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EFFECTS OF SEQUENTIAL RESISTANCE ON PESTICIDE MANAGEMENT

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Summary Some populations of key insect pests readily develop resistance to each successive insecticide used for their control. Each insecticide selects one or more mechanisms of resistance which differ sometimes very widely in the number of insecticides to which they confer cross-resistance. An incorrect or unlucky choice of compound can lead to the selection of mechanisms conferring cross-resistance to most or all chemicals of that particular group or to compounds from different groups and thus waste valuable insecticides. These factors are discussed in the light of work done on the sequential resistance in the housefly.

INTRODUCTION

Resistance is one of the most intractable problems facing insect control. It shortens the useful life of insecticides and cross-resistance eliminates not only close analogues but often unrelated pesticides. The seemingly limitless persistence of resistance genes prevents the long term re-use of insecticides against populations which have apparently reverted to full susceptibility (Keiding, 1967a). Because of this, established resistance can be dealt with only by switching to alternative pesticides to which there is no resistance. This however is a transient solution because in time resistance develops to the alternative which must then be replaced by yet another compound. Each new insecticide selects in turn one or more mechanisms of resistance, and each mechanism usually confers resistance to several insecticides. Hence sequential introduction of alternative compounds can lead to widespread cross-resistance and to the rapid elimination of possible alternative insecticides. This has already happened with some major pests of agricultural and medical importance, repeatedly treated with, and now resistant to most insecticides, e.g. the cattle tick (Boophillus microplus)(Schnitzerling et al.,1974), the cotton leafworm (Spodoptera littoralis), the housefly (Musca domestica)(Georghiou et al., 1972, Keiding, 1975a).

It is seldom realised how important the choice of the alternative insecticide can be in terms of subsequent control. An incorrect or unlucky choice can lead to the selection of mechanisms which confer resistance to all or nearly all members of the same chemical group or to widely differing chemicals and in both cases valuable materials are wasted. Unfortunately, the consequences of any particular choice cannot yet be predicted because not enough is known about sequential resistance, which is very complex. The study of sequential resistance is thus indispensable to determine the order in which insecticides should be used to minimise the effects of cross-resistance.

Sequential resistance is complex because it is the product of several different resistance mechanisms, each with its own cross-resistance spectrum. These mechanisms often interact unexpectedly and this distorts or conceals their diagnostic features so that the combined resistance spectra reveal little about the mechanisms present (Sawicki, 1974). Sequential resistance can be understood fully and typed correctly only through resolution into its individual constituents by a combination of genetic and biochemical techniques. Each isolated mechanism can then be investigated and interactions can be studied by genetically resynthesising the original resistance from its components (Sawicki, 1970; Georghiou, 1971). It is also possible to deduce from such work the approximate sequence in which the resistance genes have been selected by insecticides used in the field, and this can be useful when deciding on the choice of suitable alternatives.

Work on fundamental aspects of sequential resistance can be done reasonably easily only with insects that develop resistance to many compounds, have a well documented history of sequential resistance in the field, are easy to rear in the laboratory, and have a short life cycle, relatively few chromosomes and good visible mutant markers. Of the roughly 250 species of insect pest known to have developed resistance to insecticides, only the housefly (Musca domestica)fulfils all these conditions, and was thus used as the test species for our studies on sequential resistance at Rothamsted. Most of the strains used were sent to us by Dr. Keiding of the Danish Pest Infestation Laboratory. They had been collected at various times on farms where Keiding has been assessing the effectiveness of insecticides for fly control since 1948. Thanks to his work we were provided with very thoroughly documented material.

History of sequential resistance of the housefly on Danish farms

Keiding (1975a)reports that the DDT-resistance developed in 1947 and became widespread the following year (Fig.1). Strong resistance to lindane and chlordane soon followed and by 1950 parathion impregnated strips and/or pyrethrum/piperonyl butoxide (pb) space sprays were introduced for fly control. Resistance to diazinon, the first organophosphorus (OP) insecticide to be used in residual sprays (1953) was detected in 1955, two years after its introduction. At first resistance was moderate (10-20 fold) and regressed during the winter when insecticides were not used. Following further use however it increased generally to about x 40-60 to reach x 80-200 on farms treated very frequently with diazinon. By 1957 resistance was widespread and this compound no longer recommended. For the next 3-4 years residual sprays of fenchlorfos and fenthion and sugar baits with trichlorfon controlled the flies with varying degrees of success. The introduction of dimethoate in 1963 completely changed the prospects for fly control. This compound gave such excellent results that within two years it became the dominant insecticide on Danish farms. Resistance to dimethoate developed very slowly during the following 5-7 years, but in 1970 there was an unexplained sudden and great increase in resistance (up to x 75) on some trial farms. By the following year it had increased further and has persisted, although dimethoate has now been replaced on many farms by natural and synthetic pyrethroids. The sudden increase in resistance to dimethoate was paralleled by a corresponding increase to fenitrothion, fenthion and bromophos and where these insecticides were used resistance to dimethoate increased.

The use of pyrethrum/pb space sprays in the 1971 trials led to a 20-40 fold resistance at LD_{95} at the end of that year on a few farms and to similar strong resistance on most other trial farms by the end of the following year. Much of it persisted into 1974 showing that pyrethrum resistance can sometimes acquire considerable stability within a short period.

INSECTICIDAL USAGE AND RESISTANCE ON DANISH FARMS

from J. KEIDING (1974)

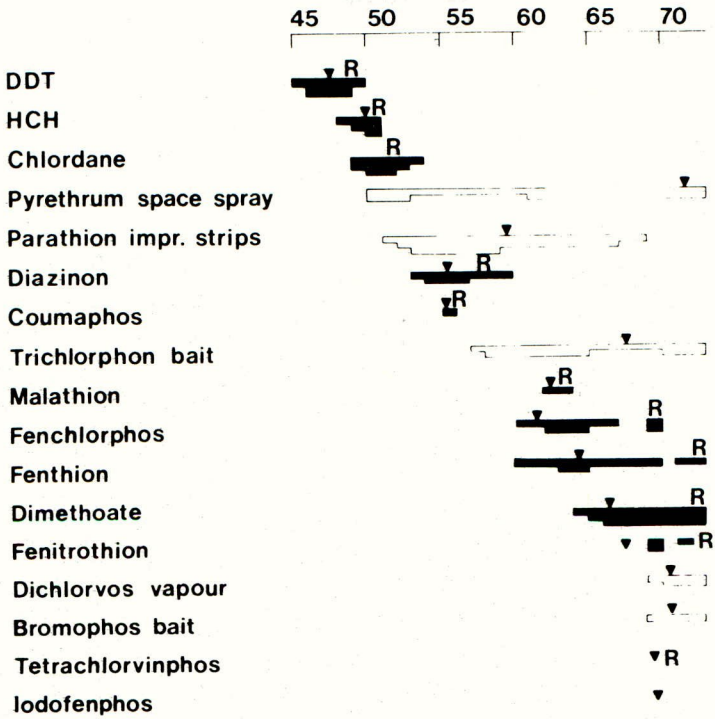

Fig. 1. Country-wide use of insecticides for housefly control on Danish farms 1945-72 and development of resistance (After J.Keiding 1974)

Treatments:

The insecticides were used as residual sprays except where other applications (impregnated strips, space sprays, paint-on baits, or vapour generators) are indicated.

The width of each band indicates the extent to which the insecticide concerned was used, from relatively few to the majority of Danish farms.

Occurrence of resistance:

▽ First confirmed case(s) of resistance of practical importance.

R Resistance causing control failures occurs on the majority of farms.

Some OP insecticides, e.g. coumaphos, malathion and tetrachlorvinphos were never widely used in Denmark because they failed to control houseflies on trial farms even during the first season, and there was usually some resistance to them before they had been used for the first time. Similarly carbamates were never introduced for fly control because resistance to these insecticides developed so readily. (Carbamates are seldom very effective as adulticides and even slight resistance can lead to lack of adequate control). Recent tests have shown also that dimethoate-resistant flies resist methoprene and dimelin. There appears to be now no effective insecticides left against houseflies in Denmark and fly control is very difficult.

Genetics of sequential resistance

We examined the genetics and biochemistry of resistance of several strains of houseflies collected by Keiding at various intervals on his trial farms and were able to correlate the presence of certain resistance genes or factors with the control measures used in the field and with Keiding's results in the laboratory. It was thus possible to establish from field data and our own and other studies of the genetics of resistance a picture of the development of sequential resistance in Denmark. Although tentative, speculative and incomplete it helps to explain many aspects of the history of fly control on Danish farms over the last 25 years.

We used for our work a diazinon-resistant strain collected in 1958 (strain 203d, a parent of strain SKA), a dimethoate-selected strain collected in 1970 (strain $49r_2b$), a tetrachlorvinphos-selected strain collected in 1969 (strain $39m_2b$) and two pyrethrum-resistant strains, one collected in 1958 (213ab), the other in 1972 (290). We are examining three additional strains collected in the 1960s.

DDT selected two mechanisms of resistance and one non specific intensifier: (i) the most important, kdr, conferring knockdown resistance (Milani & Travaglino, 1957; Keiding, 1957), (ii) DDT-dehydrochlorinase (DDT-ase) of an intermediate type (Oppenoorth, 1965). It may have also selected DDTmd, a sesamex suppressible microsomal detoxication (Oppenoorth, 1967; Sawicki & Farnham, 1968), a mechanism recovered from the diazinon resistant strain 203d. Each conferred singly weak to moderate (up to x 10) resistance but interactions between these (Grigolo & Oppenoorth, 1966) and the non-specific modifier, pen (Hoyer & Plapp, 1968; Sawicki & Farnham, 1968) an unknown mechanism which delays the entry of non-polar insecticides into insects, resulted in very strong resistance to organochlorine insecticides. The three mechanisms had very distinct cross-resistance spectra: DDT-ase detoxified only DDT-like analogues that could be dehydrochlorinated (Perry, 1964), a DDT-md conferred cross-resistance to methoxychlor and a few OPs, e.g. diazinon (Sawicki & Farnham, 1968) and kdr conferred resistance to most if not all compounds of the DDT group and to pyrethroids (Plapp & Hoyer, 1968). Almost certainly the presence of these mechanisms in Danish populations of houseflies and their selection by DDT led to unforeseen difficulties in later years when other insecticides were used for fly control.

The development of resistance to lindane and chlordane had no known subsequent repercussions because this resistance was specific only for cyclodiene insecticides and lindane.

The widespread use of parathion impregnated strips, on about 200,000 farms in 1952 (Keiding, 1957) to control cyclodiene and DDT-resistant flies led to the selection of gene a (phosphatase) (Oppenoorth, 1959) and two other OP resistant genes (Lewis & Sawicki, 1971; Oppenoorth, 1972) also on chromosome 2. These three genes are collectively referred to in this paper as 'gene a'. The parathion strips worked well for over 12 years and resistance to parathion remained low, because the resistance conferred by 'gene a' is moderate even when homozygous (Sawicki &

Farnham, 1968) and on most farms resistance was probably heterozygous because the strips control houseflies and thus select for resistance less effectively than residual sprays.

Strong resistance to diazinon occurred because diazinon selected not only 'gene a', which confers resistance to several OPs (Table 1), but also DDT-md which parathion did not (Sawicki & Farnham, 1968). Presumably DDT-md, the mechanism conferring resistance to both diazinon and DDT had been selected 7-10 years earlier during treatment with DDT. Together 'gene a' and DDT-md conferred strong diazinon resistance (Sawicki, 1973), but since DDT-md is ineffective against parathion, even flies strongly resistant to diazinon were only moderately resistant to parathion (Keiding, 1960). Where diazinon was used most frequently the flies became exceptionally resistant to this compound due to the selection of homozygosity or near-homozygosity of resistance genes on chromosomes 2 and 5 ('genes a' and DDT-md) and to the selection of the delayed penetration mechanism which strongly enhances resistance conferred by 'gene a' (Sawicki, 1970). Thus where resistance was highest many of the flies were probably homogeneous for 'gene a', DDT-md and delayed penetration. Where resistance was intermediate there was considerable heterogeneity of these three mechanisms in the fly populations, and in the early stages of selection, resistance was weakest because all the populations were heterozygous for the three resistance genes.

Delayed penetration is a very important intensifier of resistance both in houseflies (Sawicki, 1970; Hoyer & Plapp, 1971) and in other insect pests (Szeicz et al., 1973) and was probably particularly effective in magnifying the efficacy of the hydrolytic phosphatase controlled by 'gene a' which has a low Km (4×10^{-9}M) (Welling et al, 1971) and works even at very low substrate concentrations. This phosphatase was unlikely to be effective against OPs that penetrate very rapidly, and are not retarded significantly by the delayed penetration mechanisms, e.g. dimethoate (Devonshire, 1973), and this may partly explain why dimethoate controlled parathion- and diazinon-resistant flies so successfully. Dimethoate remained very effective against Danish houseflies for 5-7 years during which time the hydrolytic phosphatase declined and became rare, although we recently found it in a bromophos-resistant strain collected by Keiding in the field in 1964 (Keiding, 1967b). By 1968 it seems to have been replaced by new mechanisms selected by dimethoate which greatly extended the OP resistance spectrum of the Danish flies (Table 2). Although these flies retained resistance to diazinon and parathion, this was now conferred by dimethoate-selected mechanisms and not 'gene a' (Table 1) (Sawicki, 1974).

Dimethoate appears to have selected the following new mechanisms:-
on chromosome 2 -

(i) a modified acetylcholinesterase (AChE) less sensitive to inhibition by OPs than the susceptible enzyme, controlled by gene $AChE_R$ co-dominant with the wild-type gene (Devonshire, 1975),

(ii) a sesamex-suppressible mechanism controlled by gene D, about 20 units from the marker ar and close to $AChE_R$. Together these mechanisms confer weak to moderate resistance to many OPs (Table 2).

and on chromosome 5 -

(iii) a sesamex-suppressible mechanism conferring slight resistance to trichlorfon, DDT and barely detectable resistance (about 1.5 x) to other OPs such as parathion and dimethoate (Sawicki, 1974). This mechanism and another sesamex-suppressible mechanism of resistance to tetrachlorvinphos and DDT, isolated from a tetrachlorvinphos-selected strain (39m$_2$b) may be allelic with gene DDT-md referred to earlier. DDT-md type mechanisms probably increase the resistance of mechanisms controlled by genes on chromosome 2.

Table 1

Cross-resistance of flies with "gene a" to OP insecticides used by Keiding ("gene a" was probably the major mechanism of resistance to most OPs used up to 1965)

Insecticide	LD50 µg/fly Susceptible	"Gene a"	Resistance factor
Parathion	0.026	0.39	15
Diazinon	0.040	0.52	13
Trichlorfon	0.024	0.050	2
Malathion	0.68	0.95	1.4
Fenchlorphos	0.044	0.34	8
Fenthion	0.050	0.052	1
Dimethoate	0.010	0.0095	1
Fenitrothion	0.037	0.13	3.5
Bromophos	0.023	0.37	16
Tetrachlorvinphos	0.038	0.035	1
Iodophenphos	0.044	0.24	5

Table 2

Cross-resistance of flies with genes D and $AChE_R$ to OP insecticides used by Keiding (genes D and $AChE_R$ were probably the major genes to most OPs used after 1965)

Insecticide	LD50 µg/fly Susceptible	Genes D and $AChE_R$	Resistance factor
Parathion	0.015	0.090	6
Diazinon	-	-	-
Trichlorfon	0.024	0.14	6
Malathion	0.70	1.40	2
Fenchlorphos	-	-	-
Fenthion	0.039	0.28	7
Dimethoate	0.010	0.32	32
Fenitrothion	0.027	0.40	15
Bromophos	0.029	0.18	6
Tetrachlorvinphos	0.038	0.080	2
Iodophenphos	0.028	0.16	6

We have not been able to separate the gene controlling the modified AChE from the sesamex-suppressible gene D, but indirect evidence suggests that singly these mechanisms are weak and must be present together to confer moderate to strong resistance (Devonshire & Sawicki, 1974). It is interesting to note that $AChE_R$ and gene D confer only weak resistance to tetrachlorvinphos, and this is possibly why Keiding found dimethoate-resistant flies susceptible to tetrachlorvinphos (Keiding, 1969). The correlation between the resistance to dimethoate, fenthion and fenitrothion reported by Keiding (1975b, in press) can be explained because the same resistance mechanisms operate against these insecticides (genes D and $AChE_R$) (Table 3). The genetic analysis also explains why field populations resistant to dimethoate and fenthion were fully susceptible to sesamex, whereas those resistant to trichlorfon or fenitrothion were not; only gene D and the mechanisms on chromosome 5, both sesamex suppressible, are responsible for dimethoate and fenthion resistance. Other genes present in many of the OP resistant populations, e.g. gene M (described later) are also partly responsible for resistance to trichlorfon and fenitrothion, and the mechanisms controlled by these genes are not sesamex suppressible (Sawicki, 1974).

The persistence of resistance to diazinon and parathion in spite of the decline of 'gene a' is attributable to the cross-resistance conferred by gene D and also probably gene $AChE_R$. This diazinon resistance is sesamex suppressible, unlike the diazinon resistance controlled by 'gene a' that developed in 1955 (Sawicki, 1974). The lack of response to sesamex was caused by the opposing effects of this compound on the mechanisms controlled by 'genes a' and DDT-md; sesamex suppresses the action of DDT-md but enhances that of 'gene a' (Sawicki and Farnham, 1968). Our results thus provide a probable explanation why Yasutomi's and Keiding's (1968) observations that sesamex-synergized diazinon was effective against diazinon-resistant flies collected in 1968 but was without effect against diazinon-resistant strains collected 10 years earlier.

Attempts to control dimethoate-resistant houseflies with tetrachlorvinphos in 1969 proved impractical because houseflies became resistant very rapidly to this compound (Keiding, 1970). Resistance to tetrachlorvinphos is caused by an incompletely known mechanism, unaffected by synergists or inhibitors of mfos (mixed function oxidases) or esterases (Keiding, 1970; Sawicki, 1974) which confers slight resistance to dimethoate (Sawicki, 1974). It is controlled by gene M which maps close to the marker ar on chromosome 2 but has not yet been separated from other genes of resistance to OPs also on chromosome 2, and its complete cross-resistance has not been determined. The mechanism controlled by gene M may be homologous with that which conferred resistance to malathion in 1959 (Keiding, 1963). Preliminary work on a malathion-resistant strain (strain 153a,b) collected in the field in 1963 indicates that the malathion selected flies have normal carboxylesterase activity against α-naphthyl acetate and resist tetrachlorvinphos (Sawicki, 1975, unpublished data). Thus flies of this strain had the potential to develop resistance to tetrachlorvinphos nearly six years before it was used for fly control in Denmark. According to Keiding (1973) this resistance is not suppressed by TBTP (S,S,S,tributyl phosphorotrithioate) a carboxylesterase inhibitor. Resistance to both malathion and tetrachlorvinphos developed very rapidly in the field, and for this reason neither insecticide was ever widely used for fly control in Denmark. Gene M may have been responsible for the resistance to both malathion and tetrachlorvinphos and may have thus been selected independently on two separate occasions. If so, this gene appears to have been well established in Denmark even before malathion was first used because resistance to this compound developed very rapidly (Keiding, 1963).

The widespread resistance to dimethoate led in 1971 to a renewed interest in the pyrethroids for fly control, but quite unexpectedly strong resistance to pyrethroids

developed within one season. This was surprising because pyrethrum/pb sprays had been used on Danish farms for over 20 years either to supplement other space sprays or intermittently as the sole control agent and had not led to any significant resistance (Keiding, 1975a). With hindsight it can be seen that warning signs had occurred from time to time, but were not recognised because nothing was known about pyrethrum resistance. Already in 1951 "some DDT-resistant strains seemed to be considerably more resistant to pyrethrum sprays than DDT-susceptible lab strains" (Keiding, 1953). By 1953 aerosols used in Denmark had to contain more pyrethrins than American or British aerosols, because Danish flies resistant to organochlorine insecticides were less susceptible to pyrethroids (Keiding, 1954), and transient pyrethrum resistance developed on some Swedish farms in 1957 (Davies et al., 1958). On these farms pyrethrum/pb dust and aerosols had been used, and in 1962 and 1964 there was some slight resistance to pyrethrum on a few Danish farms on two of which automatic aerosols were used continuously for two seasons (Keiding, 1975).

Several factors contributed to the rapid increase in pyrethrum resistance. They are now apparent from knowledge gained from the genetic analysis of this resistance by Farnham (1973, 1975) and from Keiding's detailed study of the field strains. It seems that the potential for pyrethrum resistance had been present since 1950. The major mechanisms of resistance to pyrethroids, i.e. the recessive gene kdr and the major modifier, delayed penetration were well established long before pyrethroids were used intensively for fly control. Gene kdr, which confers resistance to both DDT and the pyrethroids, an observation first made 24 years ago by Busvine (1951), was selected when DDT was used for fly control in the late 1940s. Delayed penetration has been detected in Danish strains of houseflies on several occasions and must have been established for many years (Sawicki & Farnham, 1968). The continuous selection in 1971 with pyrethrum/pb space sprays (up to 50 treatments during the six-month spray season) ensured the selection of kdr and other resistance genes and intensifiers, which are all recessive and therefore expressed only when homozygous (Farnham, 1973, 1975). Resistance to piperonyl butoxide, first detected in dimethoate-resistant flies (Sawicki, 1974), weakened the killing power of the pyrethrum/pb formulations and may have helped to concentrate the mechanisms or factors of resistance which singly are weak. In contrast, it seems that in a previous case of pyrethrum resistance in Sweden in 1957, piperonyl butoxide had weakened pyrethrum resistance in that population, because the synergist had suppressed one of the pyrethrum resistance mechanisms without which resistance is only moderate (Farnham, 1973).

The intensive use of pyrethroids has been the major cause of the present strong resistance to these compounds on Danish farms, as emphasised by Keiding (1974). Such resistance is likely to develop where the kdr mechanism is present, which has so far been shown only in the housefly and Culex tarsalis (Plapp & Hoyer, 1968), the only two species examined for this mechanism of resistance. The consequences of strong resistance to pyrethroids must be considered seriously now that the extremely potent photostable synthetic pyrethroids discovered by Elliott and his colleagues (Elliott et al.,1973) are to be used extensively against agricultural pests. It would be most regrettable if the useful life of these outstanding insecticides were to be shortened by unwise or unnecessary usage, especially since this is the only group of compounds available that is really effective against some important resistant pests. Moreover, recent work by Farnham (1975, personal communication) shows that through kdr, resistance to one pyrethroid conveys simultaneous resistance to all other pyrethroids.

A combination of field and laboratory work has improved our understanding of sequential resistance. However, there are still many observations which need to be explained. For example, it is not known why many OP-resistant strains of houseflies resist carbamates, methylene dioxyphenyl compounds (Sawicki, 1974), and juvenile hormone mimics (Cerf & Georghiou, 1972). Sesamex suppressible and

non-suppressible resistance to carbamates is controlled by genes on chromosomes 2 and 5 distinct from the genes that control dimethoate or tetrachlorvinphos resistance and from the gene on chromosome 2 conferring resistance to methylene dioxyphenyl compounds (Sawicki, 1975, unpublished). Nothing has yet been published about the genetics of resistance to the juvenile hormone mimics. Strong carbamate resistance is probably endogenous in Danish strains of houseflies and this is why carbamates could not be used for housefly control. There is no information about the causes or the selection of the other types of resistance, but it is interesting to note that resistance to juvenile hormone mimics has been detected independently in two dimethoate-selected strains of houseflies, one in Denmark (Arevad, 1974) the other in California (Cerf & Georghiou, 1972). It may well be that dimethoate selects unrelated mechanisms of resistance in the same way that parathion selects for resistance to both parathion and DDT, but the inverse does not seem to occur; flies selected with DDT do not develop simultaneous resistance to parathion. The reasons for this cross-resistance are unfortunately not understood. Similarly, it is not known why the prolonged use of parathion strips led to the selection of 'gene a' but not to the selection of genes D and $AChE_R$, although both groups of genes confer resistance to parathion. The order in which insecticides are used seems of paramount importance and had dimethoate been discovered and used before parathion or diazinon it might not have been possible to use these last two compounds on Danish farms because flies would have already been resistant to them. Similarly, parathion could have led to the development of DDT-ase and prevented the use of DDT.

The fact that certain insecticides select unrelated mechanisms of resistance complicates but does not rule out the devising of optimum strategies for sequential use of insecticides. The benefits that can result from the correct sequence are real, and lessons learned from one geographical area can be applied elsewhere if the mechanisms of resistance are identified correctly. It is most probable that the geographical distribution of certain resistance hasapparently not yet been reported in American strains of houseflies and most reports of strong DDT-ase in houseflies have come from the USA; in Europe, kdr appears to be more prevalent than in the USA. In the USA therefore pyrethroids might be used more frequently and be effective longer than in Denmark. Resistance to malathion in the USA was caused mainly the the TBTP-suppressible carboxylesterase, but this type of resistance is less common in Denmark, which is why both malathion and tetrachlorvinphos could be used for housefly control in the USA but not in Denmark.

Lessons learned from the development of sequential resistance in the housefly should not be lost; although sequential resistance cannot be avoided with present control methods and there is no way of foretelling the cross-resistance of newly introduced compounds, resistance can be delayed and costly and unnecessary mistakes prevented when experience already gained is put to good use. For this, the accurate identification or typing of existing resistance mechanisms is a prerequisite. Different types of resistance have been identified very successfully and used to type resistance in the field in the cattle tick, Boophillus microplus (Schnitzerling et al., 1974), stored product pests (Green, 1975), some mosquitoes, (Georghiou et al., 1975) and in the peach potato aphid Myzus persicae (Needham & Sawicki, 1971). So far, however, very little has been done on characterising resistance in most pests of agricultural importance. Yet this is where most insecticides are used and where the incorrect choice of alternative compounds is likely to be most damaging for both insecticide users and producers.

The work on sequential resistance has shown that the correct choice of alternative insecticides is extremely important and that it is already possible to chose the right alternative. Unless this is realized problems caused by resistance will get worse.

Table 3

History of insecticidal usage and development of resistance on Danish farms

Year	Insecticide	Resistance genes			
		DDT ase	DDT md	kdr	
1946	DDT				
1948	cyclodienes	Dld_4			
1953	parathion	a			
1954	diazinon	x	x		
1953	malathion	carboxylesterase			M
1961	dimethoate	$AChE_R$ and D			
1960s	fenthion	x	x		
1969	fenitrothion	x	x		
1969	tetrachlorvinphos	x			x
1971	pyrethroids	several		x	

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References

AREVAD, K. (1974) Juvenile hormone mimics against housefly larvee. Danish Pest Infestation Laboratory Annual Report for 1973, p.42.

BUSVINE, J.R. (1951) Houseflies resistant to a group of chlorinated hydrocarbon insecticides, Nature (London), 174, 783.

CERF, D.C. and GEORGHIOU, G.P. (1972) Evidence of cross-resistance to a juvenile hormone analogue of some insecticide-resistant houseflies, Nature (London), 239, 401.

DAVIES, M. et al (1958) Resistance to pyrethrins and to pyrethrin-piperonyl butoxide in a Wild strain of Musca domestica L. in Sweden, Nature (London), 182, 1816.

DEVONSHIRE, A.L. (1973) The biological mechanisms of resistance to insecticides with special reference to the housefly, Musca domestica and aphid, Myzus persicae, Pesticide Science, 4, 521.

DEVONSHIRE, A.L. (1975) Studies of the acetylcholinesterase from houseflies (Musca domestica L.) resistant and susceptible to organophosphorous insecticides, Biochemical Journal, 149, 463.

DEVONSHIRE, A.L. and SAWICKI, R.M. (1974) The importance of the decreased susceptibility of acetylcholinesterase in the resistance of houseflies to organophosphorous insecticides, Environmental Quality and Safety (in the press)

ELLIOTT, M. et al (1973) A photostable pyrethroid, Nature, (London), 246, 169.

FARNHAM, A.W. (1973) Genetics of resistance of pyrethroid-selected houseflies Musca domestica L., Pesticide Science, 4, 513.

FARNHAM, A.W. (1975) Resistance of houseflies to pyrethroid insecticides, Rothamsted Experimental Station Report for 1974, I, p.143.

GEORGHIOU, G.P. (1971) Isolation, characterization and resynthesis of insecticide resistance factors in the housefly, Musca domestica, Proceedings Second International I.U.P.A.C. Congress of Pesticide Chemistry (A.S. Tahori, ed.) II, 77-94. Gordon and Breach Science Publishers, New York.

GEORGHIOU, G.P. et al. (1972) Insecticide resistance in houseflies in California, Californian Agriculture, Sept., 72, 4.

GREEN, A.A. (1975) Resistance as a problem in the control of insects and mites infesting stored products in Britain, Proceedings of the 4th British Pest Control Conference, St. Helier (in the press)

GRIGOLO, A. and OPPENOORTH, F.J. (1966) The importance of DDT-dehydrochlorinase for the effect of the resistance gene kdr in the housefly, Musca domestica, L., Genetica, 37, 159.

HOYER, R.F. and PLAPP, F.W. (1968) Insecticide resistance in the housefly: identification of a gene that confers resistance to organotin insecticides and acts as an intensifier of parathion resistance, Journal of Economic Entomology, 61, 1269.

HOYER, R.F. and PLAPP, F.W., Jr. (1971) Insecticide resistance in the housefly: effect of a modifier gene in combination with major genes which confer resistance, Journal of Economic Entomology, 64, 1051.

KEIDING, J. (1953) Testing of insecticides. Fly sprays. Danish Pest Infestation Laboratory Annual Report for 1950-1951, p.26.

KEIDING, J. (1954) Testing of insecticides. Insecticides against houseflies. Danish Pest Infestation Laboratory Annual Report for 1951-1952, p.25.

KEIDING, J. (1957) Control of houseflies. Danish Pest Infestation Laboratory Annual Report for 1954-1955, p.39.

KEIDING, J. (1960) Investigations of the relations between house fly control and resistance to insecticides on farms. Danish Pest Infestation Laboratory Annual Report for 1956-1957, p.54.

KEIDING, J. (1960) Resistance to organophosphorous compounds in various farms reporting unsatisfactory effect. Parathion resistance. Danish Pest Infestation Laboratory Annual Report for 1956-1957, p.58.

KEIDING, J. (1963) Investigation of housefly resistance to insecticides in Danish farms. Malathion. Danish Pest Infestation Laboratory Annual Report for 1959-1960, p.38.

KEIDING, J. (1963) Investigation of housefly resistance to insecticides in Danish farms. Parathion. Danish Pest Infestation Laboratory Annual Report for 1961-1962, p.32.

KEIDING, J. (1967a) Persistence of resistant populations after research of selection pressure, World Review Pest Control, 6, 115.

KEIDING, J. (1967b) Development of housefly resistance to fenitrothion and bromophos in the laboratory and in the field, World Health Organization/VBC/67. 2, 1-12.

KEIDING, J. (1970) Insecticide resistance in houseflies. Tetrachlorvinphos. Danish Pest Infestation Laboratory Annual Report for 1969, p.46.

KEIDING, J. (1973) Effect of synergists on resistance. Danish Pest Infestation Laboratory Annual Report for 1972, p.41.

KEIDING, J. (1974) Insecticide resistance in houseflies. Danish Pest Infestation Laboratory Annual Report for 1973, p.34.

KEIDING, J. (1975a) Problems of housefly control due to multi-resistance to insecticides, Journal of Hygiene, Epidemiology, Microbiology and Immunology (Prague) (in the press)

KEIDING, J. (1975b) (in the press)

LEWIS, J.B. and SAWICKI, R.M. (1971) Characterizations of the resistance mechanism to diazinon, parathion and diazoxon in the organophosphorus resistant SKA strain of houseflies (Musca domestica L.), Pesticide Biochemistry and Physiology, 1, 275.

MILANI, R. and TRAVAGLINO, A. (1957) Richerche genetiche sulla resistenza al DDT in Musca domestica concatenazione del gene Kdr (knock-down resistance) con due mutanti morfologici, Rivista di Parasitologia, 18, 199.

NEEDHAM, P.H. and SAWICKI, R.M. (1971) Diagnosis of resistance to organophosphorus insecticides in Myzus persicae (Sulz.), Nature (London), 230, 125.

OPPENOORTH, F.J. (1959) Genetics of resistance to organophosphorus compounds and low aliesterase activity in the housefly. Entomologia Experimentalis et Applicata, 2, 304.

OPPENOORTH, F.J. (1965) Some cases of resistance caused by the alteration of enzymes, 12th International Congress of Entomology, London 1964, 240.

OPPENOORTH, F.J. (1967) Two types of sesamex suppressible resistance in the housefly. Entomologia Experimentalis et Applicata, 10, 75.

OPPENOORTH, F.J. (1972) Degradation and activation of organophosphorus insecticides: studies of their influence in insect resistance, In: 'Toxicology, biodegradation and efficacy of livestock pesticides (Khan, M.A., Haufe, W.O. Eds.) Swets and Zeitlinger, Amsterdam, 1972, 73-92.

PERRY, A.S. (1964) In: 'The physiology of insects' (Rockstein, M. Ed.) Academic Press, New York, 285-378.

PLAPP. F.W.J. and HOYER, R.F. (1968) Possible pleiotropism of a gene conferring resistance to DDT, DDT analogs and pyrethrins in the housefly and Culex tarsalis, Journal of economic Entomology, 61, 761.

SAWICKI, R.M. (1970) Interaction between the factors delaying penetration of insecticides and the desethylation mechanisms of resistance in organophosphorus-resistant houseflies. Pesticide Science, 1, 84.

SAWICKI, R.M. (1973) Resynthesis of multiple resistance to organophosphorus insecticides from strains with factors of resistance isolated from the SKA strain of houseflies. Pesticide Science, 4, 171.

SAWICKI, R.M. (1974) Genetics of resistance of a dimethoate-selected strain of houseflies (Musca domestica L.) to several insecticides and methylenedioxyphenyl synergists. Journal of Agriculture and Food Chemistry, 22, 344.

SAWICKI, R.M. (1975) Interactions between different factors or mechanisms of resistance to insecticides in insects. Third International Congress of Pesticide Chemistry, Helsinki (in the press)

SAWICKI, R.M. and FARNHAM, A.W. (1968) Examination of the isolated autosomes of the SKA strain of house-flies (Musca domestica L.) for resistance to several insecticides with and without pretreatment with sesamex and TBTP. Bulletin of Entomological Research, 59, 409.

SCHWITZERLING, H.J., SCHUNTNER, C.A., ROULSTON, W.J. and WILDON, J.T. (1974) Characterization of the organophosphorus resistant Mt Alford, Gracemere and Silkswood strains of the cattle tick, Boophilus microplus, Australian Journal of Biological Sciences, 27, 397.

SZEICZ, F.M., PLAPP, F.W. Jr., and BRADLEIGH, V.S. (1973) Tobacco budworm. Penetration of several insecticides into the larva. Journal of economic Entomology, 66, 9.

WELLING, W., BLAAKMEER, P., VINK, G.J. and VOERMAN, S. (1971) In vitro hydrolysis of paraoxon by parathion resistant houseflies. Pesticide Biochemistry and Physiology, 1, 61.

YASUTOMI, K. and KEIDING, J. (1969) Effect of synergists on resistance to insecticides. Danish Pest Infestation Laboratory Annual Report for 1968, p.45.

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PATHOGEN RESPONSE TO FUNGICIDE USE

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Summary The response of pathogens to fungicide use is considered in terms of the availability of mutations conditioning different levels of fungicide insensitivity and subsequent selection affecting their increase in pathogen populations. Particular attention is drawn to the relative rarity of mutants conditioning insensitivity to fungicides with multisite activity, compared with those conditioning insensitivity to site-specific compounds.

Selection is considered in terms of exposure of the pathogen to the fungicide particularly in relation to persistence of the compound. Examples of more or less available fungicide responses and low and high degrees of selection are discussed. From the interaction of the availability of, and selection for, fungicide insensitivity, empirical recommendations are made for fungicide use with the purpose of avoiding insensitivity problems and increasing the durability of the fungicides in use.

Resumé La réponse des pathogènes à l'utilisation des fongicides est considérée, en termes de mutations présentes et potentielles qui sont à l'origine de différents niveaux d'insensibilité aux fongicides et de la sélection consécutive en faveur de leur accroissement dans les populations de pathogènes. La relative rareté des mutations conférant l'insensibilité aux fongicides à action multisite, comparée a celle des mutations conférant l'insensibilité aux composés à action spécifique est particulièrement soulignée.

La sélection est considérée en termes d'exposition du pathogène au fongicide qui dépend particulièrement de la persistance de ce dernier. Des exemples de réponses plus ou moins fortes et de faibles et fortes degrés de selection sont discutés. A partir de l'interaction entre le possibilité d'apparition et la sélection de formes insensibles aux fongicides, des recommendations empiriques sont faites quant à l'utilisation des fongicides dans le but d'éviter des problèmes d'insensibilité et d'accroître la durabilité des fongicides utilisés.

The most important response of pathogens to the use of fungicides is that due to changes in the frequencies of various genes in the pathogen populations subjected to fungicidal control. Such frequency changes may be due to the effects of mutation, selection and migration. Mutation provides the range of characteristics available to the organism to enhance fungicide insensitivity, whilst selection determines the rate and degree of the response. The effects of migration can be regarded to some extent as a function of the degree of selection;

with large populations or within large areas, they may be disregarded. The problem of whether the observed response is of economic concern has been considered by Fletcher (1975).

MUTATION AND THE AVAILABILITY OF FUNGICIDE RESPONSE

Against a particular fungicide or family of fungicides, a pathogen may have a range of potential mutational responses from the experimentally imperceptible to the large and obvious. If mutations which condition a large response occur at least as frequently as those which condition a lesser response, then the mutations of large effect will be selected preferentially. On the other hand, mutations of large effect may simply not occur or be non-recurrent or lethal, so that the pathogen response may be severely limited.

The overall rate of mutation towards insensitivity is dependent upon the type of fungicide involved. As a useful generalisation, Georgopoulos (1971; pers. comm.) has stressed that fungicides with multisite activity require a more complex mutational response from the pathogen than do site-specific compounds. There is thus a correlation between the ease of finding or inducing mutants in the laboratory and the occurrence of field problems due to insensitivity. Field problems occur, apparently, infrequently with heavy metal ion fungicides and the dithiocarbamates, more frequently with the protectant aromatic hydrocarbons such as dodine, and most dramatically with the site-specific systemic fungicides. However, for this generalisation to have universal validity, it is necessary to propose that the pathogen requires a mutation to overcome the effect of a fungicide at each metabolic site, analogous to the gene-for-gene theory in host-pathogen relationships. This is most unlikely, as is evident for example, in the insensitivity of Pyrenophora avenae to organo-mercurial fungicides (see Greenaway, 1971), where a compound with multisite activity has lost effectiveness in many areas through selection of a relatively simple mechanism of insensitivity. Exceptions to the generalisation among site-specific compounds include griseofulvin, for which insensitivity has not yet been detected, and the insensitivity of Ustilago hordei to carboxin, obtained by ultra-violet mutagenesis (Ben-Yephet, Henis & Dinoor, 1974), but which has not yet emerged as a field problem.

Further exceptions to the one-for-one relationship between site effect and mutational response occur where a number of loci condition the same character. For example, Srivastava & Sinha (1975) found 10 loci in Aspergillus nidulans which control insensitivity to p-fluorophenylalanine and it was estimated that there may be as many as 18 more controlling the same character. Kappas & Georgopoulos (1971) found at least four loci controlling dodine insensitivity in Nectria haematococca.

The mutation rate may also be affected directly by the particular fungicide used. Mutagenic effects have been suggested, for example, for ferbam on Aspergillus niger (Prasad & Pramer, 1968), penta-chloro-nitrobenzene on Escherichia coli (Clarke, 1971) and benomyl on Fusarium oxysporum (Dassenoy & Meyer, 1973). Hastie & Georgopoulos (1971) also pointed out the effect of mitotic disturbance due to benomyl on diploid cells of Aspergillus nidulans heterozygous for recessive insensitivity.

At very low frequencies it is difficult to determine whether a gene is being maintained polymorphically in the population by selection or whether it is being continually regenerated by recurrent mutation. For example, Wuest, Cole & Saunders (1974) found evidence of benomyl insensitivity in a culture of Verticillium malthousei which had been collected some years before benomyl was first manufactured. Similarly, Dinoor (pers. comm.) found evidence of benomyl insensitivity in samples of

Sphaerotheca fuliginea in areas isolated from sites of use of the compound. Selection for insensitivity will generally act more quickly in the case of the polymorphism than in that of the recurrent mutation.

The status of a pathogen population in terms of the frequencies of all of the characters which may directly affect insensitivity to a particular fungicide and those which affect the fitness of the insensitivity mutants, is likely to be unique at the time of introduction of the fungicide. In other words, irrespective of the subsequent use of the compound, populations of the same pathogen in different areas and at different times will respond at different rates depending on the previous history of selection on those populations.

SELECTION AND THE DEGREE OF FUNGICIDE RESPONSE

Since a fungicide is effective at the time of its introduction, it is implicit that insensitive forms of the pathogen, if viable, have a low level of fitness at that time. This may be directly due to the nature of the insensitivity mutations, but may also be due to pleiotropic or linkage effect on other characters. That is to say, the mutation may have adverse effects on characters other than fungicide insensitivity, either directly or through the effects of genes closely linked or associated with the mutation. Selection may thus be considered to have two prime effects, firstly to increase the frequency of the available insensitivity mutants, and secondly, to increase the fitness of the emerging mutant populations. Selection for increased fitness will be most rapid where there is no association between insensitivity mutations and other characters of deleterious effect. This observation was recognised by Kappas & Georgopoulos (1971) who found variation in host virulence amongst dodine - insensitive mutants of Nectria haematococca and Yamasaki, Tsuchiya, Niizecki & Suwa (1964) who found variation in virulence amongst 12 strains of Piricularia oryzae showing insensitivity to copper sulphate.

Mather (1973) recognised three categories of selection, namely, stabilising, directional and disruptive. Stabilising selection implies that the selective advantage of a small change towards increased fungicide insensitivity is less than the disadvantage that would be caused to the whole population by such a change, so that the population does not shift. This may explain the observation of Ross & Hamlin (1961) who recognised variation in Venturia inaequalis with respect to a number of fungicides but observed no differences in pathogen populations obtained from sprayed and unsprayed orchards.

Under directional selection, characters affecting fungicide insensitivity, available within the population at low frequency, now have a selective advantage, so that the whole population shifts to a new optimum. Under disruptive selection, the insensitivity character is outside the normal limits of variation, so that a second, distinct, population, based on rare mutants, becomes established. Determination of whether fungicide insensitivity lies within or outside the normal limits of variation of the pathogen population is difficult, but large and rapid alterations towards fungicide insensitivity may often be the result of disruptive selection.

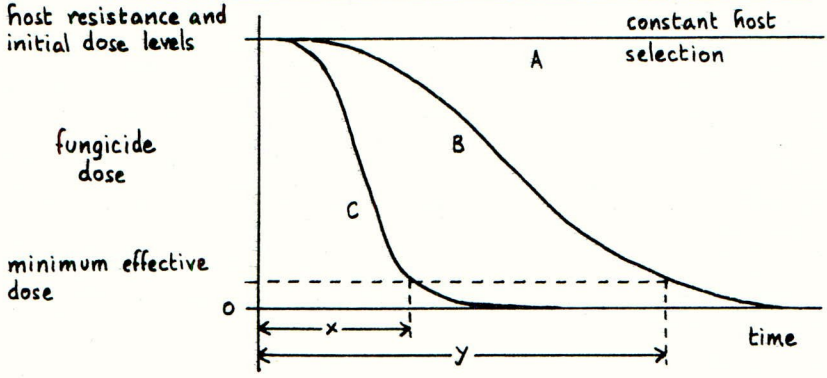
It is likely that all three types of selection occur simultaneously in the same organism for different characters associated with insensitivity and improved fitness. The relative importance of each will depend upon the availability of different kinds of mutation and the degree of selection pressure applied. Selection pressure here means the degree of exposure of the pathogen to the fungicide, where exposure comprises essentially, the level of application and the time during which the fungicide is active.

a. Level of application: the intensity of selection for fungicide insensitivity is positively related to the level of activity of a compound and the level of the dose applied. The consequence of increased selection is however, difficult to prejudge. Increasing the dose rate of some fungicides, equivalent to increasing the selection pressure, may so reduce the pathogen population that recovery is extremely delayed. This may be so for fungicides with multisite activity; for example, Szkolnik & Gilpatrick (1973) found that dodine-insensitive populations of Venturia inaequalis could be controlled by increasing the dose rate to three times the previous level. On the other hand, increasing the dose rate of other fungicides, may serve only to hasten the demise of the compounds, an effect which is probably more likely to occur with site-specific compounds such as benomyl.

b. Exposure time: the effect of exposure time as a selective influence depends on the level of use of the compound and on its persistence. If a fungicide is applied at random in space and time insensitivity may have little or no selective advantage so that it does not increase in frequency in the pathogen population. At the other extreme, if a fungicide is introduced generally and used continuously, then there will be a high degree of selection for a complete shift in the population towards insensitivity.

Fig. 1

Comparative exposure times and therefore selection intensity, of persistent and non-persistent fungicides and host resistance



In Fig. 1, with host resistance, there is constant selection for increased virulence of the pathogen (curve A). With fungicides, however, as the persistence decreases, so does the selection pressure. Thus, in Fig. 1, with the persistent fungicide (curve B), an insensitive strain has a selective advantage relative to sensitive strains during period y; with a non-persistent fungicide (curve C) the period of selective advantage is reduced to x.

In Fig. 1, curves A and C represent extremes; the position of curve B is, however, critical for different organisms. If a particular organism has a life cycle shorter than y, selection for insensitivity may cause the organism to be unsuited to the environment following disappearance of the fungicide, so that the frequency of the selected insensitive forms then declines. This effect may be reduced if the life cycle is much shorter than y, since y will then be more similar to continuous exposure. If the life cycle of the organism is longer than y, there may be a considerable selective advantage for insensitivity, since the selective organisms will presumably be well fitted to environments with and without the

fungicide. If, however, the life cycle is very long relative to y, stabilising selection may occur, where response to the fungicide has an overall disadvantage for the organism.

It is necessary here to draw the distinction between persistence and systemicity of a fungicide. Recent problems with fungicide insensitivity have been correlated with the high degree of systemicity of modern fungicides as well as with their specificity. The cause of the correlation is however, persistence rather than systemicity. Logically, the most effective and durable fungicide may be one which is highly systemic, increasing its efficiency, but of short persistence, decreasing selection for insensitivity in the pathogen. As an example, Ben Aziz, Shabi & Aharonson (1974) noted the accumulation of benomyl in the lower unimportant leaves of pear trees sprayed to control Venturia pirina, which thus caused unnecessary selection for insensitivity.

INTERACTION OF THE AVAILABILITY OF RESPONSE AND SELECTION

In Table 1, some examples to illustrate the interaction of the availability of fungicide insensitivity with the degree of selection for the response are given in an arbitrary classification.

Table 1

The interaction of the availability of fungicide insensitive response with the degree of selection for the response

Availability of insensitive forms	Selection pressure low	high
low (multisite compounds)	Venturia/dodine	Venturia/dodine
		Pyrenophora/org. mercurials
high (site-specific compounds)	Ustilago/carboxin	Venturia/benomyl
	Erysiphe/ethirimol	Cercospora/benomyl

a. Insensitivity in Venturia inaequalis to dodine and benomyl: Dodine shows characteristics of the non-persistent, multisite activity compounds against which insensitivity is rarely recognised as a field problem, even with V. inaequalis. However, Szkolnik & Gilpatrick (1969; 1973) showed that under intensive and long-persistent selection in orchards in New York State, V. inaequalis became dodine-insensitive. On the other hand, with intensive use of the site-specific compound benomyl in South Australia (Wicks, 1974), V. inaequalis became fungicide-insensitive in only 2-3 years, compared with the 10-14 years required for dodine-insensitivity in New York State.

b. Insensitivity in Pyrenophora avenae to organo-mercurials: Organo-mercurial fungicides have a multisite effect, brief persistence, high activity and are non-systemic: pathogen insensitivity may therefore be unexpected. However, selection has been applied to P. avenae for a long period, and insensitivity, due to a relatively simple pathogen mechanism, is now widespread (Greenaway, 1971; Greenaway & Cowan, 1970). Greenaway & Whatley (1975) pointed out that, nevertheless, the disease has not returned to its former importance and they argued that the increase in insensitivity has been accompanied by a loss of

agressiveness or fitness. This assumption must be treated with caution since there was a large reduction in the area of the oat host during the period of increase in insensitivity. From the data of Richardson (1974), it also appears that there are varietal differences in susceptibility to P. avenae, a further factor which may influence the increase of insensitive forms.

c. Insensitivity to Erysiphe graminis hordei to ethirimol: Although insensitivity to ethirimol is a recognised field problem in E. graminis hordei, it is not yet dominant in the field population in all areas. The fungicide is persistent but is usually applied only once during a season, so that a number of asexual, and the sexual, generations are not subject to fungicidal selection. In addition, it is only used on about one-quarter of the crop and, following an earlier suggestion (Wolfe & Dinoor, 1973), it has been little used on winter barley, which has diminished survival of the insensitive forms (unpublished data). Further, because of crop rotation, there is a reduced possibility for the build-up of insensitive populations compared with that in a perennial crop. This example is in direct contrast with that of dodine insensitivity in the intensively sprayed orchards of New York State.

In localised field situations, where there is less fluctuation, ethirimol insensitivity does appear to become established. For example, at the Plant Breeding Institute, Cambridge, ethirimol has been used annually in field trials and the recent decline in performance of the fungicide (Table 2) is closely related to lack of disease control and increased frequency of insensitive pathogen strains in the populations. A similar trend appears to be emerging nationally (Table 2) from comparisons of disease levels on ethirimol-treated and untreated fields.

Table 2

2.a). yield of ethirimol-treated cv. Golden Promise expressed as a percentage of untreated in successive field trials at the Plant Breeding Institute, Cambridge

b). mildew levels on the second leaf of ethirimol-treated crops expressed as a percentage of the levels in all crops in a national survey (data supplied by Dr. J.E. King)

	1970	1971	1972	1973	1974	1975
a). yields of Golden Promise	111	121	117	130	107	94
b). national levels of disease on treated crops	-	-	55	29	64	71

Similarly, although forms of Ustilago hordei insensitive to carboxin can be obtained (Ben-Yephet, Henis & Dinoor, 1974) the epidemiology of loose smut, and the restriction of treatment largely to seed crops, serve to reduce selection pressure so that development of insensitivity in the field will be delayed. Nevertheless, monitoring of infected seed crops at this stage may be prudent.

d. Insensitivity of Cercospora beticola to benomyl: benomyl insensitivity of C. beticola in Greece became a severe field problem shortly after the introduction of the fungicide (Georgopoulos & Dovas, 1973). After cessation of benomyl use, Georgopoulos (pers.comm.) was unable to detect a decline in the frequency of the

insensitive forms in the pathogen population. This suggests that there was a high degree of selection for a readily available character, so that the pathogen population changed rapidly and completely to the insensitive form.

The examples considered so far exhibit relatively obvious contrasts in the availability of, and selection for, an insensitivity response. However, a more detailed comparison of the response of related pathogens to benomyl may be helpful in further consideration of user recommendations. For example, benomyl insensitivity has not yet been found in Cercosporella herpotrichoides (Chidambaram & Bruehl, 1973), whereas in Cercospora arachidicola it has been found but not considered to be serious (Littrell, 1974) and in Cercospora beticola (Georgopoulos & Dovas, 1973) and many others, insensitivity has rapidly become serious as a field problem. There is also the example of Penicillium italicum and P. digitatum, growing together in the same environment, where benomyl insensitivity developed more rapidly in the first than in the second species (Cutter, 1975).

In examples where insensitivity has been detected, but has not become serious in practice, it is essential to differentiate real fitness differences in the sensitive and insensitive fractions of the populations, and what might be termed epidemiological lag, i.e. the period during which newly selected, well fitted, insensitive forms are still increasing to the density necessary to cause serious epidemics.

EMPIRICAL RECOMMENDATIONS

Detailed analyses of the population dynamics of pathogen-fungicide interactions to determine the occurrence of insensitivity factors and their subsequent fate through selection, along the lines indicated above, should lead to practical recommendations for avoiding insensitivity problems and increasing fungicide durability. Such analyses should be based on monitoring studies both in the laboratory and in the field. This may be possible for only a limited number of pathogens and fungicides, but the lessons derived may have more general application.

From the relatively casual observations so far available, however, a number of empirical recommendations can be made.

1. Availability of fungicide response

a. Careful attention to hygiene and avoiding dependence on fungicides, which restrain the use of the materials, obviously limit the potential for pathogen response.

b. Fungicides with multisite activity should be used in preference to site-specific compounds provided that they are economical and reasonably effective in comparison with the site-specific compounds.

c. Fungicide insensitivity should be monitored from the earliest possible stages of fungicide exploitation to be certain that data obtained at later stages do or do not show that a change in fungicide response has occurred. In this way the earliest possible action can be taken to deal with a developing problem.

2. Degree of fungicide response

a. High priority should be given to improvement of the technology of fungicide application in order to limit the quantity used and to obtain maximum kill. The combination of high systemicity with low persistence, to maximise the reduction of pathogen numbers whilst presenting the regenerating population with a non-selective

environment, should be considered.

b. Forecasting techniques should be further developed and applied, to reduce unnecessary fungicide application. Consideration could be given to identifying areas which tend to be more or less disease-prone. For example, Slootmaker, Wolfe, Schwarzbach & Post (in press) found that parts of Europe could be zoned for low and high risk for barley mildew. In the low risk areas, complete disease control could be obtained from reduced fungicide application. There may also be scope for identifying such areas on a more local scale, particularly for pathogens with strict environmental requirements.

c. The use of different fungicides rather than repeated use of a single compound can maintain disease control whilst reducing selection for insensitivity to individual compounds. This approach will be most effective if there is negative cross-insensitivity to the compounds used, such as that described by Ebben & Spencer (1973) for Sphaerotheca fuliginea versus dimethirimol and benomyl. Also, Littrell (1974) found that addition of chlorathonil to benomyl reduced the number of benomyl-insensitive mutants to a greater extent than any of the other additives which were used, and Lambert & Wuest (1975) found that benomyl-insensitive strains of Verticillium malthousei had increased sensitivity to zineb. The effectiveness of the approach will be reduced, of course, if there is positive cross-insensitivity to the compounds used, such as that observed in Penicillium spp.to biphenyl and sodium orthophenylphenate (Harding, 1964), or in well-known examples of cross-insensitivity to the benzimidazole fungicides.

d. Integrated control using fungicides and host resistance can serve to protect both fungicide and host by reducing selection for the pathogen response to each control measure. In this way, maximum disease control can be obtained from reduced levels of fungicide applied to partially resistant host varieties, as observed by Slootmaker, Wolfe, Schwarzbach & Post (in press) even in areas of high risk for barley mildew. A similar result was obtained for integrated control of potato late blight using a protectant fungicide and polygenic host resistance (Fry, 1975). Lowe (1975) has argued similarly for the integration of insecticide use with host resistance to pest attack for the control of pest damage and virus infection. Again, however, cross-relationships between the two types of control measure cannot be ruled out, as observed by Wolfe & Dinoor (1973) in the association between virulence for host cv. Sultan in E. graminis hordei and insensitivity for ethirimol.

References

BEN-AZIZ, A., SHABI, E. & AHARONSON, N. (1974) Persistence and distribution of three benzimidazole fungicides in pear trees sprayed for the control of Venturia pirina Phytoparasitica, 2, 117-125.

BEN-YEPHET, Y., HENIS, Y. & DINOOR, A. (1974) Genetic studies on tolerance of carboxin and benomyl at the asexual phase of Ustilago hordei. Phytopathology, 64, 51-56.

CHIDAMBARAM, P. & BRUEHL, G.W. (1973) Lack of benomyl tolerance in Cercosporella herpotrichoides. Plant Disease Reporter, 57, 935-936.

CLARKE, C.S., (1971) The mutagenic specificities of pentachloronitrobenzene and captan, two environmental mutagens. Mutation Research, 11, 247-248.

DASSENOY, B. & MEYER, J.A. (1973) Mutagenic effect of benomyl on Fusarium oxysporum. Mutation Research, 21, 119-120.

EBBEN, M.H. & SPENCER, D.M. (1973) The integrated use of two systemic fungicides for the control of cucumber powdery mildew. Proceedings of the 7th British Insecticide and Fungicide Conference, 1, 211-216.

FLETCHER, J.T. (1975) Tolerance of fungal pathogens to systemic fungicides in relation to disease control. Proceedings of the 8th British Insecticide and Fungicide Conference.

FRY, W.E. (1975) Integrated effects of polygenic resistance and a protective fungicide on development of potato leaf blight. Phytopathology, 65, 908-911.

GEORGOPOULOS, S.G. (1971) In Radiation and Radioisotopes for Industrial Microorganisms, International Atomic Energy Agency, Vienna, 129-133.

GEORGOPOULOS, S.G. & DOVAS, C. (1974) A serious outbreak of strains of Cercospora beticola resistant to benzimidazole fungicides in northern Greece. Plant Disease Reporter, 57, 321-324.

GREENAWAY, W. (1971) Relationship between mercury resistance and pigment production in Pyrenophora avenae. Transactions of the British Mycological Society, 56, 37-44.

GREENAWAY, W. & COWAN, J.W. (1970) The stability of mercury resistance in Pyrenophora avenae. Transactions of the British Mycological Society, 54, 127-138.

GREENAWAY, W. & WHATLEY, F.R. (1975) Resistance in systemic fungicides, causes and cures. Current Advances in Plant Science, 7, 335-347.

GUTTER, Y. (1975) Interrelationships of Penicillium digitatum and P. italicum in thiabendazole-treated oranges. Phytopathology, 65, 498-499.

HARDING, P.R. Jr., (1964) Assaying for biphenyl resistance to Penicillium digitatum in California lemon packing houses. Plant Disease Reporter, 48, 43-46.

HASTIE, A.C. & GEORGOPOULOS, S.G. (1971) Mutational resistance to fungitoxic benzimidazole derivatives in Aspergillus nidulans. Journal of General Microbiology, 67, 371-373.

KAPPAS, A. & GEORGOPOULOS, S.G. (1971) Independent inheritance of avirulence and dodine resistance in Nectria haematococca var. cucurbitae. Phytopathology, 61, 1093-1094.

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

LITTRELL, R.H. (1974) Tolerance in Cercospora arachidicola to benomyl and related fungicides. Phytopathology, 64, 1377-1378.

LOWE, H.J.B. (1975) Crop resistance to pests as a component of integrated control systems. Proceedings of the 8th British Insecticide and Fungicide Conference.

MATHER, K. (1973) Genetical Structure of Populations. 197 pp. London, Chapman & Hall.

PRASAD, I. & PRAMER, D. (1968) Genetic effects of ferbam on Aspergillus niger and Allium cepa. Phytopathology, 58, 1188-1189.

RICHARDSON, M.J. (1974) Response of oats to seed treatment and seed-borne inocula of Pyrenophora avenae and Micronectriella nivalis. Transactions of the British Mycological Society, 62, 567-584.

ROSS, R.G. & HAMLIN, S.A. (1961) Variation in the sensitivity of isolates of Venturia inaequalis (Cke.) Wint. to fungicides. Canadian Journal of Plant Science, 41, 499-502.

SRIVASTAVA, S. & SINHA, U. (1975) Six new loci controlling resistance to p-fluorophenylalanine in Aspergillus nidulans. Genetical Research (Cambridge), 25, 29-38.

SZKOLNIK, M. & GILPATRICK, J.D. (1969) Apparent resistance of Venturia inaequalis to dodine in New York apple orchards. Plant Disease Reporter, 53, 861-864.

SZKOLNIK, M. & GILPATRICK, J.D. (1973) Tolerance of Venturia inaequalis to dodine in relation to the history of dodine usage in apple orchards. Plant Disease Reporter, 57, 817-821.

TICKS, T. (1974) Tolerance of apple scab fungus to benzimidazole fungicides. Plant Disease Reporter, 58, 886-889.

WOLFE, M.S. & DINOOR, A. (1973) The problem of fungicide tolerance in the field. Proceedings of the 7th British Insecticide and Fungicide Conference, 1, 11-19.

WEST, P.J., COLE, H., Jr., & SANDERS, P.L. (1974) Tolerance of Verticillium malthousei to benomyl. Phytopathology, 64, 331-334.

YAMASAKI, Y., TSUCHIYA, S., NIIZECKI, H. & SUWA, T. (1964) Studies on drug resistance of the rice blast fungus, Piricularia oryzae Cav. Bulletin of the National Institute of Agricultural Science (Tokyo) Series D, 1-110 (Abstr. in Review of Applied Mycology, 44 130).