph and fenpropimorph or lacked cross-resistance. A minor part displayed negatively correlated cross-resistance to fenpropimorph (Barug & Kerkenaar 1979, 1984). Tridemorph resistant mutants of U. maydis (n = 50 - 100) were all resistant to clotrimazole and miconazole, for 75% resistant to imazalil, fenarimol, nuarimol and fenpropimorph and none were cross-resistant to triadimefon (Barug & Kerkenaar 1984). Mutants of the same fungus isolated on fenpropidin or fenpropimorph-amended agar only displayed cross-resistance to RIs but not to DMIs (Leroux & Gredt 1984). Cross-resistance to RIs in DMI-resistant laboratory isolates of other fungal species was also diverse. DMI-resistant isolates of A. nidulans showed positively correlated cross-resistance to fenpropimorph (De Waard & Dekker 1983) and those of B. cinerea lacked cross-resistance (Leroux & Gredt 1984). In P. italicum a slightly DMI-resistant isolate lacked cross-resistance to fenpropimorph, while the intermediately and highly resistant ones showed a negatively correlated cross-resistance both *in vitro* and *in vivo* (De Waard *et al.* 1982, De Waard & Van Nistelrooy 1982a).

Data on cross-resistance to DMIs and RIs have also been obtained in studies with field isolates of powdery mildews which displayed a decreased sensitivity to DMIs. In leaf disc tests with E. graminis f. sp. hordei no evident cross-resistance to RIs was observed (Hollomon 1982). Practical

experiences confirmed these data (Butters *et al.* 1983, Gilmour 1983). Similarly, in foliar spray tests field isolates of barley and wheat powdery mildews displayed no cross-resistance to fenpropimorph (Buchenauer 1984, De Waard *et al.* 1984). Field isolates of S. fuliginea from Israel where some DMIs failed to control cucumber powdery mildew were as sensitive to fenpropimorph as the wild-type. Glasshouse isolates from several locations in The Netherlands reacted in the same way (Schepers, personal communications). Such a lack of cross-resistance may be of considerable importance to design strategies aimed to extend the life-time of both types of EBI fungicides. It should be carried out with great care since selection of rare mutants with cross- (or multiple) resistance cannot be excluded.

DMI-resistant isolates of various fungi sometimes show positively or negatively correlated cross-resistance to chemicals with an unrelated mechanism of action. Positively correlated cross-resistance for a number of such chemicals have been described by Van Tuyl (1977). Negatively correlated cross-resistance has been found for acriflavin, cycloheximide and neomycin (Van Tuyl 1977) and for dodine and guazatine (De Waard & Van Nistelrooy 1983). Increased sensitivity to dodine, although relatively low, was found in the majority of DMI-resistant isolates of 5 fungal species tested. In the case of A. nidulans it has been shown that only one mutation is involved (Van Tuyl 1977). The term 'negatively-correlated cross-resistance' is, therefore, certainly applicable to this instance. It is in agreement with the observation that back-mutants selected in EBI-resistant isolates on agar amended with dodine have a wild-type sensitivity to dodine and fenarimol (De Waard & Van Nistelrooy 1983). The various results suggest that the mutations for resistance do not give rise to reduced affinity of the target site, but rather may be related with other mechanisms of resistance such as f.i. changes in membrane permeability. Resistance to fenarimol and imazalil has indeed been ascribed to decreased accumulation of these fungicides (De Waard & Van Nistelrooy 1979, 1980, 1984a, Siegel & Solel 1981). It would be interesting to study whether increased sensitivity to the various chemicals is due to enhanced accumulation. This might provide clues to synthesis of chemicals for control of DMI-resistant pathogen populations.

A variety of chemicals were reported to have synergistic activity on the toxicity of fenarimol to both sensitive and EBI-resistant mutants of A. nidulans and P. italicum in crossed-paper strip bioassays (De Waard & Van Nistelrooy 1982b, 1984a, 1984b, De Waard & Dekker 1983). Anionic and cationic agents, conventional fungicides with a multisite action (f.i. phthalimides), respiratory inhibitors and sodium orthovanadate were among the most active compounds. The synergism is ascribed to their potency to enhance fenarimol accumulation to relatively high levels in both wild-type and resistant mutants. In turn, this may be due to inhibition of energy-dependent efflux of the fungicide. Since a constitutive high energy-dependent efflux is regarded as the mechanism of resistance, the synergists somehow annihilate the mechanism of resistance. Sodium orthovanadate is also synergistic for the toxicity of other EBIs to P. italicum (De Waard & Van Nistelrooy 1984a). If these observations are of more general importance and would also apply to sensitive and EBI-resistant field isolates of pathogenic fungi, they may be a rational lead for development of synergists with practical significance.

The improved field performance of recommended mixtures of EBIs and conventional fungicides may possibly be due to a similar type of synergistic interaction (Fuchs *et al.* 1983). This has been described most clearly for a mixture of fenpropimorph and chlorothalonil in the control of Pyrenophora teres in barley under field conditions (Hampel & Lartaud 1983). If a similar

mechanism of resistance as described above also operates in resistant individuals of plant pathogens under field conditions, the mixtures released can also already be of importance in preventing or retarding the development of resistant populations. So far, no data are available in this respect. It should be stressed that the significance of synergism under field conditions should not be overestimated since, for instance, transport of EBIs into plants may result in a spatial separation of the ingredients of a formulated product. Therefore, field tests should always determine the ultimate value of mixtures.

ORGANOPHOSPHORUS COMPOUNDS

Organophosphorus fungicides used for the control of Pyricularia oryzae on rice are the phosphorothiolates (PTLs) edifenphos and S-benzyl O,O-diisopropyl phosphorothioate (IBP). Their mechanism of action is based on inhibition of the activity of phospholid N-methyltransferase. Under field conditions resistance to PTLs develops slowly since resistant isolates could only be found after 10 - 14 years of intensive use (Uesugi & Katagiri 1983). The isolates show cross-resistance to different PTLs and to isoprothiolane, a rice blast fungicide structurally unrelated to PTLs. Field isolates can be distinguished in moderately and highly-resistant categories. Most field isolates have a moderate degree of IBP resistance. They detoxify this fungicide at a faster rate than sensitive isolates by exclusive cleavage of the S-C bond into non-toxic products. Few field isolates have a high degree of IBP resistance; they resemble IBP-resistant laboratory isolates with respect to an almost complete lack of metabolization of IBP and an increased sensitivity to phosphoroamidate fungicides (PAs). The mechanism of resistance to IBP in these isolates is not understood (Uesugi & Sisler 1978). The negatively correlated cross-resistance to PAs in probably due to decreased detoxification of these chemicals into non-toxic products. Back-mutants selected on PA-amended agar were again as sensitive to IBP as the wild-type (Uesugi & Katagiri 1977). As yet, no data are available on 'resistance management' strategies based on the use of both chemicals.

Synergism between PTLs and PAs has exclusively been found for wild-type strains. It is ascribed to inhibition of PA detoxification by IBP. Synergism in highly resistant laboratory mutants was not observed since these mutants hardly metabolize PA (Uesugi & Katagiri 1977, Uesugi & Sisler 1978). IBP itself has been shown to be a strong synergist of malathion in malathion-resistant strains of the green rice leafhopper. The synergism is due to inhibition of carboxyl esterase activity (Miyata *et al.* 1980).

DISCUSSION

Strategies to cope with fungicide resistance may include the use of chemicals to which fungicide-resistant populations display negatively correlated cross-resistance. A variety of such chemicals has been reported. They can be either structure analogues (f.i. of carboxamides) or structurally unrelated compounds (f.i. in the case of benzimidazoles and EBIs). None of these chemicals has found as yet intentional application in practice. This will be mainly due to their weak field performance. A second argument may be the risk of selection of mutants which display positively correlated cross-resistance to the respective compounds. In many cases this risk is most evident since negatively correlated cross-resistance is rather exceptional. The reverse situation is almost obligatory for successful use in practice.

The negatively correlated cross-resistance to benzimidazoles and MDPC in various pathogens and the lack of cross-resistance to DMIs and RIs in several powdery mildews are by now the best examples in this field. MDPC has not yet

been licensed for use in practice but may become relevant for control of certain benzimidazole-resistant populations. RIs already play a role in the control of cereal mildew populations with decreased sensitivity to DMIs. However, with both chemicals selection of isolates which lack these favourable properties cannot be excluded. Such isolates of the same or of other pathogens have already been described. The optimal situation in resistance management would be the occurrence of reciprocal negatively correlated cross-resistance or the absence of positively correlated cross-resistance in all field isolates of all relevant pathogens. This situation is far from reality. It is, therefore, concluded that the use of MDPC or of RIs in practice will, in general, not overcome the resistance problems encountered with benzimidazoles and DMIs, respectively.

In order to discover chemicals to which fungicide-resistant pathogens display negatively correlated cross-resistance screening programs should include tests with fungicide-resistant pathogens. With certain pathogens simple *in vitro* tests may initially be sufficient. The screening should involve both chemicals with related and unrelated structures. Attention should be paid to variations in the degree of positively or negatively correlated cross-resistance. The relevance of this statement can be illustrated by the occurrence of a relatively low degree of resistance to imazalil in isolates of P. italicum which have a relatively high degree of resistance to various other DMIs. Furthermore, these isolates lack cross-resistance to fenpropimorph or even display negatively correlated cross-resistance. In other words, characterization and optimalization of cross-resistance patterns should play an important role in analogue synthesis of relevant chemicals. It will take extensive effords and investments to meet this requirement. It is beyond the scope of this paper to judge whether this is financially justified or not.

Resistance can also be counteracted with synergists which alleviate or reverse the level of resistance to a fungicide. So far, this possibility has a limited potential since it has only been described for oxadixyl and for EBIs under experimental conditions. In the latter case, the mechanism of resistance is probably based on increased efflux of the toxicant. If this mechanism of resistance is a general one, search for chemicals which inhibit the efflux may yield synergists with higher practical potential than the experimental ones described. The lack of known chemicals with synergistic activity to other important groups of fungicides like the benzimidazoles or carboxamides is probably due to the fact that decreased affinity of the target site rather than metabolic breakdown to non-toxic products or reduced uptake underlies natural insensitivity or resistance to these fungicides. In contrast, detoxification of insecticides is the major mechanism of resistance in insects. As a consequence the use of chemicals which inhibit the activity of mixed-function oxidases or hydrolases, as synergists has frequently been advocated (Georghiou 1983, Wilkinson 1983). Development of similar chemicals to suppress fungicide resistance does not seem appropriate.

It is concluded that chemicals to which fungal populations display *in vitro* negatively correlated cross-resistance or synergism do occur to a reasonable extent. However, at present only a limited number of the respective chemicals may find practical application. Their role in resistance management will be modest since they are subject to severe limitations such as for instance the selection of alternative mechanisms of resistance. A generalized recommendation for solving resistance problems in the near future only on the basis of the use of these chemicals is, therefore, difficult, if not impossible. Instead, use of such chemicals should be part of other resistance management strategies.

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REFERENCES

Arp, U.; Buchenauer, H. (1981) Untersuchungen zum Wirkungsmechanismus von RF 20615 und metalaxyl in Phytophthora cactorum und zur Resistenzentwicklung des Pilzes gegenüber diesen Fungiziden. Mitteilung Biologischen Bundesanstalt Berlin-Dahlem 203, 236-237.

Barug, D.; Kerkenaar, A. (1979) Cross resistance of UV-induced mutants of Ustilago maydis to various fungicides which interfere with ergosterol biosynthesis. Mededelingen van de Faculteit der Landbouwwetenschappen, Rijksuniversiteit Gent 41, 421-427.

Barug, D.; Kerkenaar, A. (1984) Resistance in mutagen-induced mutants of Ustilago maydis. Pesticide Science 15, 78-84.

Beever, R.E. (1983) Osmotic sensitivity of fungal variants resistant to dicarboximide fungicides. Transactions British mycological Society 80, 327-331.

Beever, R.E.; Byrde, R.J.W. (1982) Resistance to the dicarboximide fungicides. In: Fungicide resistance in crop protection. J. Dekker and S.G. Georgopoulos (Eds), Pudoc, Wageningen, pp. 101-117.

Buchenauer, H. (1984) Stand der Fungizidresistenz bei Getreidekrankheiten am Beispiel der Halmbruchkrankheit und des echter Mehltaus. Gesunde Pflanzen 36, 161-170.

Butters, J.; Clark, J.; Hollomon, P.W. (1983) Field evaluation of fungicide strategies to control barley powdery mildew. Proceedings 10th International Congress of Plant Protection 2, 644.

Davidse, L.C., Flach, W. (1977) Differential binding of methyl benzimidazol-2-yl carbamate to fungal tubulin as a mechanism of resistance to this antimitotic agent in mutant strains of Aspergillus nidulans. Journal of Cell Biology 72, 174-193.

Davidse, L.C.; Gerritsma, O.C.M.; Velthuis, G.C.M. (1984) A differential basis of antifungal activity of acylalanine fungicides and structurally related chloroacetanilide herbicides in Phytophthora megasperma. Pesticide Biochemistry Physiology 21, 301-308.

Davidse, L.C.; Hofman, A.E.; Velthuis, G.C.M. (1983) Specific interference of metalaxyl with endogenous RNA polymerase activity in isolated nuclei from Phytophthora megasperma f. sp. medicaginis. Experimental Mycology 7, 344-361.

Delp, C.J. (1980) Coping with resistance to plant disease control agents. Plant Disease 64, 652-657.

De Waard, M.A.; Dekker, J. (1983) Resistance to pyrimidine fungicides which inhibit ergosterol biosynthesis. In: IUPAC Pesticide Chemistry: Human Welfare and the Environment. J. Miyamoto and P.C. Kearny (Eds), Pergamon Press, Oxford 3, 43-49.

De Waard, M.A.; Fuchs, A. (1982) Resistance to ergosterol-biosynthesis inhibitors II. Genetic and physiological aspects. In: Fungicide resistance in crop protection. J. Dekker and S.G. Georgopoulos (Eds). Pudoc, Wageningen, pp. 87-100.

De Waard, M.A.; Groeneweg, H.; Van Nistelrooy, J.G.M. (1982) Laboratory resistance to fungicides which inhibit ergosterol biosynthesis in Penicillium italicum. Netherlands Journal of Plant Pathology 88, 99-112.

De Waard, M.A.; Kipp, E.C.M.; Van Nistelrooy, J.G.M. (1984) Variatie in gevoeligheid van tarwemeeldauw voor fungiciden die de ergosterolbiosynthese remmen. Gewasbescherming 15, 8.

De Waard, M.A.; Van Nistelrooy, J.G.M. (1979) Mechanism of resistance to fenarimol in Aspergillus nidulans. Pesticide Biochemistry Physiology 10, 219-229.

De Waard, M.A.; Van Nistelrooy, J.G.M. (1980) An energy-dependent efflux mechanism for fenarimol in a wild-type strain and fenarimol-resistant mutants of Aspergillus nidulans. Pesticide Biochemistry Physiology 13, 255-266.

De Waard, M.A.; Van Nistelrooy, J.G.M. (1982a) Toxicity of fenpropimorph to fenarimol-resistant isolates of Penicillium italicum. Netherlands Journal of Plant Pathology 88, 231-236.

De Waard, M.A.; Van Nistelrooy, J.G.M. (1982b) Antagonistic and synergistic activities of various chemicals on the toxicity of fenarimol to Aspergillus nidulans. Pesticide Science 13, 279-286.

De Waard, M.A.; Van Nistelrooy, J.G.M. (1983) Negatively correlated cross-resistance to dodine in fenarimol-resistant isolates of various fungi. Netherlands Journal of Plant Pathology 89, 67-73.

De Waard, M.A.; Van Nistelrooy, J.G.M. (1984a) Differential accumulation of fenarimol by a wild-type isolate and fenarimol-resistant isolates of Penicillium italicum. Netherlands Journal of Plant Pathology. Submitted.

De Waard, M.A.; Van Nistelrooy, J.G.M. (1984b) Effects of phthalimide fungicides on the accumulation of fenarimol by Aspergillus nidulans. Pesticide Science 15, 56-62.

Fuchs, A.; Davidse, L.C.; De Waard, M.A.; De Wit, P.J.G.M. (1983) Contemplations and speculations on novel approaches in the control of fungal plant diseases. Pesticide Science 14, 272-293.

Fuchs, A.; Drandarevski, C.A. (1976) The likelihood of development of resistance to systemic fungicides which inhibit ergosterol biosynthesis. Netherlands Journal of Plant Pathology 82, 85-87.

Georghiou, G.P. (1983) Management of resistance in arthropods. In: Pest resistance to pesticides. G.P. Georghiou and T. Saito (Eds). Plenum Press, New York, pp. 769-792.

Georgopoulos, S.G. (1977) Development of fungal resistance to fungicides. In: Antifungal Compounds. M.R. Siegel and H.D. Sisler (Eds). Marcel Dekker, New York, 2, 439-495.

Gilmour, J. (1983) Performance of barley powdery mildew fungicides in South-East Scotland. Proceedings 10th International Congress of Plant Protection 2, 643.

Gisi, U.; Harr, J.; Sandmeier, R.; Wiedmer, H. (1983) A new systemic oxazolidinone fungicide (SAN 371 F) against diseases caused by Peronosporales. Mededelingen van de Faculteit der Landbouwwetenschappen, Rijksuniversiteit Gent 48, 541-550.

Hampel, H.; Lartaud, G. (1983) The control of important cereal diseases using fenpropimorph mixtures. Proceedings 10th International Congress of Plant Protection 3, 926.

Hollomon, D.W. (1982) The effects of tridemorph on barley powdery mildew: its mode of action and cross-sensitivity relationships. Phytopathologische Zeitschrift 105, 279-287.

Hubele, A.; Kunz, W.; Eckhardt, W.; Sturm, E. (1983) The fungicidal activity of acyl anilines. In: IUPAC Pesticide Chemistry: Human Welfare and the Environment. J. Miyamoto and P.C. Kearny (Eds), Pergamon Press, Oxford 3, 233-242.

Ishii, H.; Yanase, H.; Dekker, J. (1984) Resistance of Venturia nashicola to benzimidazole fungicides. Mededelingen van de Faculteit der Landbouwwetenschappen, Rijksuniversiteit Gent. In press.

Kato, T.; Suzuki, K.; Takahashi, J.; Kamoshita, K. (1983) Negative cross resistance between benzimidazoles and methyl N-(3,5-dichlorophenyl)-

carbamate. Abstracts Fourth International Congress of Plant Pathology, Melbourne, Australia, p. 17.

Kato, T.; Suzuki, K.; Takahashi, J.; Kamoshita, K. (1984) Negatively correlated cross-resistance between benzimidazole fungicides and methyl N-(3,5-dichlorophenyl)-carbamate. Journal of Pesticide Science (Japan). In press.

Kerkenaar, A. (1983) Mode of action of tridemorph and related compounds. In: IUPAC Pesticide Chemistry: Human Welfare and the Environment. J. Miyamoto and P.C. Kearny (Eds), Pergamon Press, Oxford 3, 123-127.

Kerkenaar, A.; Van Rossum, J.M.; Versluis, G.G.; Marsman, J.W. (1984) Effect of fenpropimorph and imazalil on sterol biosynthesis in Penicillium italicum. Pesticide Science 15, 177-187.

Lambert, D.H.; Wuest, P.J. (1975) Increased sensitivity to zineb for Verticillium malthousei strains tolerant to benomyl. Phytopathology 65, 637-638.

Leroux, P.; Gredt, M. (1979) Phénomènes de résistance croisée négative chez Botrytis cinerea. Phytiatrie-Phytopharmacie 28, 79-86.

Leroux, P.; Gredt, M. (1981) Phénomènes de résistance aux fongicides anti-mildious: quelques résultats de laboratoire. Phytiatrie-Phytopharmacie 30, 273-282.

Leroux, P.; Gredt, M. (1982) Effects d'alcools primaires, de polyols, de sels minéreaux et de sucres sur des souches de Botrytis cinerea Pers. sensibles ou résistantes à l'iprodione et à la procymidone. C.R. Acedémie Science Paris 294, 53-56.

Leroux, P.; Gredt, M. (1983) Negatively correlated cross-resistance between benzimidazole fungicides and N-phenyl carbamate herbicides. In: Systemische Fungizide und Antifungale Verbindungen. H. Lyr and C. Polter (Eds), Akademie-Verlag, Berlin, pp. 297-301.

Leroux, P.; Gredt, M. (1984) Resistance to fungicides which inhibit ergosterol biosynthesis in laboratory strains of Botrytis cinerea and Ustilago maydis. Pesticide Science 15, 85-89.

Leroux, P.; Gredt, M.; Fritz, R. (1981) Resistance to 3,5-dichlorophenyl-N-cyclic-imide fungicides. Netherlands Journal of Plant Pathology 87, 244-245.

Miyata, T.; Sakai, H.; Saito, T.; Yoshioka, K.; Ozaki, K.; Sasaki, Y.; Tsuboi, A. (1981) Mechanism of joint toxic action of Kitazin-P with malathion in the malathion-resistant green rice leafhopper, Nephotetrix cincticeps Uhler. Applied Entomology and Zoology 16, 258-263.

Rosenberger, D.A.; Meyer, F.W. (1983) Use of diphenylamine with benzimidazole fungicides improves control of benomyl-resistant Penicillium expansum in stored apples. Phytopathology 73, 1346.

Rosenberger, D.A.; Meyer, F.W. (1984) Negatively-correlated cross-resistance to diphenylamine in benomyl-resistant Penicillium expansum. Phytopathology. Submitted.

Schepers, H.T.A.M. (1983) Decreased sensitivity of Sphaerotheca fuliginea to fungicides which inhibit ergosterol biosynthesis. Netherlands Journal of Plant Pathology 89, 185-187.

Schwinn, F.J. (1982) Socio-economic impact of fungicide resistance. In: Fungicide resistance in crop protection. J. Dekker and S.G. Georgopoulos (Eds). Pudoc, Wageningen, pp. 16-23.

Siegel, M.R.; Solel, Z. (1981) Effects of imazalil on a wild-type and fungicide-resistant strain of Aspergillus nidulans. Pesticide Biochemistry Physiology 15, 222-233.

Suzuki, K.; Kato, T.; Takahashi, J.; Kamoshita, K. (1984) Mode of action of methyl N-(3,5-dichlorophenyl-carbamate in the benzimidazole-resistant isolate of Botrytis cinerea. Journal of Pesticide Science (Japan). In press.

Uesugi, Y.; Katagiri, M. (1977) Back-mutation of an organophosphorus thiolate-resistant mutant of Pyricularia oryzae. Netherlands Journal of Plant Pathology 83 (Suppl. 1), 243-252.

Uesugi, Y.; Katagiri, M. (1983) Metabolism of a phosphorothiolate fungicide IBP by strains of Pyricularia oryzae with varied sensitivity. In: IUPAC Pesticide Chemistry: Human Welfare and the Environment. J. Miyamoto and P.C. Kearny (Eds). Pergamon Press, Oxford 3, 165-170.

Uesugi, Y.; Sisler, H.D. (1978) Mechanism of a phosphoroamidate by Pyricularia oryzae in relation to tolerance and synergism by a phosphorothiolate and isoprothiolane. Pesticide Biochemistry Physiology 9, 247-254.

Van Tuyl, J.M. (1977) Genetics of fungal resistance to systemic fungicides. Mededelingen Landbouwhogeschool Wageningen 77-2, 1-136.

Van Tuyl, J.M.; Davidse, L.C.; Dekker, J. (1974) Lack of cross resistance to benomyl and thiabendazole in some strains of Aspergillus nidulans. Netherlands Journal of Plant Pathology 80, 165-168.

White, G.A.; Thorn, G.D. (1980) Thiophene carboxamide fungicides: structure-activity relationships with the succinate dehydrogenase complex from wild-type and carboxin-resistant mutant strains of Ustilago maydis. Pesticide Biochemistry Physiology 14, 26-40.

White, G.A.; Thorn, G.D.; Georgopoulos, S.G. (1978) Oxathiin carboxamides highly active against carboxin-resistant succinate dehydrogenase complexes from carboxin-selected mutants in Ustilago maydis and Aspergillus nidulans. Pesticide Biochemistry Physiology 9, 165-182.

Wilkinson, C.F. (1983) Role of mixed-function oxidases in insecticide resistance. In: Pest resistance to pesticides. G.P. Georghiou and T. Saito (Eds). Plenum Press, New York, pp. 175-205.

THE EVOLUTION OF INSECTICIDE RESISTANCE AND ITS RELEVANCE TO CONTROL STRATEGY

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ABSTRACT

The evolution of resistance is traced from a single gene mutation to the point where control failure occurs. The concepts of susceptibility, tolerance and resistance are discussed from a geneticist's viewpoint. The evolutionary processes which form the basis for the development resistance and for the strategies suggested to combat the development are explained. The main conclusion is that resistance will be difficult to control unless preventitive measures are taken at a very early stage in its evolution. However to do so would require using high doses from the start and this is probably environmentally and economically unsound. The practicality of many proposed strategies to deal with established resistance would be improved by the development of more accurate methods of insecticide application.

INTRODUCTION

The appearance and spread of insecticide resistance is a classic example of evolution by natural selection. However it has been little studied as an evolutionary phenomenon. It is only within the last ten years that any progress has been made in collecting the data necessary to formulate strategies to control the spread of resistance. Many opportunities have been missed and most past data were collected and presented in such a way as to be unsuitable for evolutionary studies indeed past research on resistance has been largely limited to listing occurrences and investigating inheritance patterns and biochemical mechanisms. Such a situation is unsatisfactory; we need to understand all the processes, including the evolutionary ones, leading to failure to control pest species.

A number of authors have provided mathematical models of strategies to delay the spread of resistance (Curtis et al 1978, Wood & Mani 1981). In essence all these models act by modifying the relative fitness values in the fundamental equation of population genetics (see below). Denholm, (1981) discussed the role of mathematical models in identifying the options available for delaying resistance and concluded that general models were of little use and that detailed studies of each case were needed. I do not think he was right; if only for the reason that there are not the resources to collect such data for each pest and pesticide. We can make a small number of detailed studies on important pests, such as Sawicki and his colleagues are making at Rothamsted and my colleagues and I are making at Slough. However for most species the action taken will depend largely on theory and what we can learn from species such as Musca domestica and Oryzaephilus surinamensis.

The stage has been reached when possible strategies are being tested The strategies and experiments lean heavily on the tenets of population genetics. In order to design and understand such experiments and put the strategies into practice it is important that the processes behind the evolution of resistance are properly understood. In the following account I shall attempt to explain these processes using as an example resistance

determined by a single gene. I have chosen to restrict myself to resistance controlled by a single gene for the sake of simplicity and because most field resistances are of this kind.

RESISTANCE - A DEFINITION

Communication between those working in the various branches of pesticide resistance is hindered by a failure to agree a definition of resistance. I shall use the simplest definition which is that resistance is the ability of an insect to survive a dose of insecticide that would be lethal to members of a normal population. A normal population can be defined as a population never subjected to insecticidal pressure and in which resistant individuals are rare (WHO 1981). I would ammend this definition to read 'and in which resistant individuals do not occur'. The suggestion by Sawicki and Denholm (1984) that the WHO definition of a normal population should be abandoned in favour of designating current field strains as normal seems unhelpful. For then normality would depend on which populations were examined and base line data could differ from year to year and from locality to locality. Comparisons with field strains could mask high but still controllable levels of resistance and lead to a failure to identify an incipient control problem.

In spite of a recommendation to the contrary (FAO 1979) the term tolerance is widely used in the herbicide and fungicide literature to denote a state somewhere between resistance and susceptibility (eg. Gressel 1983). I find this concept unacceptable, as in this usage the terms merely represent the extremes of a continuum and, with selection, tolerance may become resistance without any further change in the genetical nature of the phenomenon. There is a real danger that this use of the term tolerance will lead to a belief that resistance is not present when in fact the early stages in its development have already been passed.

BEFORE RESISTANCE - THE NORMAL POPULATION

Variability in response to an external influence is an intrinsic characteristic of all populations and is the basis behind the use of dose-response lines to characterise populations. A linear response following probit transformation implies that the dose response follows a normal distribution. For a susceptible population there may be a wide range of variation in response to insecticide and at the extremes a small number of individuals may tolerate high doses or be susceptible to low doses (Such a range of variation in response may be referred to as the natural tolerance of the population and is a property of resistant as well as susceptible populations. This would be my definition of tolerance, but perhaps we should avoid the use of the word altogether). If, however, we select a group of survivors from one end of the dose response range of a susceptible strain and breed them up, the dose response should be the same as that of the original population. Thus for such strains an individuals ability to survive a particular dose is not inherited by its offspring.

RESISTANCE - THE FIRST STEPS

It is generally accepted that resistance normally arises as a result of a chance mutation enabling an individual to survive in the presence of a pesticide. The rate at which resistance mutations arise is not known but by 1982 432 species of arthropod had been found to be resistant to one or more pesticides (Conway 1982). Mutations conferring resistance

must therefore be recurrent and fairly common. A gene mutation rate of 1 in 10^5 is often quoted in this context and is usually the basis for the start of computer simulations of the evolution of resistance.

It is likely that when they first appear, most resistance genes impart a selective disadvantage to the individuals carrying them. Thus once the resistance gene has appeared in the population it will be rapidly eliminated unless the individual carrying it comes into contact with the insecticide. These selective disadvantages have been measured on only a few occasions, but such estimates as are available suggest disadvantages of up to 56% for DDT and dieldrin-resistant Anopheles mosquitoes (Curtis et al, 1978), up to 39% for dicofol-resistant strains of the mite Panonychus citri Inoue, 1980) and up to 34% for malathion resistant grain beetles, O. surinamensis Muggleton (1983). So resistance genes will have been appearing and disappearing for thousands of generations prior to the introduction of insecticides.

RESISTANCE - THE DEVELOPMENT

We can now consider the process by which resistance develops from a single gene mutation to a situation where there is a breakdown in control. Each gene is represented in an organism by two copies (alleles). If both copies are identical the individual is homozygous for that gene, if not it is heterozygous. The initial mutation will have occurred in only one copy and so at the start there will be a population containing a single heterozygous animal. If this population is then challanged by a dose of insecticide insufficient to kill the heterozygote then that individual will be more likely to produce progeny than the others, and the gene will spread by natural selection. An increasing proportion of the population will possess the resistance gene and the population can then be said to be polymorphic for resistance. Within this population there will be some individuals that are fully susceptible, some that are heterozygous and others that are homozygous for resistance. Continued selection would eventually lead to the whole populations becoming homozygous for resistance, but this could take tens, hundreds or thousands of generations (see Figure 1). Most field populations will be in a transient stage and necessarily heterogeneous. Thus, unless it is known that a population is made up wholly of individuals homozygous for the resistant gene it is wrong to refer to the population as resistant. Rather we should talk about a population containing resistant individuals or a population with a given frequency of resistance at a particular dose. To do otherwise leads to a number of misconceptions, the most frequent being that because a strain is labelled 'resistant' every individual in it has the property of resistance. This is rarely the case.

The speed at which resistance develops is determined by many factors, of which, the proportions of each genotype killed by the insecticide, the intrinsic fitness of each genotype, the proportion of the population never reached by the insecticide and the rate of immigration, are probably the most important. All these factors need to be considered when planning to control the spread of resistance as indeed will any factor that can increase the survival of the susceptible gene relative to the resistance gene. For each genoytpe the effect of the various factors can be simplified to a single value (w) which is the proportion of that genotype relative to the others which will survive to the next generation. In each generation the frequency of the susceptible gene (s_x) will be determined by the equation,

$$s_x = \frac{w_2 rs + w_3 s^2}{w_1 r^2 + w_2 2rs + w_3 s^2}$$

where, r^2 frequency of the resistant homozygote) in the
$2rs$ is the frequency of the heterozygote) previous
s^2 is the frequency of the susceptible homozygote) generation
and w_1, w_2 and w_3 are the fitnesses of the three genotypes.

The spread of a gene through a population follows a sigmoid curve. Figure 1 shows an example for a population exposed in each generation to a dose of insecticide sufficient to kill all the susceptible animals and all the heterozygotes; no homozygous resistant animals are killed. The intrinsic fitness of each genotype is assumed to be the same and ten per cent of the population escapes treatment (i.e. $w_1 = 1.0$, $w_2 = 0.1$, $w_3 = 0.1$). Note that even when starting from a resistant gene frequency of 0.0005 there is a considerable lag before resistance reaches an appreciable frequency, but thereafter the increase is very rapid indeed (218 generations to reach a resistance gene frequency of 0.005, 240 generations to reach 0.05 and 247 generations to reach 0.995). This type of curve demonstrates what is only too often true, which is that by the time resistance is detectable it will have already reached the rapid phase of its development. Note that the annual increase in resistance will be determined by the number of generations a year.

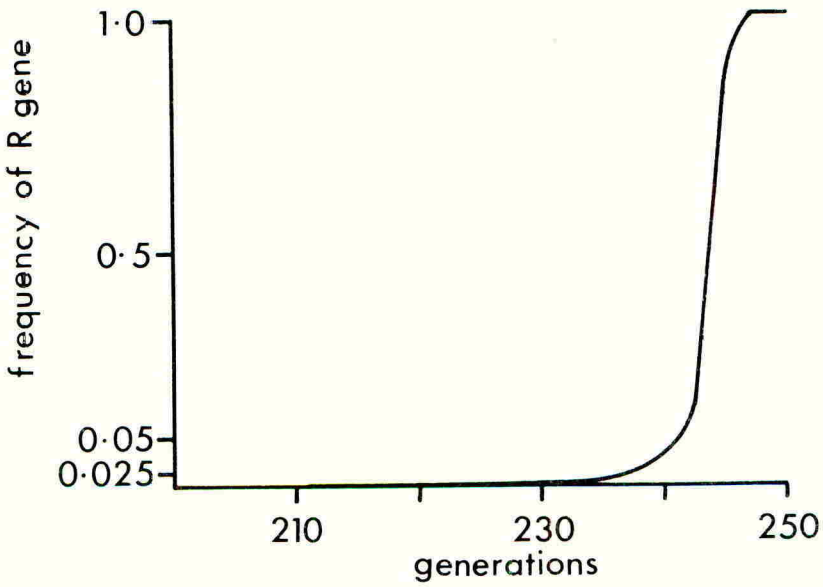

Fig. 1. Computer simulation showing the increase in frequency of a resistance gene in an infinite population treated with a dose which kills both susceptibles and heterozygotes. 10% of the population escapes treatment. The starting frequency of the resistance gene was 0.0005.

In this account I have only dealt with resistance under the control of a single gene and this is the easiest situation to explain. Resistance could however be polygenic and be controlled by a large number of genes each with a small effect. Whilst such a system would be subject to natural selection, the interaction between the genes would make predicting the rate of spread very complicated. However, in spite of earlier predictions to the contrary it now seems that resistance in the field is more usually under the control of one or several major genes each with a large effect (Whitten and McKenzie 1982). What one might expect to see as resistance develops, is selection for minor genes which enhance the effect of the major gene or increase the fitness of the resistant individuals.

RESISTANCE - CONTROLLING THE DEVELOPMENT

Firstly it is important to realise that for the purposes of management, resistance has three or possibly four phases. The first of these is the period up to and including the initial mutation to resistance, the second is the period when resistance is spreading in the presence of insecticide but is not yet detectable and the third is the period after which it has become detectable and when it is causing control problems. The period following the abandonment of the insecticide may be considered a fourth phase. As can be seen from Figure 1, the same treatment is not appropriate for each phase. A policy of using dosages high enough to kill all heterozygotes will work well if the frequency of the resistance gene is below 0.005. If the same policy were adopted when the resistance gene frequency was 0.025 it would be followed by a rapid rise in resistance gene frequency and control failure after a small number of generations.

In practice the first two phases cannot be distinguished as the chances of detecting resistance at very low frequencies would require very large sample sizes. It is therefore fortunate that the tactics that can be used in phase 1 are also appropriate to phase 2. The necessity to adopt different strategies in phase 3 in the spread of resistance is not always appreciated and some criticisms (e.g. those of Comins and Conway (1982) on the use of mixtures and high doses) seem to arise from using strategies, developed to cope with the first two phases, when resistance has reached the third phase.

As resistance arises as the result of a chance mutation it follows that the chances of two different resistance mutations arising at the same time will be much less than for one alone, perhaps as low as 1 in 10^{10}. It therefore makes sense to use a mixture of unrelated compounds rather than a single compound so that an allele resistant to one of the compounds but not to the other is eliminated directly it appears. This supposes that the potential mechanisms to break down each of the compounds are different so that there is no cross-resistance. A variation on this theme is to alternate the use of two compounds in time or space but such strategies are unlikely to be as effctive as the use of mixtures.

We have seen that in the early stages of resistance, all the resistant animals will be heterozygotes, and homozygotes will not be present. In these circumstances it is possible to use doses high enough to kill the heterozygotes without the danger of selecting for homozygous resistants. Even when the frequency of resistant individuals reaches ten percent the frequency of homozygous resistants will be less than half of one percent.

Thus population size will also play a part in determining whether homozygous resistants are present. Providing a proportion of the other genotypes escape treatment high doses can be used even if homozygous individuals are present. In effect escape preserves susceptible genes and dilutes the pool of resistant genes during the next round of breeding. If the presence of homozygous resistants is uncertain treatments should be planned to allow for escape. In fact very few treatments will be completely effective and so escape is probably the normal situation. As the frequency of homozygous individuals rises so must the proportion of the whole population allowed to escape treatment. Obviously a point will be reached when the number allowed to escape leads to an unacceptable breakdown of control. The importance of high dosages and escape has been emphasised by Wood and Mani (1981). Comins and Conway (1982) have argued against the use of high dosages on two grounds. They point out that if the high dose is not evenly applied some heterozygous individuals that would otherwise be killed will receive a low dose and survive. They overlook the fact that this argument applies to the susceptibles as well as to the heterozygotes. Even with an uneven treatment the spread would still be slowed although less so than if the treatment was even. The detrimental effect of an uneven treatment would depend on the overlap of the dose response of the susceptible and heterozygous animals; the larger the overlap the smaller the effect of an uneven treatment. The effects of even and uneven treatment on the spread of resistance are illustrated in Figure 2, where the curved lines (i), (ii) and (iii) show the decrease in the susceptible gene if the treatment is even (i) or uneven (ii and iii) (see caption for full explanation). The greatest delay in the loss of susceptibles results from the even treatment regardless of whether the biological fitness differences are taken into account (Fig. 2b) or not (Fig. 2a).

Comins and Conway (1982) also suggest that high doses will select for genes giving high levels of resistance. Such genes will appear by chance and not as a result of using high doses; if they do appear while insecticide is being used they will spread unless the dose used is sufficient to kill the heterozygous combination. Low doses are unacceptable as they will allow the heterozygotes to survive and ensure the survival and gradual increase of resistance at all levels. If the doses are sufficiently low they will, by allowing susceptibles to survive, undoubtedly slow the spread of resistance but they will also fail to control the pest population. The logical outcome of Comins and Conway's argument is not to use insecticide at all, that way resistance genes will stay at the mutation frequency.

Is there anything that can be done once resistance is detectable as a control problem? The practical answer may be, very little. The only way of slowing the spread at this stage is to increase the survival of susceptible animals at the expense of effective control. However there may be situations where this becomes acceptable and then we can make use of the probable biological disadvantage of the resistant genotypes compared to the susceptibles. Taking this into account means that smaller levels of escape can be contemplated, but such a strategy depends on an intrinsic property of the resistant genotypes which would have to be measured and which we may not be able to alter. At best it may be possible to place the resistant heterozygotes at a disadvantage compared to other genotypes. If this can be achieved the spread of resistance could be reversed while

continuing to use the insecticide to which resistance is present (Muggleton 1982). Figure 2 gives examples of the development of resistance under differing regimes and taking into account the biological fitness differences. These strategies together with some others described above require quite accurate dosing. There is little doubt that one of the greatest aids in the fight against resistance would be the development of techniques that would ensure even and accurate application. The biological fitness differences between resistant and susceptible individuals could be utilised in another way. If the reason for this fitness differential could be identified

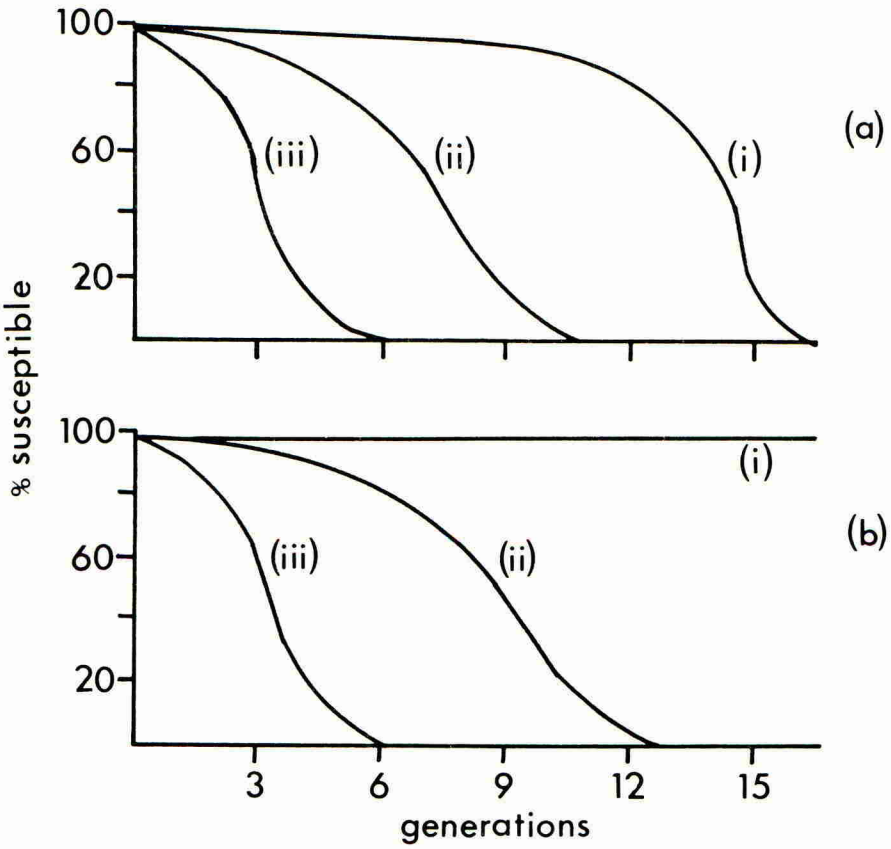

Fig. 2. A computer simulation showing the effects of various treatments on the proportion of homozygous susceptibles in a population where 10% of the individuals escape treatment and, (a) there is no biological fitness difference between the genotypes or (b) the resistant homozygote and the heterozygotes have a 10% fitness disadvantage compared to the susceptible homozygotes, and where (i) the treament is applied evenly and is sufficient to kill all heterozygotes and susceptible homozygotes or (ii) there is uneven treatment and 25% of the heterozygote and 12.5% of the susceptibles survive treatment or (iii) there is uneven treatment and 25% of the heterozygotes but none of the susceptibles survive treatment. The frequency of the susceptible homozygotes at the start of the treatment is 98%.

it might be possible to accentuate it as part of the control programme. Thus there is some evidence that malathion resistant O. surinamensis are less fit and less tolerant of insecticide at lower temperatures, and therefore by integrating cooling of grain with pesticide treatment a more effective control might be achieved.

Since most of the proposed strategies will only work effectively if resistance is at a low level, it should be apparent that we need to detect resistance at the earliest stage possible. Paradoxically it is these low levels of resistance that we are unlikely to be able to detect unless monitoring is frequent and uses as large sample sizes as possible. Waiting, indeed not even defining the pheonmenon as resistance, until there is a control failure is to adopt an ostrich-like attitude, by then it will be too late. An alternative attitude would be to assume that resistance will eventually arise everywhere and therefore to treat with large doses from the very beginning. Such an approach would probably be environmentally and economically unsound.

REFERENCES

Comins, H.N.; Conway, G.R. (1982) Strategies for delaying resistance: mathematical models. In: Pesticide Resistance and World Food Production G. Conway (Ed.). London: Imperial College Centre for Environmental

Curtis, C.F.; Cook, L.M.; Wood, R.J. (1978) Selection for and against insecticide resistance and possible methods of inhibiting the evolution of resistance in mosquitoes. Ecological Entomology, 3, 273-287.

Denholm, I. (1981) Present trends and future needs in modelling for the management of insecticide resistance. Proceedings 1981 British Crop Protection Conference - Pests and Diseases 3, 847-855.

FAO (1979) Report of 2nd session of the FAO panel of experts on pest resistance to pesticides, held in Rome 18 August - 1 September 1978. FAO plant production and protection paper 6/2.

Gressel, J. (1983) Spread and action of herbicide tolerances and uses in crop breeding. Proceedings 10th International Congress of Plant Protection, 2, 608-615.

Inoue, K. (1980) Relationship between Dicofol resistance and fitness in the Citrus Red Mite, Panonychus citri (McG.). Journal of Pesticide Science, 5, 165-175.

Muggleton, J. (1982) A model for the elimination of insecticide resistance using heterozygous disadvantage. Heredity, 49, 247-251.

Muggleton, J. (1983) Relative fitness of malathion-resistant phenotypes of Oryzaephilus surinamensis L. (Coleoptera : Silvanidae). Journal of Applied Ecology, 20, 245-254.

Sawicki, R.M., Denholm, I. (1984) Adaptation of insects to insecticides. In : Origins and development of adaptation D. Evered and G.M. Collins (Eds). London : Pitman, pp. 152-162.

Whitten, M.J.; McKenzie, J.A. (1982) The genetic basis for pesticide resistance. In : Proceedings 3rd Australasian Conference on Grassland Invertebrate Ecology K.E. Lee (Ed.), Adelaide: Government Printer.

WHO (1981) Instructions for determining the susceptibility for resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides - diagnostic tests. WHO/VBC/81.806, World Health Organisation.

Wood, R.J.; Mani, G.S. (1981) The effective dominance of resistance genes in relation to the evolution of resistance. Pesticide Science, 12, 573-581.

INSECTICIDE RESISTANCE : AN INDUSTRY VIEWPOINT

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ABSTRACT

Resistance is discussed from the technical, commercial and political perspective of the agrochemical industry. Major areas of concern are the misuse of resistance terminology, interpretation of simulation models, and field research needs, in particular inadequate monitoring and the relating of laboratory results to field performance. Multidisciplinary and interagency research matrices are essential and should include industrial representation. Industry has formed a Pyrethroid Efficacy Group and an Insecticide Resistance Action Committee to give technical advice on resistance.

INTRODUCTION

My objective in this paper is to re-assess our approach to problems arising from insecticide and acaricide resistance in the field. My viewpoint is that of an industrial biologist concerned with the responsible, international development of insecticide products. I do not intend to discuss the genetic, biochemical, toxicological or other basis of any particular resistance problem. I do intend to put our approach to these and other disciplines impinging on the study of resistance into a realistic technical, commercial and political perspective.

There are five major areas of concern to the agrochemical industry:

1. Terminology
2. Interpretation of simulation models
3. Field research needs
4. Multidisciplinary and interagency research
5. Industry actions and policy.

TERMINOLOGY

Resistance is a term that can easily be misused in an emotive or unintentionally misleading manner. Reports of resistance in insects are often exaggerated. Literature citations (e.g. Georghiou 1980) are not a reliable basis for assessing the quality of reports of resistance and usually do not place them in a realistic economic context.

It is important to distinguish between field resistance and laboratory resistance. There is no case for equating relatively minor variations in susceptibility with commercial field failure. Nevertheless, laboratory resistance data are important indicators of potential problem areas.

"Field resistance" is the failure of a product, applied correctly at the recommended rate, to give adequate control, due to a heritable genetic change allowing survival of pest numbers above an economic threshold. The term "Laboratory resistance" should be used to refer to other studies in which susceptibility is shown to be significantly reduced. This terminology is consistent with that used by the Fungicide Resistance Action Committee (Delp 1984). Unfortunately it does little to resolve the grey areas where inadequate control due to resistance is impossible to prove because of the absence of or lack of correlation with laboratory baseline data. Resistance and its control are variables depending, within limits, on economic as well as biological and operational factors (see Sawicki and Denholm 1984 for an examination of the criteria for defining resistance).

"Resistance risk" is another term which could be misused or misunderstood. According to Georghiou (1980) we must develop the capability of quantifying the risk of resistance to a chemical by a pest in a given situation. We should be wary of assuming that we already possess this capability. Although laboratory and simulation studies can give an indication of relative "risks", we are some way away from confidently assessing the genetic, biological and operational factors contributing to the risk of resistance arising in most field resistance situations. It is as much the way it is used as a property of the chemical itself. There is no case for incorporating resistance risk criteria in registration protocols, given these uncertainties.

INTERPRETATION OF SIMULATION MODELS

Simulation modelling has contributed significantly to our general understanding of resistance dynamics (Georghiou 1980). Models are essential. In some situations, such as assessing strategic options before resistance has developed, there is no other way as we cannot induce resistance in the field. However, strategic options may become tactical recommendations in a specific resistance situation. In the absence of field data, conclusions from mathematical models are frequently drawn upon in devising resistance management strategies. Agrochemical Companies have voluntarily complied with such strategies even where they have not been imposed by a regulatory authority. (I will give some examples later.) It is therefore pertinent to question the extent that general assumptions about pest characteristics affect the conclusions or recommendations made for specific management strategies. The relative importance of such factors in the build up of resistance could influence the choice of strategy as we may have to select only one from several options.

Despite obvious difficulties, assumptions must be tested where possible in the field. Or, preferably, information gathered in the field used to input real values into models. For example, most models assume constant fitness sets. But, following extensive field estimates of the trend in relative viabilities of insecticide resistant genotypes of the Australian sheep blowfly, Lucilia cuprina, McKenzie and Whitten (1982, 1984) concluded that models which assume constant fitness sets may have limited usefulness in ecological genetic studies on pesticide resistance.

Consequently, there is a lack of real data, a lack of agreement on how to use these data, and therefore a limited usefulness for general assumptions. Even so these have had to be used in practice. Hopefully these comments will stimulate suggestions for future areas of research.

FIELD RESEARCH NEEDS

A basic problem is the inadequacy or total absence of monitoring data where field failures occur and resistance is suspected. It is essential to establish realistic baselines from field monitoring under average operational conditions when control is adequate. At least, this will provide a baseline in the field. At best, it may be possible to predict the onset of resistance. But even rudimentary discriminating dose studies are beyond the resources of many countries. The agrochemical industry has recognised this problem and begun development of simple test kits suitable for unsophisticated field use (Collins, unpublished; Watkinson et al. 1984). Discriminating dose tests are relatively insensitive, have a short lead time and are not very predictive. A considerable refinement in monitoring techniques is required to overcome the problem of detecting very low resistant gene frequencies in the field. We are also ignorant of appropriate methods for determining resistant gene frequency thresholds in the field. By threshold, I mean a level of control below which chemical control is uneconomic. This may well be variable depending on economic or operational factors such as commodity market price or availability of alternative control agents (Sawicki & Denholm 1984). One objective of future research should be to establish the relationship between laboratory test results and field performance. I do not offer any solution to these problems but highlight them as worthy of investigation.

Operational factors relating to resistance are fully reviewed by Georghiou (1983). Ideally these should be tested in field trials but practical constraints are limiting. However, sufficient input from field data is needed to make models useful. Particular practical questions which always arise and need fuller field evaluation are those relating to insecticide dose rate (high or low), mixtures and alternation of chemical groups in insecticide spray programmes.

MULTIDISCIPLINARY AND INTERAGENCY RESEARCH

The problem of resistance is too vast, expertise too limited and time too short to allow significant progress by individual effort. A multidisciplinary research matrix is needed. (Such a team might include industrial chemists, field biologists, toxicologists, population geneticists, mathematical modellers, commercial co-operators and so on. Last but not least, scientific teams always seem to ignore the despised economist!).

Industry has co-operated successfully in such schemes; a particularly good example being the joint working party convened by the Australian Wheat Board (AWB) to evaluate alternative grain protectants on wheat and sorghum following widespread resistance to malathion in the early 1970's.

The co-operative programme has been successful in realising its objectives, replacing malathion with organophosphate/pyrethroid mixtures. Alternative chemicals have also been developed as a reserve (Bengston 1981). It is hoped that this tactical approach will bridge the gap until the longer term objective of insecticide-free storage is achieved. The organisational matrix of the working party is shown in Figure 1.

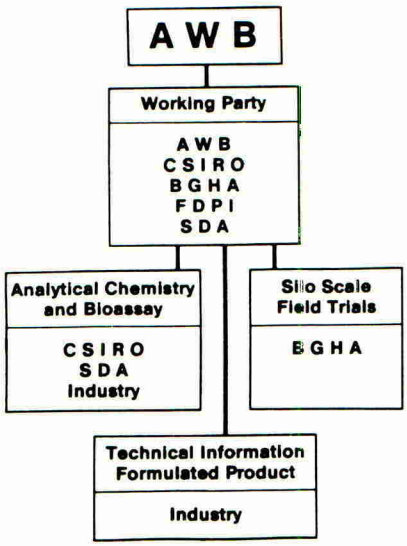

Fig. 1. Australian Wheat Board (AWB) interagency working party.
Key: BGHA - Bulk Grain Handling Authorities
CSIRO - Commonwealth Scientific and Industrial Research Organisation
FDPI - Federal Department of Primary Industries
SDA - State Departments of Agriculture

Rather than develop a specific strategic model to combat resistance, the objective of the programme was to meet the nil-tolerance imposed by the Australian Department of Primary Industries, for live insects in export grain. The time limit was short as major export markets were threatened by infested grain. In order to meet these short-term objectives research and development was target orientated. Biologists developed bioassays of direct relevance to the field. Chemists developed the appropriate analytical techniques. Industry co-operated as new candidate compounds became available. The scale of any particular contribution varied according to the stage of the project with the Queensland Department of Primary Industry and CSIRO shouldering responsibility for co-ordinating biological and chemical assays.

There were three major characteristics of the working party:

1. The objective and time limit were clearly defined
2. The structure was temporary and flexible
3. Although the agrochemical industry contributed significantly by supplying information, compounds, bioassay and residue analysis, it was not included in the working party.

The benefits of the first two characteristics should not be underestimated. Those of the third are questionable. It meant that whilst there was full and frank discussion of results at the individual working scientist level, industry was excluded from participating in joint discussion of results. Clearly the reason was to exclude industrial pressure for any particular compound from recommendations to the AWB. However, this could have been achieved by convening a separate advisory panel. Allocating material, time and resource to such a scheme can involve considerable investment risk. The conditions of participation need to be clearly stated at the outset. There should be a high level of information exchange between working scientists.

It is notable that industry representatives were also excluded from the Council for Agricultural Science and Technology (CAST) task force which reviewed resistance for Congress and the Environmental Protection Agency in the USA (Anon. 1983). This is regrettable as industrial compliance, preferably by co-operation, is imperative for the scientific success of resistance management strategies.

Another example of co-operation in Australia followed pyrethroid resistance developing in the field to Heliothis armiger in 1983 (Gunning et al, 1984). A rotational three stage strategy was devised which limited pyrethroid use to a 42 day period in Stage 2, conventionally a peak period for H. armiger attack on cotton. This was an example of an intuitive strategy based on general assumptions from simulation modelling.

Although voluntary, compliance with this strategy was substantial in 1984 by both the agrochemical industry and end users. In the 1984 season there were no failures attributed solely to resistance, although resistant individuals were found in one crop which had clearly not been sprayed correctly. Despite the lack of commercial field failures, sampling showed an overall level of 10 - 15% individuals surviving a discriminating dose for pyrethroids in laboratory tests, although results were very heterogeneous (Gunning, Forrester, unpublished). The percentage of resistant individuals sampled remained comparable throughout stages 1, 2 or 3 of the strategy. When resistance occurred in the field in 1983 Australia was in the grip of severe drought. In contrast, the 1984 season was cool and wet, and pest pressure relatively low to moderate. We do not know if the sampling accurately reflects the situation in the field. The major climatic changes which occurred may well have been responsible for any success attributed to the strategy, although the a reduction in pyrethroid use is claimed as a benefit. Industry has agreed, not without dissent, to a similar strategy for the 1985 season. However, it is essential that more

sensitive methods be developed for monitoring gene frequency and establishing the influence that operational factors have on its manifestation and expression in the field.

A similar co-operative response was obtained from Industry in the UK when photostable pyrethroids were voluntarily withdrawn from intensive animal houses to assist rational management of pyrethroid resistant houseflies (Denholm et al, 1983).

This is not to say that the agrochemical industry necessarily agreed amongst themselves over the wisdom of the proposed measures or were not reluctant in some instances to participate or will continue to cooperate if results are not positive. Nevertheless the long term benefits of short term restraint are accepted by the agrochemical industry sector, at the international level, who have taken positive actions to contribute. These economic benefits are perceived to be in product defence and safeguarding the good name of companies or their products. However, if longer term economic benefits are not apparent, or if the scientific basis of any proposed strategy is questionable, there is no particular reason why voluntary compliance with such endeavours should continue.

INDUSTRY ACTIONS AND POLICY

Industry cannot complain of being excluded from co-operative activity on resistance if it lacks appropriate representation and policy. In 1979 pyrethroid manufacturers established a Pyrethroid Efficacy Group (PEG) of technical representatives to examine technical information and produce recommendations which it believed would extend the life of pyrethroids in the event of resistance arising.

This group was active in securing compliance in the UK animal house situation and advising on Heliothis resistance in Australia in 1983. The International Group of National Associations of Manufacturers of Agrochemical Products (GIFAP) has recently formed an Insecticide Resistance Action Committee (IRAC) which will complement the fungicide committee (FRAC). The objectives of IRAC are to provide technical advice to GIFAP; to develop relationships with non-industrial researchers; to advise and assist GIFAP in representing an industry view of resistance; and to coordinate industry efforts to prolong the life of insecticide and acaricides by recommending appropriate technical strategies to combat resistance. One proposal is that IRAC undertake to classify economically relevant and clearly proved cases of resistance according to their local or inter-regional importance, rather than rely on a compilation of literature citations, (Zoebelein, unpublished). Clearly, these two bodies will provide an industrial technical resource which should be utilised by those in the public sector involved in resistance studies.

It would be idle to pretend that such technical co-operation between otherwise competing industries is not subject to constraints. Company co-operation in any sphere lends itself to suspicion of the motives of

those involved. Whilst these groups tread a narrow line as far as restrictive practices legislation is concerned; paradoxically, the more that is achieved technically the less likely it is that any such problems will arise. I do not, however, offer this as a considered legal opinion. More relevant perhaps are intra-company problems. Whilst the technical side of a company may agree on the wisdom and good sense of a measure, their commercial colleagues may not quite see it that way. This however, is not as great a problem as influencing the technical recommendations at the independent distributor levels whose salesman in the field may have a totally different time perspective. Nevertheless, the examples I have quoted demonstrate that these constraints can, and have been, overcome.

CONCLUSION

Insecticides and acaricides will be essential for agriculture and public health in the forseeable future. The principle of conserving pesticides as a non-renewable resource is accepted by the agrochemical industry. It is essential that research on resistance management strategies be targetted on resolving particular problems in the field. Resistance is a challenge to the applied biologist. It is a rare example within the evolutionary paradigm where a major factor, pesticide use, is totally controllable by man. It is essential for the resolution of these problems that interdisciplinary research matrices are established which include industry. I hope that I have been able to suggest a framework for such future interaction.

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REFERENCES

Anon. (1983) The resistance of agricultural pests to control measures. Council for Agricultural Science and Technology Report No 97.

Bengston, M. (1981) Recent developments with grain protectants. Proceedings First Australian Stored Grain Pest Control Conference 7, 1-5.

Delp, C. J. (1984) Industry's response to fungicide resistance. Crop Protection 3, (1), 3-8.

Denholm, I.; Farnham, A. W; O'Dell, K.; Sawicki, R. M. (1983) Factors affecting resistance to insecticides in house-flies, Musca domestica L. (Diptera: Muscidae). 1. Long-term control with bioresmethrin of flies with strong pyrethroid resistance potential. Bulletin of entomological Research 73, 481-489.

Georghiou, G. P. (1980) Insecticide resistance and prospects for its management. Residue Reviews 76, 131-145.

Georghiou, G. P. (1983) Management of resistance in arthropods. In Georghiou, G. P. and Saito, T. (Eds) Pest Resistance to Pesticides Plenum Press, New York, 769-792.

Gunning, R. V.; Easton, C. V.; Greenup, L. R.; Edge, V. E. Pyrethroid resistance in Heliothis armiger (Hubner) (Lepidoptera: Noctuidae) in Australia. Journal of Economic Entomology in press.

McKenzie, J. A.; Whitten, M. J. (1982) Selection for insecticide resistance in the Australian sheep blowfly, Lucilia cuprina. Experentia 38, 84-85.

McKenzie, J. A.; Whitten, M. J. (1984) Estimation of the relative viabilities of insecticide resistance genotypes of the Australian sheep blowfly, Lucilia cuprina. Australian Journal Biology Sciences (37), 45-52.

Sawicki, R. M.; Denholm, I. (1984). Adaptation of insects to insecticides. In Evered, D. and Collins, G. M. (Eds.) Origins and Development of Adaptation, Pitman Books, London (Ciba Foundation Symposium 102) 152-166.

Watkinson, I.A.; Wiseman, J.; Robinson, J. A simple test kit for field evaluation of the susceptibilities of insect pests to insecticides. Proceedings British Crop Protection Conference Pests and Diseases 1984 (in press).