SESSION 8A

THREATS TO THE EFFICACY OF AGROCHEMICALS – RESISTANCE AND DEGRADATION

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INVITED PAPERS 8A-1 to 8A-4

MOLECULAR APPROACHES TO UNDERSTANDING THE MECHANISMS OF FUNGICIDE RESISTANCE

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ABSTRACT

Molecular biology has allowed studies on the mechanisms of fungicide resistance to focus on changes at the DNA level. Much of this work has concentrated on resistance to carbendazim and triazole fungicides, and through cloning, sequencing and expression of genes, has generated considerable structural information about both ß tubulin and sterol 14 α demethylase target genes. Although practical benefits are yet to emerge from this research, detailed knowledge of fungicide binding sites at the molecular level might prompt synthesis of novel fungicides less prone to resistance. Knowledge of nucleotide changes linked with resistance opens the way for more rapid biochemical detection methods, which should improve understanding of how resistance spreads within field populations, and how best to combat it.

INTRODUCTION

Molecular genetics has significantly expanded basic knowledge of fungal biochemistry and metabolism, and there is increasing interest in using these molecular techniques to address more applied problems in crop protection research. Gene transfer, DNA sequencing, and gene expression systems augment biochemical and genetic approaches, providing more detailed information about fungicide target proteins, mode of action and resistance mechanisms. Research in these areas is advancing rapidly, and this short review will concentrate on recently published work, together with results from current work in our laboratory.

GENE CLONING

Transformation

Isolating genes involved in fungicide resistance, and comparing them with wild-type alleles, provides one way to identify changes that have occurred. It avoids protein purification, which can be difficult where the protein is either membrane bound, or present in only small amounts. In those fungi where a transformation system has been developed, resistance genes may be cloned by screening a library of DNA fragments from a resistant mutant in a recipient wild-type strain of the fungus. By selecting transformants which grow in the presence of the fungicide, DNA fragments containing the resistance gene can be identified and, if needs be, cut into smaller pieces, until a fragment is obtained which is likely to contain just a single gene. This approach requires an efficient

transformation system, and the ability to select clearly for fungicide resistance against the background of a wild-type sensitive strain. Unfortunately, transformation efficiencies achieved in model systems with *Neurospora crassa*, *Aspergillus nidulans* and *Ustilago maydis* (up to 10,000 transformants per ug DNA), using vectors with autonomously replicating sequences and strong promoter regions, are yet to be achieved with most plant pathogenic fungi, where rates seldom exceed 20 transformants per μg DNA (Hargreaves & Turner, 1990). Recent developments in cloning technology using the polymerase chain reaction (PCR), and oligonucleotide probes to amplify directly genes of interest, may avoid the need for transformation systems (McPherson & Gurr, 1990). Errors may readily accumulate in PCR cloned sequences and, in the absence of any selection for fungicide resistance, extreme caution is needed with this approach.

Heterologous probing

If only small DNA fragments (less than 5 Kb) are used to construct a library, screening the whole genome becomes unwieldy. Good quality DNA is often difficult to prepare, as in the case of powdery mildews, and alternative strategies must be adopted to ensure that libraries are made from DNA fragments enriched with the gene of interest. Many proteins and their corresponding genes are highly conserved, so that genes isolated from *N. crassa* or *Saccharomyces cerevisiae* may be used as heterologous probes to isolate the equivalent gene from restriction digests of total genomic DNA extracted from a plant pathogenic fungus. For example, we have cloned a DNA fragment from a wild-type *Erysiphe graminis* f.sp *hordei* strain using a probe for sterol 14 α demethylase (14DM) obtained from *S. cerevisiae* (pVK III) (Kalb *et al.*, 1987). The heme binding site, and at least two hydrophobic transmembrane sequences, are conserved in cytochromes P450, and this allowed us to detect in mildew genomic DNA fragments that hybridised, at moderate stringency, to the yeast 14DM probe and both the 3' and 5' ends of the coding region (Broomfield *et al.*, 1990). A mini-library was constructed in *Escherichia coli* using the plasmid vector pUC 9, and a positive clone identified by colony hybridisation using pVK III as the probe. The cloned mildew DNA fragment was transferred to a plasmid sequencing vector (pUBS III), and is now being sequenced (Chen & Seeberg, 1985). A similar strategy is being followed to clone the same fragment from a triazole resistant mildew strain.

Carbendazim binding region of ß tubulin

Probes have also been used to identify and clone the ß tubulin gene from several fungi, and to obtain structural information of both the wild-type and carbendazim-resistant alleles. To determine the region of the ß tubulin gene in *N. crassa* responsible for both carbendazim resistance, and negatively correlated sensitivity to diethofencarb, Fujimura *et al.* (1990) constructed a number of chimeric ß tubulin genes between an allele conferring resistance to both carbendazim and diethofencarb, and a second mutant allele conferring resistance to carbendazim only (Figure 1). Chimeric genes were inserted into wild-type *N. crassa*, and transformants screened for growth on media containing carbendazim alone, or together with diethofencarb. All chimeras produced carbendazim-resistant transformants, indicating that the mutations for carbendazim resistance in both alleles were very close together. However, only two chimeras generated transformants showing negatively-correlated cross resistance between carbendazim and diethofencarb. This located the

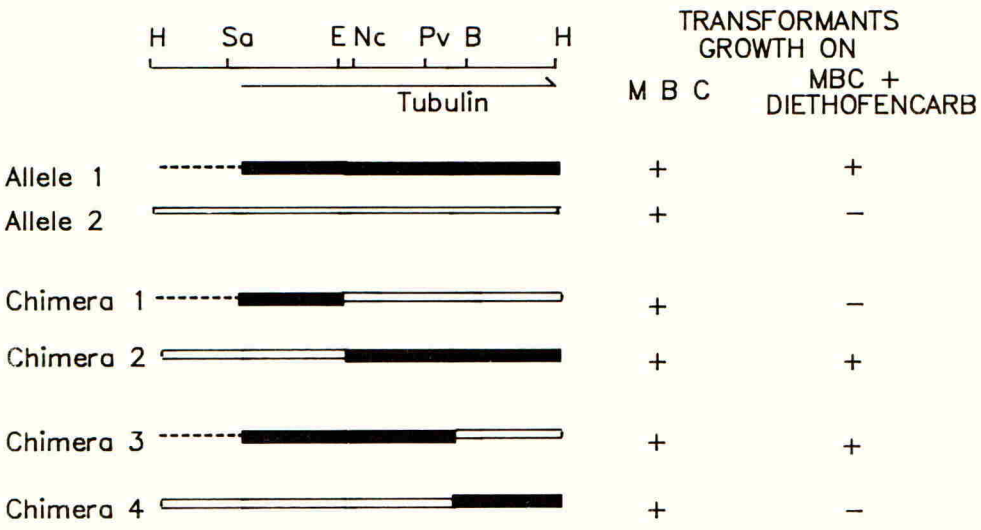

Figure 1. Characterisation of plasmids carrying chimeric ß tubulin genes. Top line is a restriction map of a DNA fragment containing the ß tubulin gene. Restriction sites are as follows: H, Hind III; Sa, Sal I; E, EcoRI; Nc, Nco I; Pv, PvuII; B, BamHI. Redrawn with modification from Fujimura *et al.* (1990)

region involved in carbendazim and diethofencarb binding between the EcoRl and BamHl restriction sites of the ß tubulin gene (Figure 1), and identified which region should be sequenced.

SEQUENCING

Sequence data

Once a gene has been cloned, the next logical step is to sequence it. Although difficulties may be encountered with some DNA seqments because of secondary structure, many different approaches backed by easy-to-use kits from several manufacturers, make sequencing a routine task capable of generating a great deal of information, and which may identify precisely amino acid changes conferring resistance. The ß tubulin gene has been sequenced from several fungi, and where resistant and wild-type alleles have been compared, single base changes have been linked to single amino acid changes in the corresponding protein (Table 1). Sequencing the region of the ß tubulin gene involved in carbendazim and diethofencarb binding revealed an amino acid change from glutamic acid to glycine at position 198 (Fujimura *et al.*, 1990). In another carbendazim-resistant allele of *N. crassa* phenyalanine at 167 was changed to tyrosine (Orbach *et al.*, 1986). In *S. cerevisiae* yet another change was observed at 241, where arginine was changed to histidine. Sequence analysis also revealed that a single nucleotide change, resulting in substitution of aspartic acid for

glycine at position 310 of 14DM, was responsible for inactivation of this enzyme in a mutant of *S. cerevisiae* (SG1) (Ishida *et al.*, 1988). Substitution of asparagine for lysine at position 433 in another yeast mutant had no effect on 14DM activity.

TABLE 1. Amino acid substitutions and carbendazim resistance

	Position of amino acid substitution		
	167	198	241
N. crassa wild-type	phe	glu	arg
N. crassa tub-2-R	tyr	glu	arg
N. crassa F914-R	phe	gly	arg
S. cerevisiae wild-type	phe	glu	arg
S. cerevisiae ben-R	phe	glu	his

From Fujimura *et al.* (1990)

Sequence analysis of genes cloned by transformation, and selection for fungicide resistance, may reveal that resistance is not associated with the target site at all, but with some other protein. Comparison with known sequences in a database, such as EMBL, may help identify the protein, but these databases are limited in extent at present, and may not give a reliable guide to the nature of the protein involved.

Modelling of active sites

Sequence data alone provide limited information on protein structure, and any altered binding sites that may confer fungicide resistance. Considerable effort is currently directed toward developing computer programmes that adequately describe protein tertiary structure from DNA sequence data, and where crystallographic information is available, reasonable predictions may be possible. Amino acid sequences are known for at least 53 cytochromes P450 (Nelson & Strobel, 1989), and by comparing some of these sequences with that of *S. cerevisiae*, Ishida *et al.* (1988) aligned residue 310 to a conserved helical region adjacent to the heme-binding site (Table 2). Using crystallographic data for the soluble cytochrome $P450_{cam}$ from *Pseudomonas putida* (Poulos, 1986), which hydroxylates camphor, computer modelling indicates that insertion of a hydrophilic aspartate residue would shift Asp-310 toward the protein surface, thrusting histidine 317 sufficiently close to the heme allowing its imidazole group to form the sixth ligand with the heme iron. This blocks the incoming oxygen and explains why mutant SG1 lacks 14DM activity.

TABLE 2. Alignment of partial sequences from cytochrome P450s

$P450_{cam}$	G	L	244	L	L	V	G	G	L	D	251	T	V	V	N	F	L	S	F
P450 *S. cerevisiae* WT	I	G	310	V	L	M	G	G	Q	H	317	T	S	A	A	T	S	A	W
P450 *S. cerevisiae* SG1	I	D	310	V	L	M	G	G	Q	H	317	T	S	A	A	T	S	A	W

From Ishida *et al.* (1988)

Unfortunately, soluble $P450_{cam}$ may be an inadequate model for the less polar, membrane-bound, cytochrome P450 14DM, and where resistance to triazole and related fungicides perhaps involves changes at the substrate binding site rather than the heme-binding region. It will be interesting to see whether sequence data now being generated in our laboratory for 14DM from cereal mildews, will contribute to understanding the molecular basis of resistance to DMI fungicides, and explain the activity of less polar triazoles, such as tebuconazole, against triadimenol resistant strains. As confidence grows in computer modelling of proteins, it may be possible to confirm the suggestion of Fujimura *et al.* (1990) that the imidazole of carbendazim binds to glutamate 198 in wild-type ß tubulin, and when it is absent diethofencarb fits in the open space.

EXPRESSION

Complementation

Sequence analysis may be used to indicate the function of a gene by comparison of sequences of known proteins already loaded in databases. Where a known gene has been used as a heterologous probe to isolate and clone a DNA fragment, sequence similarities are to be expected, but confirmation requires expression of the DNA fragment. This can perhaps best be achieved by complementing a mutant lacking the enzyme of interest. For example, the DNA fragment from barley powdery mildew thought to code for 14DM might be expressed in yeast (SG1; Ishida *et al.*, 1988 or JLD 101-1A; Kalb *et al.*, 1987) or *Ustilago maydis* (Erg 40; Walsh & Sisler, 1982) mutants, which are known to lack 14DM. Synthesis of desmethyl sterols in mutants transformed with the mildew DNA fragment would confirm that it contained the coding region of 14DM. Where appropriate null-mutants are unavailable, these may be constructed using molecular techniques. Kalb *et al.* (1987) confirmed the identity of a cloned 14DM gene from *S. cerevisiae* by inserting it into a yeast strain disrupted by inserting a uracil gene(URA3) at a unique Hinc II restriction site in the 14DM gene. A more powerful technique has recently been exploited in *N. crassa* where the function of unknown DNA sequences can be determined by disrupting the corresponding *N. crassa* sequence in the dikaryotic ascogenous hyphae prior to meiosis (Marathe *et al.*, 1990). We are exploring the use of this rearrangement phenomenon induced pre-meiotically (RIP) in our work with mildew 14DM.

Expression in wild-type strains

The identity of a cloned fungicide resistance gene may be confirmed by insertion into a wild-type strain. However, resistance levels will generally not be as great as in the mutant from which the resistance gene was originally isolated, since the resistance allele must be expressed against the background of the wild-type allele of the recipient strain. The *tub*-2 carbendazim resistance gene from *N. crassa* (Orbach *et al.*, 1986) inserted into both *Neurospora* and *Septoria nodorum* did not show as great a resistance to carbendazim as resistant mutants generated in either fungus (Cooley *et al.*, 1990).

Isolation of resistance genes by overexpression

Expression linked to selection for fungicide resistance has been used

successfully to clone 14DM from *S. cerevisiae* (Kalb *et al.*, 1986). By screening a genomic library in a multi-copy expression vector, a 4.7 Kb DNA fragment was isolated which increased the levels of cytochrome P450 in transformants, and caused resistance to ketoconazole. In principle, at least, if resistance can arise through overproduction of the target-site protein, this approach may lead to the isolation of the target gene even if its function is unknown. Searching a database for a similar sequence may provide a guide to its mode of action.

DIAGNOSTICS

Oligonucleotide probes

Precise identification of changes at the genomic level causing resistance provides a basis for developing rapid biochemical methods for detection of fungicide resistance. Recent advances in medical diagnostics have led to techniques capable of detecting single base changes associated with genetic disorders (Caskey, 1987). Once a resistance allele has been sequenced, two oligonucleotide probes can be synthesised which base-pair exactly with the resistant sequence, and can be joined head to tail to make a longer probe. With the wild-type allele ligation does not occur because of the mismatch at the junction between the two probes (Landegren *et al.*, 1988). When automated and linked to the PCR to amplify specific DNA regions, point mutations can be detected in as few as 100 cells (Sakai, 1985). Application of these techniques in agriculture is clearly some way off, but since each diagnostic probe would be specific for a single resistance mechanism, they would be valuable research tools to establish the extent of different fungicide resistance mechanisms within pathogen populations.

Restriction Fragment Length Polymorphisms (RFLPs)

More immediate application of molecular techniques to charting the spread of fungicide resistance within field populations may be achieved by RFLP analysis. DNA changes are detected by altered fragment size of genomic DNA after it has been digested with a restriction endonuclease. Banding patterns are visualised after hybridisation with various DNA probes, commonly labelled with either P^{32} and detected by radioautography, or non-radioactively with enzyme-linked reagents. An altered restriction site will create polymorphism and altered banding patterns. Modified restriction sites seldom occur within resistance alleles, and as RFLPs are not generally specific for a particular resistance mechanism, correlations must be established between any RFLP and fungicide resistance. The number of hybridising bands, and the complexity of RFLP patterns, depend on the probe used and the frequency with which its complementary sequences occur within the genome.

DISCUSSION

Molecular genetics has added several dimensions to studies on the mechanism of fungicide resistance, but practical benefits of this research are still to emerge. As more precise information is assembled about protein structure in resistant strains and changes governing fungicide binding, synthesis of new fungicides that avoid existing resistance

mechanisms may be possible. But the challenge facing chemists is how to utilise this structural information, and to identifiy protein domains or amino acid residues which might provide novel target sites, and justify a synthesis programme. Cytochromes P450 are perhaps the best understood eukaryotic proteins, and many conserved regions are found adjacent to the coupled NADPH reductase. Fungicides acting at novel sites within these conserved regions may be less prone to generate resistance (Goodford, personal communication 1990), but they may also lack the necessary specificity and be subject to toxicological constraints.

Detection of fungicide resistance using bioassay methods may not alert growers before resistance emerges as a serious practical problem. More sensitive and rapid biochemically based diagnostic methods would certainly be of benefit, but in the foreseeable future both oligonucleotide probe and RFLP methods are likely to be too expensive for routine monitoring programmes. Instead, new diagnostic methods, including perhaps ELISA systems, have a valuable part to play as research tools to elucidate the genetics of resistance more clearly, and to describe more accurately the epidemiology and spread of fungicide resistance within field populations of plant pathogens. Better quality validation of different fungicide strategies would provide the basis of better programmes to combat resistance.

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BIOCHEMICAL AND MOLECULAR GENETIC ANALYSIS OF INSECT POPULATIONS RESISTANT TO INSECTICIDES

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ABSTRACT

The role of biochemistry and molecular biology in characterizing insecticide resistance is reviewed, with emphasis on their exploitation for monitoring the mechanisms in large numbers of individual insects. The advantages and limitations of present and prospective methods are discussed.

INTRODUCTION

Resistance to insecticides is a consequence of genetic changes mediated through qualitatively or quantitatively altered biochemical processes. Although such changes occur at the level of the individual, they become apparent at the level of the population as the proportion of resistant insects increases in response to insecticide selection, leading eventually to control failure. Accurate bioassays are of fundamental importance for diagnosing resistance and establishing cross-resistance spectra. They form the basis of monitoring programmes aimed at the early detection of resistance (Roush and Miller, 1986) which is vital since resistance management tactics are likely to have only limited impact once the resistance gene frequency reaches a level that impairs control in the field. Monitoring programmes have generally involved comparisons of LD50s or LD90s between field populations and laboratory strains, and whilst useful for identifying a high frequency of resistant individuals in a population such bioassays are very inefficient for detecting incipient resistance. The use of a discriminating dose of insecticide based on the LD99 for susceptible insects is better, but even then as many as 1500 insects must be examined to be sure of detecting resistance at a frequency of 1% in the population, and this is critically dependent on the accuracy of the LD99 estimate. The number of insects would fall dramatically (5-fold) if the dose were perfectly diagnostic so that all susceptible insects died and all resistant ones survived (Roush and Miller, 1986), but this is rare in practice, and is unpredictable for each new species to be studied.

Whilst bioassays are essential for studying resistance, they can be complemented by other methods that throw light on the underlying biochemical and genetic changes responsible, and how these changes develop within insect populations:-

Formal genetic studies establish inheritance patterns and can isolate resistance genes in homozygous strains for detailed toxicological and biochemical analysis.

Population genetics and modelling describe and predict the build-up of resistance. Population cages that simulate field conditions can be especially valuable for such studies.

Studies of insecticide metabolism can identify the type of enzyme(s) involved in resistance.

The use of synergists in bioassays can give *indications* of the biochemical mechanisms involved, although this can be misinterpreted due to the so-called opportunity factor (Oppenoorth, 1985).

Protein purification and characterization can establish whether insecticide-degrading enzymes change qualitatively or quantitatively in resistant insects, and determine the range of insecticidal substrates on which they act.

Electrophysiological and enzyme kinetic studies describe changes in the interaction between insecticides and their targets.

Molecular biological techniques provide direct access to the genes responsible so that their structure and organization can be established.

Biochemical, immunological and molecular biological studies can provide accurate and sensitive methods for monitoring resistance genes or their protein products in insect populations. However, these methods are still subject to the statistical considerations on sampling outlined above.

It is only by exploiting all these approaches to understand the factors influencing the build-up of resistance that we can hope to limit its impact on chemical pest control. However, the present paper focuses on the need to understand the underlying biochemical and DNA changes so that *in vitro* monitoring techniques can be used with confidence.

BIOCHEMICAL MONITORING OF RESISTANCE MECHANISMS

When resistance has been confirmed by bioassay, and its biochemical nature established, biochemical assays on individual insects can be reliably employed to monitor resistance gene frequence (Brown and Brogdon, 1987). However, it is important to remember that, unlike bioassays, which assess the insects' response to the toxicant and thereby all the contributing biochemical and physiological factors, biochemical assays can only give information about the particular mechanisms chosen for study. As with resistance monitoring based on bioassay, *in vitro* techniques must be capable of analysing many insects if a low frequency of resistance genes is to be detected. This is now possible by exploiting 96-well microplate technology, originally developed for immunoassay, but also well suited for biochemical microanalysis.

Three major groups of detoxifying enzymes, the hydrolases, glutathione transferases and mixed-function oxidases, contribute to the degradation of insecticides, and resistance can arise from changes in any of them (Oppenoorth, 1985). Unfortunately, none is readily assayed in large numbers of individual insects with insecticides as substrates and model substrates are therefore used *in lieu* since they give products that are readily assayed, usually spectrophotometrically.

Although a thoroughly established correlation between resistance and such biochemical markers can support their use in resistance studies, it is preferable to confirm by detailed biochemical analysis that resistance is caused by the enzyme(s) being assayed (Devonshire and Moores, 1982). This is especially important for polymorphic enzymes such as esterases and glutathione

transferases, and should be done for each insect species to be studied if the credibility of this approach is to be established and maintained.

Esterases are the enzyme group most widely assayed in resistance monitoring programmes, usually with naphthyl esters as substrates. Resistance can be associated with substantial changes in activity of these enzymes and in aphids (Field *et al.*, 1988) and mosquitoes (Mouchès *et al.*,1986) this is known to involve quantitative changes in the titre of an enzyme due to gene amplification. The esterase levels of insects taken from treated crops should be interpreted carefully since they can be influenced by *in vivo* inhibition of the enzyme(s). For example, resistant whitefly with intrinsically high esterase activity that have survived exposure to an organophosphate residue on the crop have inhibited esterase levels characteristic of untreated susceptible insects (Byrne *et al.*, 1990). This inhibition can persist for several days even after the insects have been transferred to clean plants. Such effects must be fully characterized if esterase measurements are to be used with confidence.

Besides those enzymes involved in insecticide degradation, resistance often involves changes in the target proteins themselves, rendering them less sensitive to disruption by the insecticide (Oppenoorth, 1985), as is well established for acetylcholinesterase. Enzyme assays that measure directly this interaction in individual insects can unequivocally identify this resistance mechanism without needing further biochemical validation. Although there is electrophysiological evidence for comparable changes in the sodium channels of insects showing nerve insensitivity to pyrethroids and DDT (often referred to as *kdr* or knockdown resistance), the biochemical basis remains to be established in each insect species (Oppenoorth, 1985), and only then can we hope to develop reliable and generally applicable methods for diagnosing it *in vitro*.

The attributes of the methods already in use for resistance monitoring, or likely to become available in the foreseeable future, are summarized in Table 1. A major consideration is that they must be robust, especially if intended for 'field' use, and this can be a limitation since the most incisive information comes from the more demanding biochemical techniques; 'spot tests', including enzyme activity measurements in microtitre plates, give limited insight of the potentially diverse resistance mechanisms to be found in the field. Ideally, biochemical assays should give quantitative data in order to identify the multiple components in the population that can arise from both multiple alleles and multiple loci, together with their heterozygotes. The identification of heterozygotes as well as homozygotes for a resistance mechanism is particularly important since the former predominate in the critical early stages of resistance build-up (Roush and McKenzie, 1987).

'Spot' tests have been used extensively to detect esterases using substrate-impregnated filter papers (Pasteur and Georghiou, 1981) and assays done in microplates (Brogdon *et al.*, 1988) that are assessed visually. They are primarily for field use where they can give clearcut results if resistant insects differ dramatically in enzyme content. However, for insects with intermediate enzyme activities commonly found in the field, these simple assays must be assessed quantitatively, e.g. by using a portable battery-operated microplate reader, but this dramatically reduces throughput. Spot tests can be very dependent on temperature; in whiteflies the esterase activity doubles on increasing the temperature from 20° to 30° (Byrne and

TABLE 1. Attributes of *in vitro* methods for monitoring insecticide resistance[a]

Method	Effort/cost to: develop	validate	use	Robustness for field use[b]	Throughput	Sensitivity	Quality of information Precision	Distinguishing Enzyme forms	heterozygotes
Enzyme assays									
'Spot tests'									
filter paper	+++	+	+++	+++	+++	++	+	-	-
microplates[c]	+++	+	+++	+++	+++	+++	+++	-	-
Spectrophotometry	++	+	++	+	+	+++	+++	-	-
Electrophoresis[d]	+	+	++	-	+	+++	+++	++	++
Immunoassay[e]	-	+	+	+	+++	+++	+++	-	-
AChE kinetics[f]	+	+++	+	-	++	+++	+++	+++	+++
DNA probing[e]									
Dot blots	-	-	+	-	++	+	+	-	-
Southern blots	-	-	-	-	-	+	+	+++	+++

[a] worst, - + ++ +++, best; scores are based on a combination of experience and educated guesses, averaged over various circumstances (insect, enzyme, qualitative, large or small quantitative differences, precision required etc.).

[b] includes rudimentary laboratory facilities.

[c] attributes for microplates will vary greatly according to whether colour is assessed visually or by which type of microplate reader.

[d] scanning of electrophoresis gels is not practicable on the large scale required for such studies.

[e] data are available only for insects with amplified esterase genes; scores therefore represent the author's present perception of these techniques' wider potential.

[f] using kinetic microplate reader; end point assays are simpler and more robust but the quality of information they give is not so good.

Devonshire, unpublished). Although corrections can be made, this requires careful prior calibration for each esterase form and insect species to be studied.

Spectrophotometry has long been used to assess enzyme activities in individual insects under temperature-controlled conditions. All three relevant enzyme groups can be assayed in this way, mixed function oxidases by the dealkylation of p-nitroanisole (Plapp *et al.*, 1976), glutathione transferases (Oppenoorth *et al.*, 1979) using CDNB (1-chloro-2,4-dinitrobenzene) or DCNB (3,4-dichloronitrobenzene), and esterases by the hydrolysis of naphthyl esters (Devonshire and Moores, 1982). Although conventional spectrophotometric assays should remain a standard with which other methods are compared, they are being replaced by the higher throughput assays in microplates, accelerated by the recent availability of temperature-controlled kinetic microplate readers operating at wavelengths down to 340 nm.

Electrophoresis is also used for resistance monitoring, primarily for studying esterases (Ozaki and Kassai, 1970; Sawicki *et al.*, 1978); it can identify qualitative variants that might be toxicologically significant but be indistinguishable in an assay of total esterase activity, and it can detect small quantitative changes in one particular isoenzyme that would otherwise be imperceptible against the greater activity and variability of other isoenzymes. For example in the peach-potato aphid, moderate levels of resistance are caused by a four-fold increase in one insecticide-detoxifying esterase (FE4) which cannot be unequivocally distinguished in assays of total esterase activity but is clear when the enzymes are visualized on electrophoresis gels (Devonshire, 1989). Allozymes of the esterases associated with resistance in aphids (Devonshire, 1989) and mosquitoes (Pasteur *et al.*, 1981) are also revealed by electrophoresis. Similarly in houseflies and blowflies, the presence or absence (null allele) of esterases ($E_{0.39}$ or E_3, respectively) associated with resistance to some organophosphorus insecticides is only discernible after electrophoresis (Sawicki *et al.*, 1984; Hughes and Raftos, 1985).

Disadvantages of electrophoresis are the time, equipment and expertise required for reliable analysis, coupled with the semiquantitative nature of the results which must be assessed subjectively. Although it readily identifies 'allozyme heterozygotes' it does not reliably distinguish 'null allele heterozygotes' (Sawicki *et al.*, 1984), nor individuals with similar degrees of gene amplification.

Immunoassays offer an attractive prospect for monitoring proteins involved in resistance, but require much more effort to develop; it is necessary not only to identify the biochemical basis of resistance, but to purify sufficient of the protein for preparing antibodies. This effort would not be justified for enzymes that are readily measured by conventional colorimetric assays, unless it were necessary to eliminate the background contribution of other isoenzymes to achieve adequate resolution. So far, this approach has been adopted only for the amplified esterases of aphids (Devonshire *et al.*, 1986) and mosquitoes (Beyssat-Arnaouty *et al.*,1989), perhaps reflecting the large amounts of these proteins available from the very resistant insects. This has established that immunoassays can contribute to resistance monitoring, but it remains to be seen what impact they will have for other less amenable resistance mechanisms. A feature of immunoassays is their specificity, and

this could be a disadvantage since each mechanism will require individual development work for each species.

Acetylcholinesterase inhibition kinetics can now be characterized in individual insects in order to measure resistance gene frequency by using kinetic microplate readers which give a throughput of 100-200 insects per hour. The acetylcholinesterase in each insect homogenate is assayed colorimetrically in the absence and presence of a diagnostic inhibitor concentration and an on-line microcomputer fits linear regressions to the reaction curves; this *average* activity in the presence of inhibitor is then expressed as a percentage of the uninhibited rate, and distribution histograms generated to distinguish homozygotes and heterozygotes. By determining the inhibition by more than one insecticide for each insect and expressing the data as bivariate plots instead of histograms, it is possible to distinguish all six genotypic combinations of a sensitive and two insensitive forms of the enzyme (Moores *et al.*, 1988a). These methods have established that the gene for this key enzyme in the nervous system can exist in multiple allelic forms within houseflies (Moores *et al.*, 1988a), whiteflies and aphids (Moores *et al.*, 1988b). For arrhenotokous species such as whiteflies, analysis of the haploid males gives clearest results since they contain only one enzyme form. Whilst the method will clearly identify *known* heterozygotes, data for field-collected insects must be interpreted carefully since there may be other forms of the enzyme present.

DNA probes clearly hold great potential for identifying resistance genes, but this technology is not a panacea. The mechanisms to be analysed will require thorough characterization not only at the biochemical level but also by molecular biological methods, and in many cases it will be simpler to continue using biochemical monitoring techniques to complement bioassays. However, DNA probes will be an automatic spin-off from the increasing fundamental research effort worldwide to understand the molecular genetics of resistance. This approach should benefit from developments in medical diagnostics (Landegren *et al*, 1988), although the economics of applying such techniques to many thousands of insects instead of people will not be so easily justified.

As with immunoassays, DNA probing of resistance genes has so far been developed only for insects producing very large amounts of esterase protein as a consequence of gene amplification. There is no advantage in adopting this approach purely as an alternative to esterase assays, but in *M. persicae* the two methods are complementary, enabling the identification of individuals with amplified but non-expressed esterase genes (Field *et al.*, 1989). These genes are occasionally 'switched on' again in some offspring (ffrench-Constant *et al.*, 1988), and insecticide selection can then act on the consequent intraclonal variability leading to rapid build-up of resistance which does not occur in truly susceptible clones.

The broader potential of DNA diagnostics for resistance genes is yet to be realized, but this work on *M. persicae* can give some indication of the applicability of the approach. Approximately 0.5 μg DNA is extracted from each aphid (i.e. 1 μg/mg insect), sufficient to detect, but not quantify accurately, the highly amplified genes in crude homogenates of individual insects by using dot blots. If the DNA is to be digested with restriction enzymes and analysed by Southern blotting, more stringent cleanup is necessary so that approximately 5 mg insect material is required. If single-copy genes

were to be studied, the specific sequences would probably need amplifying *in vitro* by the polymerase chain reaction (PCR) and this would add further complication and cost to the assays (Landegren *et al.*, 1988). Detecting qualitative changes in genes due to point mutations would then be feasible, but this would constitute the ultimate in specificity and be of very restricted value. DNA hybridization can be detected colorimetrically, but ^{32}P labelling still gives the best sensitivity (Landegren *et al.*, 1988) and this is a further constraint on the broad applicability of DNA diagnostics.

CONCLUSION

These *in vitro* techniques can make a major contribution to studies of insecticide resistance, but they can only complement bioassays and not replace them. They will only detect particular resistance genes, a major limitation when used to monitor insect populations from the field, and one that will become more important for increasingly specific methods such as immunoassays and DNA diagnostics. Their greatest impact is likely to be on studying the build-up of known resistance genes in defined insect populations as a means of developing and evaluating resistance management strategies.

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THE THREAT OF ACCELERATED DEGRADATION OF PESTICIDES - MYTH OR REALITY?

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ABSTRACT

The current status of accelerated degradation of pesticides in previously-treated soils is reviewed and the extent to which it might be threatening the production of some of the UK's major horticultural crops is discussed. Some strategies are proposed for defining the practical impact of the phenomenon and for more fundamental studies.

INTRODUCTION

The application of synthetic organic pesticides to soils to control a wide range of insect pests, weeds and diseases has formed an essential part of agricultural practice for almost half a century. Since the phasing out of the chlorinated hydrocarbons, only rarely has it been shown that significant residues persist into the year after application or are taken up by subsequent crops. While such extensive degradation results from a variety of factors, it is recognised that the major pathway is microbiological (Felsot, 1989).

The development of adaptive mechanisms by soil microorganisms following their exposure to hitherto extraneous organic chemicals is an essential and inevitable ecological process. The fundamental development of the phenomenon currently described as accelerated, or enhanced, biodegradation should not, therefore, be considered as novel. It was first demonstrated with pesticides more than 40 years ago in soil perfusion experiments with the herbicide 2,4-D (Audus, 1949) and confirmed in subsequent studies with different herbicides and insecticides. However, most of these experiments were done under controlled conditions in the laboratory, often at dose levels substantially greater than would occur in field soils in practice, and it was long thought that microbial adaptations were unlikely to occur at the low dose levels resulting from recommended soil treatments. Only during the last 15 years has it emerged as a significant threat to the efficacy of soil-applied pesticides.

So why the relatively sudden interest? Is the threat to crop protection in the developed world really as significant as has been suggested (Kaufman, 1987)? Or is it, as more than one agrochemical company has declared (Fox, 1983), predominantly mythical and of limited practical reality to the vast majority of pesticide users? With the emphasis of this Conference on looking forward to the year 2000, it would seem to be an appropriate moment to assess the extent to which the phenomenon might be jeopardising or limiting the potential of chemical-based crop protection systems which, despite many advances in alternative procedures, will need to continue providing the principal means of crop protection until at least the end of this century.

THE PROBLEM IN PERSPECTIVE

The phenomenon of accelerated degradation first attracted the attention

of research and extension workers in the corn-belt of the USA in the mid-1970s. Then, failures in the field performance of the herbicide EPTC and of the insecticide carbofuran were correlated with the rapid loss of their residues in previously-treated soils (Felsot, 1989; Tollefson, 1986). Corn continues to be the largest single crop in the USA, with 30 million ha projected by USDA to be grown in 1990 (Anon, 1990a), much of it under a non-rotational cropping system. With several hundred million $ spent by farmers each year to control corn rootworm (Diabrotica spp.) alone, the threat to agrochemical sales was thus abrupt and real and was soon to be exacerbated by evidence of the development of accelerated degradation of most of the carbamate and organophosphorus alternatives to carbofuran. It is hardly surprising, therefore, that during the past decade increasing numbers of North American researchers have embarked upon studies of the scientific and practical aspects of the phenomenon.

Despite the prolonged and well-publicised interest in accelerated degradation in North America, it has aroused only sporadic concern in the UK and the rest of Europe. In the UK the failure of diazinon to control the lettuce root aphid (Pemphigus bursarius) after 3 successive annual applications was associated more than a decade ago with the increased rate of degradation of the insecticide (Forrest et al., 1981). More recently, similar reports from Belgium (Van de Steene & de Smet, 1988; De Proft, 1989; Pussemier, 1990), West Germany (Hommes & Pestemer, 1985), the Netherlands (Smelt et al., 1987), France (Naibo, 1988) and the UK (Suett, 1986; Suett & Jukes, 1988a, 1988b; Walker et al., 1986) have associated failures in the field performance of a number of insecticides, nematicides and fungicides with their repeated use in the same soils. Various crops have been deemed to be at risk in these studies but, as yet, there has been no indication of a major threat to cereals, Europe's most economically important crop. In the UK, cereals account for 75% of the total cropped area (Anon, 1989a) and for a similar proportion of total pesticide expenditure (Griffiths, 1988).

In contrast with the North American situation, therefore, the potential threat to the UK agrochemical market is so far small and limited to relatively minor crops. Furthermore, with the phenomenon restricted almost by definition to those pesticides which are applied directly to soil, only a small proportion of compounds is likely to be exposed to its consequences. The great majority of pesticide applications are made to plant foliage and it seems likely that 80-90%, and possibly more, of the 277 individual crop protection chemicals and other agents currently available to the UK grower (Anon., 1990b) will continue to be unaffected by the activities, modified or otherwise, of soil microorganisms.

This estimate may offer much consolation to agrochemical companies active in the UK market but it puts into perspective the severity of the threat to those farmers and growers whose crops are exposed to soil-inhabiting pests and, to a lesser extent, diseases. The armoury of pesticides available to control them is not large and is unlikely to increase significantly in the near future. Furthermore, only rarely can more than a few of the available compounds be considered to be fully interchangeable alternatives for specific pest control situations, especially with the demands for high-quality, blemish-free produce continuing to escalate (Griffiths, 1988). In such circumstances, the loss of even one or two pesticides could be catastrophic. Recent studies of some of the factors influencing the development of accelerated degradation of soil-applied insecticides illustrate the extent to which the phenomenon might already be threatening the reliable commercial

production of some of the UK's principal horticultural crops.

Cabbage root fly control on brassicas

Virtually all the UK's horticultural brassica crops, with an annual value of more than £180 million (Anon, 1989b), are liable to damage by larvae of the cabbage root fly (Delia radicum). The major periods of infestation and damage occur in May and July and protection is usually achieved by incorporating insecticide into the soil at sowing or planting. In most instances, this necessitates application several weeks before infestation, so that any significant reduction in the stability of these soil insecticides as a result of their accelerated degradation could threaten the effective control of this pest.

Current recommendations (Anon., 1990b) specify 12 insecticides for control of the pest, some of which are formulated with an aphicide as dual-purpose granules and others as WP or EC formulations. Eight compounds are formulated as single-component granules for soil application, with chlorfenvinphos and carbofuran, together with the carbofuran pro-insecticide carbosulfan, accounting for >75% of these soil treatments on commercially-grown brassicas (Garthwaite et al., 1990). In contrast with the North American corn crop, brassica crops are usually grown under a wide rotation in order to limit the development of club root (Plasmodiophora brassicae). Thus frequent application of the same insecticide to the same soil is much less common with these crops than with corn. Nevertheless, in Belgium (Van de Steene & de Smet, 1988) and Germany (Hommes & Pestemer, 1985), it was found that control of cabbage root fly with chlorfenvinphos was less satisfactory in previously-treated than in previously-untreated soil. Subsequent residue studies showed that the insecticide was lost more rapidly from the previously-treated soil (Pestemer et al., 1989). Similar evidence of accelerated degradation of chlorfenvinphos in field soils has been obtained in the UK but has not yet been correlated with loss of biological performance (Suett & Jukes, unpublished results).

With carbofuran, most reports associating its accelerated degradation with repeated applications have resulted from its deteriorating performance against corn rootworm. However, a decline in its efficiency against Delia radicum has been observed in Canada by Harris et al. (1988) and by Read (1986). In the UK, a study of 6 farm soils revealed that, despite evidence of accelerated degradation of carbofuran in soils taken from fields where brassicas were growing, only one of the growers was dissatisfied with its performance against Delia radicum (Suett, 1986). There are a number of reasons for discrepancies between the behaviour of an insecticide in the laboratory and its performance in the field but the observation stressed the importance of quantifying the practical implications of accelerated degradation. At the Institute of Horticultural Research, Wellesbourne, therefore, subsequent studies of the phenomenon have wherever possible included assessments of the performance of soil-applied insecticides against field populations of cabbage root fly, using a log-dose evaluation technique (Thompson, 1984) and radish as the indicator crop. These studies have shown that, one year after a single application of the recommended dose of a granular formulation of carbofuran, a second application was totally ineffective against cabbage root fly (Suett & Jukes, 1990a). Furthermore, a recently-completed study of the performance of carbofuran against this pest at 18 previously-treated sites has shown that it may be 5 years before efficacy is restored fully (Suett & Jukes, unpublished results). It should

be noted also that the latter field observations were made on the relatively short-term radish crop, which requires protection for only a few weeks after sowing. The implications for other brassicas, most of which need protecting for longer periods, are therefore at least as serious.

Most of the other insecticides in current use against this pest have also, at some time or other, been associated with the phenomenon of accelerated degradation but their reduced stability has not yet been correlated with inadequate control of cabbage root fly.

Carrot fly control on carrots

Most of the UK carrot crop, with an annual farm-gate value of more than £80 million (Anon. 1990b), is liable to damage by larvae of the carrot fly (Psila rosae). In contrast with brassicas, many of which can tolerate a limited amount of root damage without a significant adverse effect on yield or quality, even a light infestation of carrot fly can reduce drastically the market value of the crop.

Initial control is achieved by applying granular formulations of insecticides at sowing but most problems of inadequate efficacy occur when treatments fail to control second-generation larvae, which are active from about mid-August onwards, often several months after sowing. Most of the insecticides recommended currently for carrot fly control have been in use for 15-25 years and it is significant that, during the past 10-15 years, it has proved increasingly difficult to maintain the high levels of control that were achieved formerly (Wheatley & Percivall, 1974). The problem has been particularly acute in the organic soils of East Anglia and has led to increasing use of mid-season supplementary applications of liquid formulations of organophosphorus insecticides.

Since its introduction in 1966, phorate has been the most widely-used insecticide against carrot fly in these soils and it is currently applied to more than 50% of the UK carrot-growing area (Garthwaite et al., 1990). However, recent studies with organic soils from carrot-growing regions in East Anglia and Lancashire have revealed that the rates of formation and degradation of the major phorate residue components, phorate sulphoxide (PSO) and sulphone (PSO_2), are now very different from those reported 20 years ago (Suett, 1971). In the earlier work, prolonged activity against carrot fly was attributed to the persistence of PSO and, especially, PSO_2, which accumulated to as much as 60% of the applied dose of parent phorate before declining very slowly. In the recent studies (Suett & Jukes, 1988a), this pattern of degradation was found only in soil from sites where the insecticide had never been used. In all the previously-treated soils, parent phorate remained the principal residue component, with only small and transient proportions of PSO and PSO_2 detected. Perhaps more ominously, this modified behaviour was found also in soils from hedgerows and ditches which had never been treated directly with phorate. However, exposure of these soils to gamma-irradiation restored the previously-observed pattern of prolonged PSO and PSO_2 stability. None of the other soil insecticides available for carrot fly control has proved as effective as phorate in these organic soils and the now extensive use of supplementary treatments to protect main- and late-crop carrots suggests that accelerated degradation may have led to the loss of a once uniquely-effective control procedure.

Other insecticides used widely on mainly mineral soils for long-term

carrot fly control include chlorfenvinphos, disulfoton, carbofuran and carbosulfan. Of these, only carbofuran has so far been associated with failures in performance against carrot fly as a result of accelerated degradation. In the UK, pre-treatment of a mineral soil with carbofuran reduced the performance of subsequent applications of carbofuran and carbosulfan against the pest (Suett, 1987) and, in Canada, Harris et al. (1988) reported a gradual decline in the effectiveness of carbofuran in soils where it had been used for several years.

Lettuce root aphid on outdoor lettuce

The outdoor lettuce crop in the UK has a current annual value in excess of £50 million (Anon., 1989b). Since the mid-1980s, the lettuce root aphid (Pemphigus bursarius) has become an increasingly significant problem (Luers, 1990) and it now threatens the major proportion of the mid-season crop (J. Blood-Smyth, personal communication). Only phorate, as an off-label recommendation, and diazinon are currently available for control of this pest and, as shown above, both insecticides are prone to accelerated degradation. Unlike diazinon (Forrest et al., 1981), deficiencies in the performance of phorate against lettuce root aphid have not yet been correlated with its accelerated degradation, although it has been suggested that this may be a reason for its limited efficacy (Luers, 1990). However, the extensive modification in its behaviour and persistence reported above is likely to have less impact on the short-term lettuce crop than on carrots. Nevertheless, the lack of available alternatives illustrates the dilemma faced by growers of many "minor" crops.

Damson-hop aphid on hops

The UK hop area has a current annual value of £12 million (Anon., 1989b). Since 1975, the organophosphorus insecticide mephosfolan has provided the only soil treatment available for controlling damson-hop aphid (Phorodon humuli) on the crop. The insecticide has therefore been applied annually to the same soils for many years and the extent to which this might be affecting its persistence and efficacy was studied in a survey of 42 soils from UK hop farms (Suett & Jukes, 1990b). Although this study confirmed that accelerated degradation had been induced in many of the soils, there was little correlation between the stability of freshly-applied insecticide and the frequency of pre-treatment. It was also evident that adequate control was being achieved in the field despite, in some cases, near-instantaneous loss of mephosfolan in a laboratory incubation. Subsequent studies revealed that site-to-site differences in the development of accelerated degradation were related to soil pH, with few problems evident in soils of pH 5.5 or less and most rapid loss at pH 6.5 and above. However, even in a highly-active soil this rapid degradation did not occur until concentrations had declined to <100 mg/kg. It was concluded that similarly limited degradation was likely to occur in the field following the localised application of large doses and that this could explain how acceptable performance of mephosfolan against the aphid was achieved in rapid-degrading soils.

Aldicarb has been evaluated as a possible alternative to mephosfolan but reports of its accelerated degradation following its use as an insecticide and/or nematicide on a range of crops (Read, 1987; Suett & Jukes, 1988b; Smelt et al., 1987) as well as on hops (Suett & Jukes, 1988b) suggest that its long-term potential might be limited. Furthermore, although dose-related delays in degradation have been observed with aldicarb in previously-treated soils

(Read, 1987), these did not occur to any marked extent at doses <750 mg/kg and thus seem unlikely to influence its availability in these soils.

White rot disease of onions

The UK green and dry bulb onion crop is valued currently at >£45 million per annum (Anon., 1989b). Although only a small proportion of the total crop is exposed to white rot disease (Sclerotium cepivorum), on some sites >70% of the crop may be lost (Entwistle & Munasinghe, 1978). For some time, the dicarboximide fungicides iprodione and vinclozolin provided the only treatments available in the UK to control this disease. The sudden loss of efficacy of iprodione and then vinclozolin (Entwistle, 1986) was associated subsequently with their accelerated degradation in previously-treated soils (Walker et al., 1986). Further studies with iprodione, summarised recently by Walker & Welch (1990), showed that its rapid degradation could be induced by a single pre-treatment with as little as 0.5 mg/kg. Furthermore, incorporation of only 0.1% of a pre-treated, rapid-degrading soil was sufficient to increase markedly the iprodione-degrading ability of a previously-untreated soil. The rapid and widespread development of the accelerated degradation of iprodione and vinclozolin clearly has limited growers' options for controlling this disease.

Soil-inhabiting pests of sugar beet

Sugar beet is one of the UK's major arable crops, with an annual value of >£240 million. Principal seedling pests include several nematodes (Trichodorus spp., Heterodera schachtii), pygmy beetle (Atomaria linearis) and springtails (Onychiurus spp.), control of which is currently achieved by applying pesticides either as seed treatments or in the seed-furrow at sowing. Carbamates are the most commonly-used insecticides. Recent reports have correlated inadequate control of nematodes and arthropod pests of sugar beet in Belgium with the development of accelerated degradation of carbofuran in soils which have been treated previously with the compound (De Proft, 1989; Pussemier, 1990). In the UK, most of the crop is grown under a rotation of at least 3 years, with <1% following a break of less than 2 years (Cooke et al., 1989). Furthermore, some of these rotations may include crops which also have been treated with soil pesticides. With increasing evidence of cross-adaptations, in which accelerated degradation has been induced by pre-treating soil with a different chemical, it is important that this aspect of crop rotation should receive full consideration.

Potential impact of cross-adaptations

Studies of cross-adaptation (Chapman & Harris, 1990; Felsot, 1989) have shown that, although this can be relatively common between different pesticides within the same chemical group, it is encountered rarely between compounds from different groups. Cross-adaptation seems to be especially characteristic of carbamate pesticides and is much less extensive with organophosphorus compounds (Racke & Coats, 1990b). Furthermore, even within groups, cross-adaptations are unpredictable and may occur in only one direction. Thus the stability of iprodione was reduced in soil pre-treated with vinclozolin but vinclozolin did not exhibit accelerated degradation in iprodione-treated soils. Also there was no evidence of significant direct- or cross-adaptations with other dicarboximide fungicides such as myclozolin, procymidone or metomeclan (Walker & Welch, 1990). In other instances, it has been found that accelerated degradation of one compound can be induced more

readily by pre-treatment of soil with a different pesticide. Thus the degradation of aldicarb was faster after a carbofuran pre-treatment than after an aldicarb pre-treatment (Suett & Jukes, 1988b).

The potential for some cross-enhancement of degradation between the many different pesticides applied to soils is enormous. If the practical consequences are to be fully rationalised, the hitherto largely empirical approach to the phenomenon will need to be reinforced with intensive, and extensive, studies of the relevant enzyme processes associated with soil microbial activities.

MICROBIAL ASPECTS

It has long been established that the phenomenon of accelerated degradation is the result of modifications in the metabolic capacities of soil microorganisms and, during the past decade especially, much has been learned about specific pesticide-microorganism interactions. Continued investigations of the genetic and molecular aspects of these processes are therefore essential and some recent evaluations of the role of degradative plasmids are discussed elsewhere in this Session. Nevertheless, it is equally essential that the microbial geneticist maintains awareness of the practical aspects of these degradative mechanisms. Despite continued insights into the biochemical processes associated with metabolism, there remains an enormous gulf between the behaviour of microorganisms in optimally-maintained laboratory cultures and their behaviour under constantly-fluctuating environmental conditions. There are, for example, numerous reports of the isolation of cultures which can use specific pesticides, or groups of pesticides, as their sole source of energy but it is rare indeed for this specific activity to be retained fully, or even at all, following transfer of the culture into a natural soil environment.

However, the impact of such unpredictable characteristics on the practical consequences of accelerated degradation is not necessarily important. The initial stimulation of the phenomenon, together with its subsequent persistence, are of little practical significance if, in the field, it fails to be expressed actively enough to reduce pesticide availability against a specific pest. Furthermore, it now seems increasingly likely that, in a microbial context at least, the phenomenon has existed for as long as pesticides have been applied to soils (Chapman & Harris, 1990) and that it is simply its sporadic expression, and its relatively recent recognition as a field occurrence, that have led to its perception as a threat to crop protection. Continued evidence of satisfactory performance in some demonstrably "active" soils suggests that the practical impact of accelerated degradation will be rationalised only by fuller evaluation of the many factors which influence its expression. Thus, even a relatively modest decline in soil moisture, to levels which are not uncommon under crops in summer, can reduce drastically the degradative capacity of a hitherto very active soil (Chapman & Harris, 1990; Suett & Jukes, unpublished data). Other factors include: application variables, which determine pesticide concentration and distribution; field variables, such as soil pH; and other edaphic factors, such as soil temperature. All of these interact in the field to yield constantly fluctuating gradients with, as yet, largely undefined effects on microbial activity and pesticide degradation. Such evaluations will not be simple. However, without them, studies of the practical consequences of accelerated degradation will remain predominantly empirical and, in many

cases, retrospective.

PRACTICAL ASPECTS

Strategies for suppressing or limiting the effects of accelerated degradation include the use of biocides, extenders and soil fumigants. They have recently been discussed elsewhere (Racke & Coats, 1990a). Such approaches are invariably expensive and somewhat unpredictable in their efficacy and there is little doubt that it is far more practical to minimise the development of accelerated degradation than it is to attempt to eradicate or suppress it. Despite its limitations, therefore, the maximum rotation of chemicals remains the first essential strategy for managing accelerated degradation.

CONCLUSIONS

The above summary suggests that, for most of those involved in the supply and use of pesticides, the phenomenon of accelerated degradation will remain of little practical consequence. For others, however, it is already limiting performance and has led to significant changes in some established procedures. It is evident that a substantial majority of the high-value horticultural crops produced in the UK could, as a result of deteriorating pesticide activity, be exposed to damaging pest infestations. It is, I feel, not unduly optimistic to suggest that the extent to which this occurs should eventually be predictable from knowledge of pesticide characteristics, treatment histories and variables, and of environmental fluctuations.

There are two other aspects of accelerated degradation which would seem to hold some significance for those associated with current crop protection measures. The first concerns the currently-available range of soil pesticides, which is not large. For crops such as lettuce and onions, it numbers no more than 1 or 2 compounds, with the result that the sudden loss of even a single pesticide as a result of its accelerated degradation could threaten the continued production of some crops. The interests of the producer and the agrochemical companies, and ultimately the consumer, would seem to be ill-served by such a potentially fragile system.

The other major concern is with the potential impact of accelerated degradation on the current trend towards reducing pesticide usage. There seems little doubt that the continued efficacy of many treatments has been maintained in active soils only because recommended doses are generally many times greater than those actually required to control target pests. Some recently-developed crop protection systems with greatly-reduced pesticide inputs are proving highly effective but, inevitably, they place much greater demands on the consistent availability of the pesticide component. Although, in the longer term, such systems will probably limit the stimulation and development of accelerated degradation, during the interim they may encounter unduly adverse pressures in previously-treated soils.

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MOLECULAR ASPECTS OF ENHANCED MICROBIAL DEGRADATION OF PESTICIDES

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ABSTRACT

The microbiology and the genetic basis of enhanced microbial degradation of 2,4-D, parathion and carbofuran were briefly reviewed and the importance of the powerful techniques of molecular biology for gaining an understanding of the underlying processes leading to enhanced degradation were highlighted.

INTRODUCTION

The microbial basis of pesticide degradation in the environment has been extensively documented over the years. More than any other group of microorganisms, bacteria have demonstrated the greatest versatility in extensively catabolising these xenobiotic chemicals (Lal & Saxena, 1982). It was logical therefore that when enhanced pesticide degradation emerged as a potential problem for pest and weed management that the microbial basis of the phenomenon was investigated.

Microorganisms have been isolated which can degrade many of the compounds known to be subject to enhanced degradation (see Roeth, 1986; Walker & Suett, 1986; Felsot, 1989 for reviews). The list encompasses 2,4-D (Loos *et al.*, 1967), chloridazon (Engvildt & Jensen, 1969), dalapon (Berry *et al.*, 1979), carbofuran (Karns *et al.*, 1986b), EPTC (Tam *et al.*, 1987) and organophosphorus compounds (Racke & Coats, 1987).

The microbiological, biochemical and genetic aspects of 2,4-D, carbofuran and organophosphorus compound degradation by microorganisms have been investigated extensively and will form the basis of this short review.

2,4-DICHLOROPHENOXYACETIC ACID DEGRADATION

2,4-D was the first synthetic pesticide demonstrated to be susceptible to enhanced degradation (Audus, 1949). Since this discovery the microbial basis of 2,4-D degradation has been comprehensively studied. Biochemistry and the

powerful tools of molecular biology provided substantial insight into the catabolic mechanisms responsible for degradation of the herbicide and have given clues to the evolution of the degradative pathway. In the late 1960's and early 1970's classical biochemistry elucidated the pathway of 2,4-D catabolism (Tiedje & Alexander, 1969; Evans *et al.*, 1971). Subsequently the genes encoding the catabolic enzymes were located on a plasmid (pJP1) harboured by a strain of *Alcaligenes paradoxus* (Pemberton & Fisher, 1977). This plasmid and other related plasmids were characterised (Fisher *et al.*, 1978; Don & Pemberton, 1981) and other plasmids were identified in several genera of 2,4-D degrading bacteria which carried the genes neccessary for degradation of the herbicide (Pierce *et al.*, 1981; Chaudhry & Huang, 1988). Southern hybridisation analysis revealed substantial homology between the 2,4-D degradation genes present on plasmids isolated from different sources (Chaudry & Huang, 1988). This suggested an evolutionary relationship between 2,4-D degradation genes; this was already established for genes responsible for the metabolism of naturally occurring aromatic compounds (Heinaru *et al.*, 1978).

The implication of these results was that either:

1. the genes responsible for 2,4-D catabolism had evolved independently from ancestral chromosomally located genes involved in the catabolism of unchlorinated natural analogues of the herbicide or

2. that the genes were transmitted through the microbial population on a conjugal plasmid.

In support of the latter possibility the 2,4-D degrading plasmid pJP4, belongs to the highly promiscuous P1 incompatability group and is known to transfer freely between several bacterial genera (Don & Pemberton, 1981).

DNA and protein sequence comparisons between the genes and gene products encoded on pJP4 that were responsible for dichlorocatechol degradation, and the analogous genes of chlorocatechol degradation found on plasmid pAC27 revealed substantial sequence homology (Gohsal & You, 1988a; 1989; Perkins *et al.*, 1990). Interestingly when similar analyses were performed to compare the sequences of genes involved in catechol metabolism with those of chlorocatechol metabolism a comparable degree of homology was observed (Frantz *et al.*, 1987). These studies provided evidence that genes required for the degradation of chloro-aromatic herbicides had evolved from catabolic pathway genes involved in the dissimilation of naturally occurring aromatic compounds.

Duplication of key genes in the 2,4-D catabolic pathway was also demonstrated (Ghosal & You, 1988b; Perkins & Lurquin, 1988), a similar observation was previously made with plasmid encoded genes for the oxidation of substituted aromatic compounds (Chatfield & Williams, 1986). This was compatible with the earlier discovery that a single point mutation in the *tfdA* gene encoding 2,4-D monooxygenase on plasmid pJP4 prevented *Alcaligenes eutrophus* JMP143 from growing at the expense of 2,4-D, but the bacterium was able to grow on phenoxyacetic acid even though prior to the mutation it could not. It was further discovered that mutants of strain JMP143 in which *tfdA* was duplicated on plasmid pJP4 and one of the copies subsequently underwent a mutation that the organism harbouring the plasmid could grow on both 2,4-D and phenoxyacetic acid. (Pemberton *et al.*, 1979). Gene duplication followed by divergence could thus play an important

role in the evolution of novel catabolic activities such as those for enhanced pesticide degradation.

2,4-D degradation has provided some useful clues to the development and spread of pesticide degrading genes. From the evidence gained with this system it would be reasonable to assume that duplication of genes for the catabolism of natural substrates followed by divergence of the duplicate gene or genes could lead to the evolution of novel catabolic sequences. Dissemination of these sequences on broad host range plasmids could result in the rapid spread of degradative ability observed with enhanced degradation. This scenario is likely to be a recurrent theme in studies of enhanced pesticide degradation.

ORGANOPHOSPHORUS INSECTICIDE DEGRADATION

The first instance of pest control problems arising as a result of enhanced degradation was reported for the organophosphorus compound diazinon (Sethunathan, 1971) and a *Flavobacterium* sp. isolated for its ability to hydrolyse diazinon and also parathion (Sethunathan & Yoshida, 1972) has been one of the microorganisms most intensively studied in relation to the molecular aspects of enhanced degradation. The purified parathion hydrolase from this organism catalysed the hydrolysis of several related compounds (Brown, 1980) and its use in an integrated toxic waste disposal system was investigated (Karns *et al.*, 1986a). A strain of *Pseudomonas diminuta* was also isolated which had similar hydrolytic activities towards organophosphorus compounds (Serdar *et al.*, 1982). Since only a single enzyme was involved in the inactivation of these insecticides it provided a simple model of the development of enhanced pesticide degradation.

Like 2,4-D degradation the parathion hydrolase activity of both *Pseudomonas diminuta* MG and *Flavobacterium* sp. ATCC 27551 was associated with distinct plasmids present in these organisms (Serdar *et al.*, 1982; Mulbry *et al.*, 1986). It was in fact the homology between a fragment of the *Pseudomonas* plasmid and one of the plasmids present in the *Flavobacterium* sp. that first indicated the plasmid-borne nature of the hydrolase gene in *Flavobacterium* sp. ATCC 27551. The immediate conclusion which was drawn from this was that the parathion hydrolase genes were evolutionarily related. Restriction mapping of the two plasmids which harboured the parathion hydrolase genes showed however, that the plasmids were totally unrelated apart from the presence of the homologous hydrolase genes (Mulbry *et al.*, 1987). Sequencing of the individual genes revealed a remarkable degree of homology between the hydrolase genes. Over the entire 975 nucleotide open reading frame of the genes there was a single base pair difference (Harper *et al.*, 1988). The almost complete homology between the two genes indicated strongly that the genes were recruited from a common source. Recently a methyl-parathion degrading *Pseudomonas* sp. was isolated, this too contained DNA related to the parathion hydrolase gene (Chaudhry *et al.*, 1988). *Flavobacterium* sp. ATCC 27551 was isolated in the Philippines and *Pseudomonas diminuta* MG was isolated in the United States prompting comparisons with the global spread of antibiotic resistance genes. Although at present the threat of an epidemic spread of catabolic genes responsible for enhanced pesticide degradation seems remote, it is essential that the potential for widespread dissemination of these genes is not ignored.

CARBOFURAN DEGRADATION

While 2,4-D and parathion degradation were the best documentated cases of the genetic basis of enhanced microbial degradation of pesticides and have indicated some of the fundamental processes which underpin the phenomenon, they are of little practical importance. 2,4-D is a foliar acting herbicide thus its efficacy is not compromised by its rapid degradation in soil. The parathion detoxifying system is of little commercial importance since parathion has been largely superceded by new organophosphorus compounds and is rarely used in contemporaray pest management. Isofenphos, an organophosphorus insecticide used to control soil-borne pests of corn, is now however causing concern due to its accelerated degradation in some soils (Racke & Coats, 1987). The N-methylcarbamate carbofuran however, is used extensively for the control of soil borne pests. In the United States and Canada it has found application in the control of corn rootworm, in Asia it is used against brown planthopper a serious pest of rice and in the United Kingdom it is used to control soil-dwelling pests of a number of root crops. Studies of enhanced degradation of carbofuran are therefore of direct relevance to commercial agriculture.

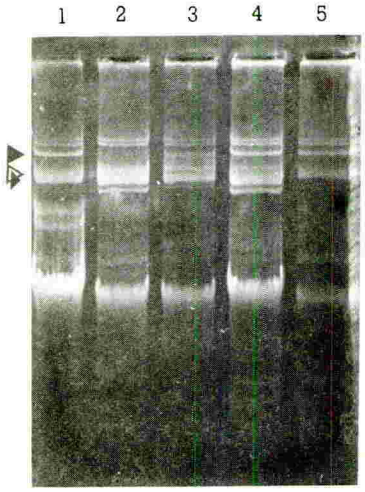

Figure 1. Agarose gel electrophoretogram of plasmid DNA from strains of *Flavobacterium* sp. MS2d. Lane 1. wild-type (CFH+, CFP+) 2. cured derivative (CFH+, CFP-) and lanes 3-5. transconjugant strains produced by mating wild-type MS2d with a cured rifampicin resistant derivative of strain MS2d. Strains capable of degrading carbofuran phenol (CFP+) always harboured plasmid pIH3(▶) and those unable to degrade carbofuran phenol (CFP-) always lacked this plasmid. Plasmids pIH4(▷) and pIH5(◗) were unresolved on this gel.

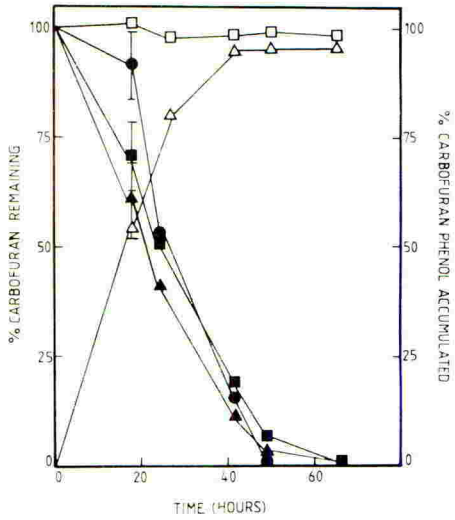

Figure 2. Metabolism of carbofuran (0.25mM) by strains of *Flavobacterium* sp. MS2d. Wild-type (■), cured strain (▲), transconjugant strain (●) and carbofuran in an uninoculated control flask (□). Carbofuran phenol (△) in medium inoculated with a cured strain (CFH+, CFP-).

It was only recently that widespread reports of reduced performance of carbofuran as a result of enhanced degradation began to appear in the literature (Felsot *et al.*, 1981;1982; Read, 1983, Harris *et al.*, 1984; Suett, 1986). Consequently work on the microbial basis of carbofuran metabolism is less advanced than it is with 2,4-D and parathion degradation. Several groups isolated bacteria which rapidly degraded carbofuran (Karns *et al.*, 1986b; Chaudhry & Ali, 1988; Head *et al.*, 1988; Ramanand *et al.*, 1988). Some of these strains completely mineralised the carbofuran molecule while other strains hydrolysed the carbamate ester linkage and utilized the methylamine generated as either a nitrogen or carbon source. The latter transformation was sufficient to render carbofuran biologically inactive. *Achromobacter* sp. WM111 isolated by Karns and his colleagues was found to contain a large (>100kb) plasmid, pPDL11, but initial attemps to associate the plasmid with carbofuran hydrolysis failed (Karns *et al.*, 1986a). The carbofuran hydrolase gene from this strain was cloned and by means of Southern hybridisation, using the cloned gene as a probe, the gene was detected on plasmid DNA but not chromosomal DNA from the wild type bacterium. *Flavobacterium* sp. MS2d isolated from a soil exhibiting enhanced degradation of carbofuran (Head *et al.*, 1988) carried up to seven plasmid species. Curing and conjugation experiments showed that plasmid pIH3 from this strain contained the genes necessary for catabolism of carbofuran phenol produced on cleavage of the carbamate group from the insecticide molecule (Head *et al.*, 1989)(figure 1.).

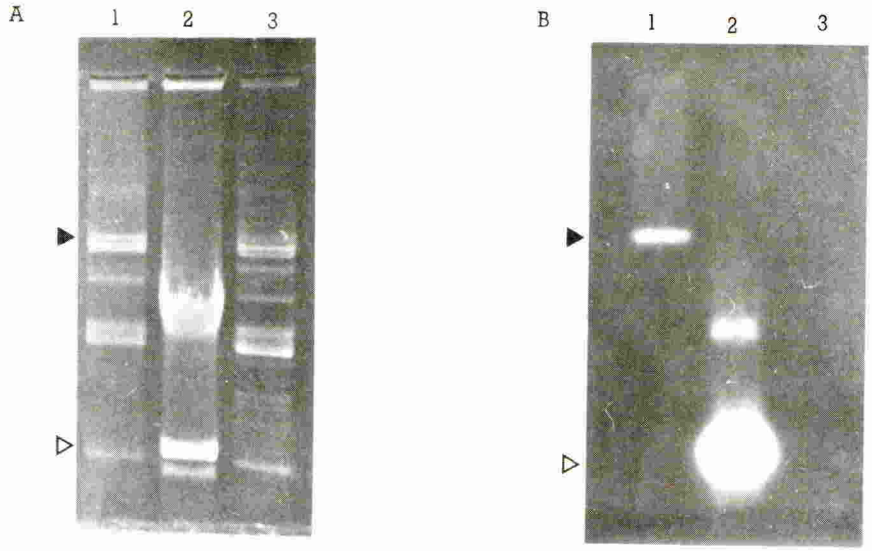

Figure 3. A. Agarose gel electrophoretogram of DNA from, lane 1. A Transposon Tn5-*mob* insertion mutant of *Flavobacterium* sp. MS2d (CFH-, CFP-). 2. *Escherichia coli* Sm10 (carries plasmid pSUP5011, Tn5-*mob* donor - positive control) and 3. wild-type *Flavobacterium* sp. MS2d.

B. Autoradiograph of DNA from gel A. which was transferred to a nylon membrane and hybridised with plasmid pSUP5011 labelled with α32P dCTP by nick translation.

pSUP5011(▷) which carried the transposon Tn5-*mob* hybridised with intact plamid and sheared plasmid and chromosomal DNA from the positive control *E. coli* strain but not with DNA from the wild type *Flavobacterium* sp.. The radiolabelled probe DNA did, however hybridise with plasmid pIH4(▶) present in the mutant strain, this indicated that the transposon had inserted in to pIH4 and inactivated the carbofuran hydrolase gene.

Cured strains which lacked pIH3, although unable to grow at the expense of carbofuran retained the ability to hydrolyse the molecule (figure 2.). Since carbofuran hydrolysis was an unselectable phenotype and attempts to cure carbofuran hydrolase activity failed, transposon mutagenesis and Southern hybridisation were used to identify the site of the carbofuran hydrolase gene in the genome of *Flavobacterium* sp. MS2d (Head *et al.*, submitted for publication)(figure 3.). As with *Achromobacter* sp. WM111 the carbofuran hydrolase gene in *Flavobacterium* sp. MS2d was present on a plasmid, pIH4 (79kb).

It is tempting to draw analogies with the parathion hydrolase genes of *Pseudomonas diminuta* MG and *Flavobacterium* sp. ATCC 27551. Evidence from the substrate specificity of carbofuran hydrolase activity from *Achromobacter* sp. WM111 and *Flavobacterium* sp. MS2d, however suggested that the enzymes involved are distinct. Whole cells of strain WM111 and purified carbofuran hydrolase from this organism were capable of hydrolysing several carbamate compounds including aliphatic carbamates and dimethylcarbamates (Derbyshire *et al.*, 1987) whereas *Flavobacterium* sp. MS2d hydrolysed a number of aromatic *N*-methylcarbamates but not dimethylcarbamates or aliphatic carbamates (Head *et al.*, manuscript in preparation). It would seem, therefore that the carbofuran hydrolase genes in these organisms were unrelated.

RELEVANCE OF A MOLECULAR APPROACH TO THE STUDY OF ENHANCED DEGRADATION OF PESTICIDES

It is largely accepted that the reason for enhanced degradation of pesticides in soil is the development and stimulation of microbial populations with the ability to catabolise the problem chemical rapidly. A logical first step in understanding the phenomenon is to examine the behaviour of soil microorganisms, particularly those responsible for degradation of the compound of interest, in response to treatments which induce enhanced degradation. The methods of microbial ecology conventionally used to this end are unreliable (Brock, 1987) and thus the use of nucleic acid based methodologies to detect and enumerate microbial cells in environmental samples is now widely advocated (Atlas & Sayler, 1988). The main advantage of this approach is that the need to culture microorganisms is obviated. The growth media used in the enrichment of bacteria from soil exert a significant selective pressure and enrichment of fast growing organisms is favoured. The organisms likely to be active in oligotrophic environments are slow growing and generally go undetected by standard methods. The use of nucleic acid probes to specific genes overcomes these problems and in addition would allow the spread of a gene to be detected even if the gene was transmitted to a non-culturable organism.

Nucleic acid probes and molecular biological techniques have already been applied to the detection of *Pseudomonas cepacia* AC1100, a 2,4,5-T degrading organism, in soil (Steffan & Atlas, 1988). This strain was used in the decontamination of soil contaminated with the highly recalcitrant herbicide. Further reports demonstrated the feasibility of such an approach (Holben *et al.*, 1988; Chaudhry *et al.*, 1989; Barkay *et al.*, 1985). The application of these methodologies to enhanced pesticide degradation will provide data on the dynamics of the development and spread of the phenomenon in the environment. It may be possible to identify factors which regulate enhanced

degradation allowing the formulation of strategies aimed at prevention or remediation of the problem.

Study of the catabolic mechanisms of pesticide degrading strains might also lead to the discovery of specific enzyme inhibitors which have the ability to halt enhanced degradation once it has become established. Precedents for this approach have been reported (Kaufman *et al.*, 1970) and the use of specific enzyme inhibitors has met with some success in overcoming antibiotic resistance in bacteria (Schindler, 1980).

Now that a carbofuran hydrolase gene has been cloned (Tomasek & Karns, 1989) and attempts have been made to clone a fragment of a second carbofuran hydrolase (Head *et al.*, submitted for publication), the possibility of applying molecular approaches to the study of enhanced pesticide degradation is becoming a reality. The understanding of enhanced degradation obtained by this approach promises to supplement the meagre arsenal at present available to combat the problem. While there are at present few documented cases of large scale pest control problems resulting from enhanced pesticide degradation complacency would be ill advised. The lessons learned from the worldwide spread of antibiotic resistance plasmids should be considered when addressing the potential of enhanced degradation as a major cause of reduced pesticide efficacy in the future. It is likely that enhanced degradation will assume a higher profile alongside pest resistance as a major cause of economic losses in agriculture, and as such a thorough knowledge of the microbiology which underpins the problem will be essential if its effects are to be minimised.

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SESSION 8B

DEVELOPMENT AND USE OF HOST PLANT RESISTANCE IN CROP PROTECTION

CHAIRMAN DR O. M. B. DE PONTI

SESSION ORGANISER DR P. R. ELLIS

INVITED PAPERS 8B-1 to 8B-4

ASPECTS OF DISEASE RESISTANCE IN FIELD BEANS (*VICIA FABA*)

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ABSTRACT

Progress has been made in the development of screening procedures for resistance to some of the major diseases of field beans and the identification of sources of resistance. Resistance to chocolate spot (*Botrytis fabae*) has been found in accessions which originated in Ecuador, supplied by the International Center for Agricultural Research in the Dry Areas (ICARDA). This resistance is being combined with winter hardiness in adapted genotypes. Resistance to ascochyta blight (*Ascochyta fabae*), identified in PBI Cambridge breeding lines, has been incorporated into advanced breeding material; new sources of resistance from ICARDA are also being used. White-flowered, tannin-free beans have potentially greater establishment problems than coloured-flowered beans due to seedling infection by species of *Fusarium* and *Pythium*, but variation in resistance to these pathogens has been identified and screening of early generation material is now possible.

The role of disease resistance in producing healthy bean crops is discussed.

INTRODUCTION

In the UK, field beans (*Vicia faba*) are affected by numerous diseases. These include fungal diseases: chocolate spot (*Botrytis fabae*), ascochyta blight (also known as leaf, stem and pod spot, caused by *Ascochyta fabae*), rust (*Uromyces viciae-fabae*), downy mildew (*Peronospora viciae*), stem rot (*Sclerotinia trifoliorum* and, less importantly, *Sclerotinia sclerotiorum*), foot rot and establishment diseases (caused principally by *Fusarium* spp. and *Pythium* spp.), and virus diseases: bean leaf roll, bean yellow mosaic, pea enation mosaic, broad bean true mosaic and broad bean stain are probably the most important.

Some diseases are of more importance in autumn-sown crops, for example chocolate spot and ascochyta blight, and others in spring-sown crops, for example viruses. The importance of the disease can also be associated with a particular growth habit or quality character. For example, determinate genotypes tend to be more susceptible to fungal foliar diseases than conventional beans and tannin-free beans tend to have more establishment problems than those containing tannin. At PBI Cambridge, techniques for screening for resistance to chocolate spot and ascochyta blight have been developed over the past decade and these have been used, in collaboration with the International Center for Agricultural Research in the Dry Areas (ICARDA), to identify resistant genotypes and also to assist the breeder in a disease resistance programme. In addition, research on establishment problems in tannin-free beans has been initiated recently because of the interest in

varieties with improved nutritional characteristics.

CHOCOLATE SPOT

This is generally regarded as being the most serious disease of field beans. The disease can totally destroy crops in some years, although severe epidemics occur only in Eastern England about one year in six. The disease has two phases, the non-aggressive phase which gives the classic chocolate spot appearance, with discrete reddish-brown spots, and the aggressive phase during which lesions become much larger and darker and coalesce, leading to foliage death and leaf drop. Flowers and pods are also affected. It is the aggressive phase of the disease which is associated with substantial yield loss, particularly if it occurs before pod formation and filling. This phase occurs when the relative humidity of the microclimate is close to 100% and a drop in humidity for even a short period can substantially reduce the rate of lesion expansion (Harrison, 1980). This means that much selection for resistance and assessment of varieties has been based on the intensity of non-aggressive spotting, which occurs more frequently than the aggressive stage. This may be of value but Bond et al. (1981) have demonstrated that some genotypes differ in resistance to the two phases; those which show a low frequency of non-aggressive lesions are not necessarily those which are resistant to the aggressive phase of the disease and vice versa.

In order to maintain plants at high humidity for long periods, tests at PBI Cambridge are carried out by inoculating plants in polythene tunnels with mist irrigation (Jellis et al., 1982). Using such a system, the Chocolate Spot International Nursery supplied by ICARDA has been tested for a number of years. The most interesting sources of resistance identified by this technique are two closely related lines designated BPL 710 and BPL 1179. These lines originated in Ecuador and have now been rated as resistant at locations in Syria, Egypt, Qatar, Ethiopia, Tunis, Algeria, Morocco, France, The Netherlands, Poland, Italy, Portugal, China, Bolivia, Chile and Canada (S.B. Hanounik, pers. comm.).

BPL 710 and BPL 1179 are poorly adapted to UK growing conditions. In particular they are not winter-hardy, and as chocolate spot is more frequently a problem in winter beans (i.e. autumn sown) than spring beans it was necessary to combine the resistance from ICARDA lines with winter hardiness. This was achieved by crossing the ICARDA lines with UK winter beans with moderate resistance to chocolate spot and then either backcrossing to other winter-hardy, well-adapted lines, or selfing. At various stages throughout the breeding programme, material combining chocolate spot resistance with winter hardiness was selected by growing plants over winter and then covering with polythene and inoculating with B. fabae. This material is now being trialled in inoculated field plots for selection of suitable lines for composite crosses. It is not easy to get consistent results in field trials, even with irrigation, but some lines have shown moderately high resistance over 3 years. The inheritance of resistance of the ICARDA lines has not been studied in any detail but results suggest that there are a number of genes involved and that it is difficult to retain high resistance and also select for good agronomic characters.

There is no evidence of race specificity to BPL 710 or BPL 1179 but it is obviously desirable to use different sources of resistance. To

some extent, this has already been done by using established lines with some resistance to chocolate spot in the backcrossing programme, but new sources of resistance from ICARDA are also being evaluated.

ASCOCHYTA BLIGHT

Ascochyta blight can cause heavy losses of yield on occasions but these are usually confined to local outbreaks. Lesions occur on leaves, stems and pods and result in leaf death, lodging, pod abortion and seed staining. The pathogen can be seed transmitted and this has been assumed to be the major source of inoculum although carry-over may also occur on plant debris or through volunteer plants (Gaunt, 1983). However, recently Jellis (unpublished) has found the previously unknown teleomorph of A. fabae on overwintering bean stems and this may be a major source of wind-dispersed inoculum.

In recent years, ascochyta blight has been a serious problem for seed producers because of failure to meet certification standards. This situation is particularly serious during the early years of a new variety when seed stocks are very limited. Chemical control is not very effective and so improved resistance to A. fabae is seen as highly desirable. Work at PBI Cambridge began in 1980 and screening techniques were developed. Young plants were inoculated in the field using pycnidiospores of A. fabae and irrigation was used to maintain leaf wetness and to ensure splash dispersal of spores (Lockwood et al., 1985; Jellis et al., 1985). Varietal differences were found in the incidence of disease in the foliage, pods and seed. Among plants with a similar growth habit, the incidence of disease in pods and seeds reflected that in young plants, demonstrating that in such material foliage scores will give a reasonable forecast of resistance. However, when varieties and lines with a range of growth habits were assessed there was no association between foliar and pod infection. The large-podded broad bean types had a higher incidence of pod infection relative to foliar infection than the smaller-podded field beans. As might be expected with a splash dispersed organism, there was also a strong correlation between straw length and incidence of pod disease, taller varieties having the lower incidence of disease. The reliability of using scores on young plants, at least for the traditional small-podded field beans, means that glasshouse tests can be used as a reasonable guide to field performance, and screening of early generation material is now carried out routinely.

Resistant lines have been identified in both UK winter-hardy material (e.g. IB18-1/3, now know as Quasar, Jellis et al., 1984, and line 224) and in the ICARDA International Nursery. The PBI Cambridge lines have now been used widely in crossing programmes and in the production of composite varieties. In order to broaden the genetic base, four of the resistant ICARDA lines have also been introduced into the breeding programme recently.

DISEASES AFFECTING ESTABLISHMENT

Recently there has been considerable interest in field beans with virtual freedom from condensed tannins in the testa. Tannins bind with protein and decrease its digestibility, limiting the use of beans in animal feeds. This character is controlled by a single recessive gene

which also controls flower colour; tannin-free beans are completely white flowered. However, some tannin-free lines have been found to establish poorly under certain conditions and this has resulted in poor yields (Bond et al., 1987). We have been examining the causes of poor establishment and looking for variation in this character within white-flowered, tannin-free material.

Pairs of near-isogenic winter and spring lines developed by the Pulses Group at PBI Cambridge, with or without the gene which confers virtual freedom from tannin in the testa, were used in comparisons of emergence and plant growth in a number of soils (Pascual Villalobos and Jellis, 1990). It was found that the tannin-free lines were shorter and had more disease on the radicle and plumule than tannin-containing lines. Fusarium solani and Fusarium culmorum were isolated from lesions and when re-inoculated into seedlings were found to be highly pathogenic. In later work Pythium sp. was also found to be associated with seedling infections.

Glasshouse tests using surface sterilized seed dipped in a spore suspension of Fusarium sp. or Pythium sp. showed that whereas tannin-free members of the two near-isogenic lines were always severely affected, other tannin-free lines and varieties differed in their resistance. By using a simple glasshouse test, breeders should be able to select tannin-free beans which establish satisfactorily.

THE CONTRIBUTION OF DISEASE RESISTANCE TO HEALTHY CROPS

Field beans have traditionally been regarded as a low input, low cost crop but also as somewhat unreliable. A number of approaches to improving the crop are being made, one of which is to incorporate better disease resistance in new varieties. Chocolate spot is the disease most feared by farmers and many now spray crops with fungicide to try and contain it. However in wet years, which are most conducive to chocolate spot, crops grow very tall and fungicide application at flowering time can be difficult and not very effective. It seems unlikely that the sources of resistance available at present will be adequate to prevent disease outbreaks without any crop protection in wet years, but a strategy of integrated control, i.e. resistant varieties with carefully timed fungicide application, should give substantial improvements in disease control.

Ascochyta blight has traditionally been controlled by using healthy seed but in recent years this does not seem to have been adequate, at least for seed growers trying to meet stringent health requirements. Whereas control of A. pisi in peas has proved very effective, on field beans fungicides currently on the market used as seed dressings or foliar sprays only seem capable of reducing the incidence of disease by approximately fifty percent (Jellis et al., 1988). Advanced lines and composite varieties currently in the programme have greatly improved resistance to ascochyta blight and it is already evident that multiplication of such material is much more trouble-free.

An advantage of currently grown varieties of field beans over peas is that they do not require seed dressing for good establishment. It would be unfortunate if an improvement in quality characteristics of the crop

could only be achieved at the expense of reliability of emergence. Recent work suggests that this need not be the case if screening tests for resistance to diseases affecting establishment are introduced.

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MECHANISMS OF RESISTANCE IN CEREALS TO PESTS

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ABSTRACT

A research programme to detect resistance to pests in a crop should ideally address at the outset the following questions: How wide a genetic range of plant material should be screened? Should the mechanisms of resistance be investigated? Should the biological components (antibiosis, antixenosis, tolerance) be studied? At what point should plant breeders be involved? An alternative to the above approach is the crude but rapid screening of large numbers of plants by releasing pests and assessing plant survival after an interval. Examples of both approaches are given, concentrating on cereals, with a discussion of the advantages and limitations of each strategy.

INTRODUCTION

Host plant resistance to insects has the potential, acting alone, or with insecticides and/or with natural enemies, to keep pest numbers below economic thresholds and thereby reduce pesticide inputs. Pesticides are not always used rationally on U.K. cereals, for instance; economic losses (Wratten & Mann, 1988; Wratten et al., 1990) and undesirable side-effects can occur even when products are applied in response to spray thresholds (Vickerman & Sunderland 1987; Vickerman et al., 1987; Sotherton et al., 1987; Wratten et al., 1988). In addition to the above economic and environmental advantages, simple cost-benefit analysis relating research and development costs for a new resistant cultivar to its economic value following release can be 1:300 (Luginbill, 1969). The development of a typical new pesticide, however, may incur an expenditure of $50m, with little prospect of recouping this outlay for many products before patent expiry (Finney, 1988). Because of these high costs, return on investment has been delayed, raising doubts in some companies about long-term investment in agrochemical R & D (Finney, 1988). Against this must be weighed the limitations of host-plant resistance as a major pest-control method. These limitations will be discussed in this paper, which will use case studies of cereal pests to illustrate prospects for the use of host plant resistance.

APPROACHES TO THE INVESTIGATION OF RESISTANCE

Screening via crude bioassays

The simplest way of searching for useful levels of resistance is to inundate a wide range of replicated plant material in the laboratory, glasshouse or field, with large numbers of the pest and leave the system for

a few days or weeks. All that need be assessed after the chosen interval has elapsed is the condition and number of surviving plants. In theory, this method can identify highly resistant lines which survive the high pest pressure and has been used for aphids by Reinink & Dieleman (1989) in a glasshouse screen of lettuce cvs., by Birch (1989) for turnip root fly (Delia floralis) in brassicas, and by Dunbier, Kain & McSweeney (1977) in New Zealand; the latter led to the release of a new, aphid-resistant lucerne cultivar within three years of initial screening. Although this method is intuitively attractive, it does have potential drawbacks, especially if the initial screening is not conducted under realistic conditions. The main limitation is that no information on the classical components of resistance (antibiosis, non-preference, tolerance; Painter, 1951) is obtained, or on resistance mechanisms. This also means that screening has to be based on bioassays using the pest, rather than via a short-cut involving screening for cuticular hair density, levels of secondary plant compounds, etc. Also, should the resistance break down in the field, the lack of a detailed understanding of its nature means that an explanation for the breakdown is unlikely to be found. Because of this method's broad approach, however, it has the advantage that it is not biased in favour of only one component; some laboratory screens concentrate on antibiosis only, for instance the work by Bohidar et. al., (1986) and Spiller & Llewellyn (1987) on seedling resistance in wheat to aphids.

Evaluation of the biological components and mechanisms of resistance

The advantage of investigating all potential resistance components is that there is less likelihood that a key insect-plant interaction will be overlooked. If, in addition, a major mechanism of resistance is identified, the possibility exists for a rapid biochemical or physical (e.g. leaf hairs) screen of a very large range of plant material without the time-consuming elements of insect culturing and bioassays. Of the three components proposed by Painter (1951), antibiosis (defined in this case as an effect on the performance of the insect, via fecundity, mortality, development rate, etc.) is the commonest in the literature, especially among laboratory studies. It often involves the caging of single insects on plants, and in the case of aphids on cereals, of recording such parameters as intrinsic rate of increase (r_m; Birch, 1948), relative growth rate (van Emden, 1969), rate of honeydew production (Spiller & Llewellyn, 1987), number of embryos in the adult parthenogenetic female (Bintcliffe & Wratten, 1982), adult weight (Lee, 1983) etc. Host non-preference (= antixenosis) is a less relevant measurement for laboratory screening since it is likely to be greatly influenced by environmental conditions in the field, including the crop's agronomy and the areas of monoculture over which it is grown. The relevance of simple laboratory choice experiments involving cereal aphids (e.g. Leather & Dixon, 1982; Dent, 1986; Dent & Wratten, 1986) to single-cultivar monocultures is questionable. Tolerance, whereby certain cvs. yield better than do others per unit pest number, is a realistic measurement only under field conditions. Even then, unless it operates below or near the pest's economic threshold, it would be unlikely to replace or even supplement pesticide use. Although tolerance is unlikely to promote the development of resistance - breaking pest biotypes, if employed as the major component it could lead to high pest levels on a regional basis, leading to future problems.

Screening of a wide genetic range of plant material

Concentration on commercial cvs. has the potential disadvantage that they may have low levels of resistance compared with their ancestors and wild relatives. This decline in resistance with cultivation history is well

illustrated by the black bean aphid, Aphis fabae on Vicia (Fig. 1). The disadvantage of screening plant material which is genetically remote from commercial cultivars is that crosses may be difficult to make, due to 'premating' and/or 'postmating' problems (Stalker, 1980).

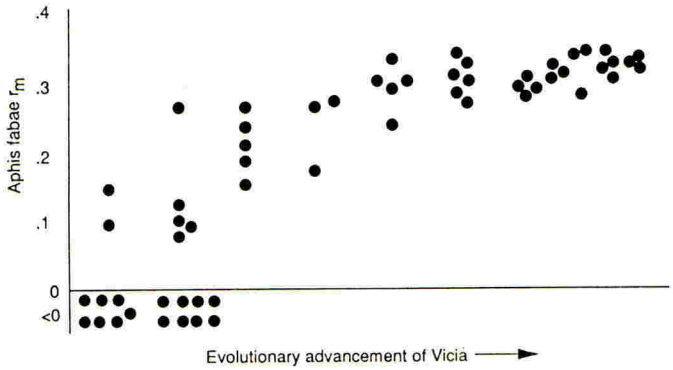

FIGURE 1. Performance of Aphis fabae in relation to evolutionary advancement of Vicia hosts (from Holt & Birch, 1984)

CASE STUDIES OF RESISTANCE IN CEREALS

The role of hydroxamic acids in conferring resistance to Lepidoptera and aphids

The structure of hydroxamic acids (Hx) is similar to that of amides with a hydroxyl group rather than a hydrogen atom attached to the nitrogen atom. Hx from cereals are benzoxazinone type cyclic Hx, i.e. benzene ring is fused to a 6-membered ring containing an oxygen atom besides the hydroxamic acid function. They share much of their biosynthetic pathway with tryptophan. DIMBOA (2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one) is the most abundant compound among the Hx which have been isolated from maize, wheat and several related wild Gramineae. They are found in the plant as glucosides which are enzymically hydrolysed to the corresponding aglucones when the plant tissue is injured (see review by Niemeyer, 1988).

Early work on Hx concentrated mainly on resistance in maize. Klun, Tipton & Brindley (1967) reported significant correlations between resistance to the European corn borer (Ostrinia nubilalis) and levels of benzoxazolinones (hydroxamic acid breakdown products) in maize extracts and followed this with bioassays using artificial diets which showed hydroxamic acids to be the most active plant component. Robinson, Klun and Brindley (1978) demonstrated feeding deterrent effects both in field and laboratory tests. In the 1980's, recurrent selection based either on DIMBOA levels or on insect damage led to improved first-brood resistance.

Long, Dunn, Bowman & Routley (1977) reported a highly significant negative correlation (r = 0.72) between the level of infestation of the corn leaf aphid (Rhopalosiphum maidis) and the concentration of DIMBOA in maize tissue. Argandona, Luza, Niemeyer and Corcuera (1980) found similar

relationships for the rose-grain aphid Metopolophium dirhodum on wheat. In addition hydroxamic acids have been implicated as resistance factors in several wheat cultivars to the aphids Schizaphis graminum and R. maidis (Corcuera, Argandona & Niemeyer, 1982). S. avenae was investigated in this context in preliminary work by Bohidar et. al. (1986) who found that 96% of the variance in the resistance of six Chilean wheat cultivars was explained by Hx levels. Although Hx levels decline as plants age (Thackray et. al. 1990), the youngest leaves of seedlings had high Hx levels, while the levels in young flag leaves were similar to those of some seedlings. The latter data are important as S. avenae causes yield loss when feeding on flag leaves and ears of mature wheat plants (Wratten, 1978) and is not a seedling pest in the U.K. other than as a BYDV vector in N. England (McGrath & Bale, 1989). Leszczynski et. al. (1989) demonstrated a highly significant negative correlation ($r = -0.905$) between S. avenae intrinsic rate of increase (r_m) and Hx concentration in flag leaves of wheat. Screening mature plants for Hx or via an insect bioassay is time-consuming, however, as at least two months are needed to obtain wheat in ear under U.K. glasshouse conditions. If there was a correlation between seedling Hx or aphid resistance and their values in mature plants, this would be of value in accelerating a screening programme. In a series of papers by Lowe in the 1980s (e.g. Lowe, 1984 a,b,c) aphid bioassays were used to rank a large number of cvs. of winter wheat in relation to their resistance as semi-mature plants to the grain aphid. When these were evaluated as two-leaf seedlings for DIMBOA content, using high performance liquid chromatography (Fig. 2 a,b; see Niemeyer et. al., 1989 for methods), a wide range of DIMBOA content was obtained (Fig. 3). Resistance to the grain aphid was measured in six of these cvs. which showed the greatest range in Hx concentration and the correlation was very high ($r = -0.94$; Fig.4). However, there was no significant correlation between the rankings of these cvs. as seedlings, based on Hx levels and aphid relative growth rate, and the rankings of Lowe(loc. cit. There was also no significant correlation between the Hx ranking of all 36 cvs. as seedlings and their ranking in Lowe's mature plant bioassays. Hydroxamic acids can therefore explain a high proportion of the variation in resistance to S. avenae in wheat, but plants need to be screened for Hx levels and resistance at the same growth stage. The effect is mainly via antibiosis, although the fact that inverse relationships exist between DIMBOA levels in aphids feeding on wheat p lants and DIMBOA levels in the plants themselves suggest that feeding deterrency may be involved (Niemeyer et. al., 1989).

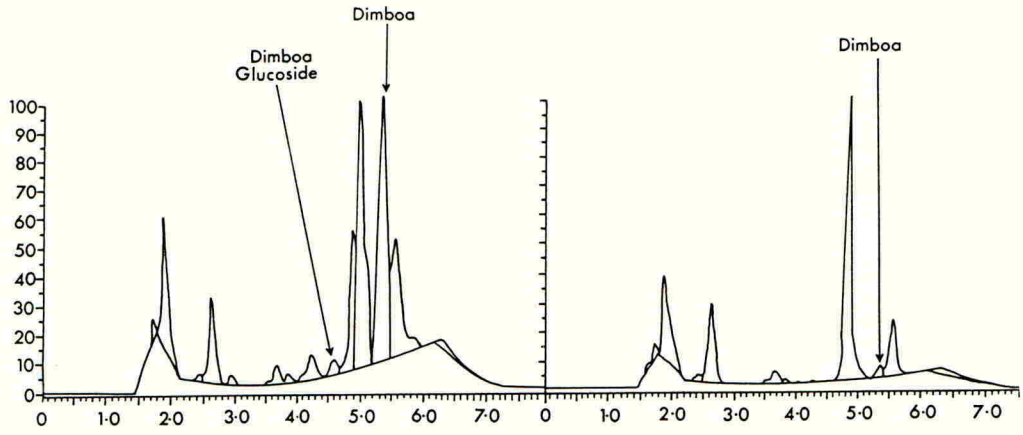

FIGURE 2. HPLC analyses of high and low DIMBOA wheat cultivars.

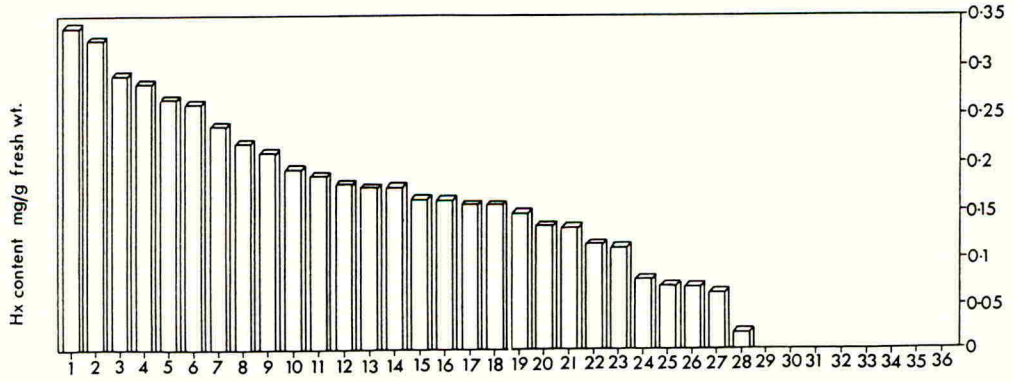

FIGURE 3. Range of DIMBOA concentrations in seedlings of cultivars screened by Lowe as mature plants via aphid bioassays.

The role of physical factors in aphid resistance in wheat

Lowe et al. (1985) demonstrated a role for antixenosis in wheat against S. avenae, with the possibility that non-glaucous cvs. may be avoided by the aphid. Acreman & Dixon (1986) examined six genotypes of awned spring wheat and showed that aphids were up to 22% less fecund, and were more likely to be dislodged than aphids feeding elsewhere on an ear. These two factors reduced aphid population growth to one third of that on awnless plants. Population modelling by Acreman (1984) revealed that with the regular reductions in aphid numbers of cereals by 85% which are feasible via host plant resistance, the three outbreaks recorded over a five-year study period would not have occurred.

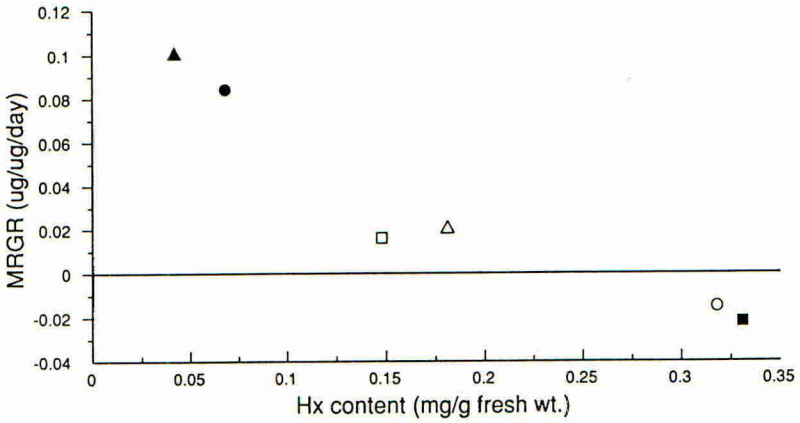

FIGURE 4. Relationship between mean relative growth rate of Sitobion avenae and seedlings selected from those in Fig. 3 representing a high range in hydroxamic acids.

Resistance to aphids as virus vectors

This is usually biologically less tractable than is the production of resistance against direct-damaging pests as resistance is usually only partial and thresholds for virus vector control are usually low. However, Givovich & Niemeyer (1991) showed that in wheat seedlings with high DIMBOA levels, fewer plants were infected with BYDV by R. padi than on low DIMBOA cvs. Fewer aphids reached the phloem on the high DIMBOA cvs. and those which did reach the phloem took longer on high DIMBOA cvs. In W. Europe, R. padi feeds low down on wheat and barley seedlings, often on the leaf sheaths at or below ground level. In view of the above results for S. avenae (Fig. 4; which showed the importance of evaluating the appropriate growth stage, it is relevant to examine leaf sheath Hx levels in seedling wheat. This was done for R. padi on a range of Triticum material, using relative growth rate and HPLC analysis of DIMBOA, as described above. A correlation coefficient of - 0.794 was obtained (Fig. 5). This evidence, and that of Givovich & Niemeyer (1991) suggests that Hx levels may be a good indicator of pest performance in plant breeder's screening, not only for direct-damaging pests such as S. avenae but for virus-vectors such as R. padi.

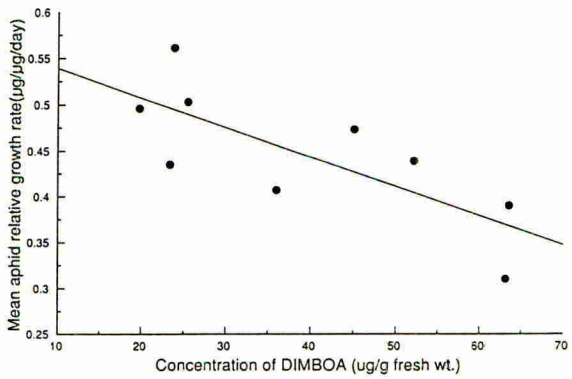

FIGURE 5. Relationship between mean relative growth rate of Rhopalosiphum padi and seedling sheath hydroxamic acid levels.

Resistance to other cereal pests

Silica has been implicated in the resistance in grass to frit fly (Oscinella frit) attack (Moore, 1984), and the distribution and quantity of silica within the plant may make a major contribution to control of this fly in grassland (Clements & Henderson, 1983). Silica was also implicated in resistance in oats and wheat to Hessian fly (Mayetiolia destructor; Miller et. al., 1960), and in rice resistance to mollusc grazing (Wadham & Wynn-Parry, 1981). The addition of sodium silicate to host plants reduced growth rates of populations of frit fly and S. avenae (Hanisch, 1981). The structure and persistence of the coleoptile (in oats) and colour (in ryegrass) determine levels of oviposition by the frit fly.

CONCLUSIONS AND PROSPECTS

Even a relatively small increase in resistance can reduce the probability of a pest outbreak, especially in combination with the action of natural enemies with or without insecticide use (see van Emden, 1987 and van Emden & Wratten, 1990 for reviews). However, Burn (1987) in a review of integrated pest management in cereals, concluded that for most insect pests in these crops the ' ... use of varietal resistance seems remote, and will remain so while so little attention is given to it in plant breeding programmes'. This is in contrast to resistance to fungal pathogens, where varieties exist which offer some resistance to eyespot, powdery mildew, Septoria and yellow and brown rust. It is interesting to speculate whether the absence of aphid-resistance breeding programmes leading to the growing of aphid-susceptible cvs. (such as Maris Huntsman in the 1970s) over large areas, may have influenced grain aphid abundance at field or regional 'meta-population' (Hanski, 1989) scales. The reason for this lack of progress in insect resistance is likely to be a combination of a) arable pests are easily controlled chemically, with no serious insecticide resistance in cereal pests b) there has been no suitable biochemical or physical factor in cereals which could be used as an indicator of pest performance, to speed up the screening process c) bioassays were often laboratory based and concentrated on modern cvs. which did not reveal large differences in resistance for use in breeding programmes d) environmental awareness of insecticide side-effects has been much higher recently than in the 1970s, leading to moves in some European countries to reduce these inputs substantially.
e) some plant breeding concerns have been taken over by agrochemical companies; this may make it less likely that pest-resistant cvs. will be produced from this source as insecticide sales may suffer as a consequence.

Despite the above five partly historical reasons, in the 1980s and 1990s a general desire to reduce pesticide inputs has arisen alongside the demonstration that some physical (e.g. awns) and biochemical (e.g. DIMBOA) factors can explain high proportions of the variation in aphid numbers on wheat. It seems that it is now timely to exploit this information in directed screening and plant breeding programmes, possibly via technological development agencies such as the British Technology Group in the U.K. or via agrochemical companies.

ACKNOWLEDGEMENTS

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BREEDING FOR RESISTANCE TO VIRUS VECTORS

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ABSTRACT

Most plant viruses are transmitted by invertebrate vectors and the control of such vectors is an important strategy in the control of virus infection in some crops. The genetic control of resistance to virus vectors offers a potentially inexpensive and effective way of decreasing or delaying virus spread in many crops. However, identifying and incorporating the best sources of resistance into plants can be difficult due to the complex interactions that exist between the host plant, virus, vector and environment. This article identifies some of the mechanisms involved in vector resistance in plants, highlights some of the problems encountered in breeding plants for increased resistance to virus vectors, and touches on some possible solutions.

INTRODUCTION

Unlike most other plant pathogens, viruses cannot be controlled in crop plants directly by chemicals. Control measures therefore aim to evade or delay infection and/or to minimise the effects of infection on marketable yield. As most plant viruses are transmitted by invertebrate vectors, one important way to decrease virus incidence is to control vector populations. For some viruses and some crop species, chemical control of vector-mediated virus spread is an important and effective strategy but for some others it is either ineffective, difficult or too costly. The increasing public awareness of the financial and possible ecological costs of repeated pesticide applications to crops has generated renewed interest in devising a more biological approach to controlling virus infections in crop plants. Until very recently, little or no direct attention has been given to breeding plants for resistance to virus vectors. Most instances in which such resistance has been introduced into plants has been a bonus from breeding for pest resistance. Nevertheless, several well documented instances (see review by Jones, 1987) already exist where virus spread in commercial crops has been effectively controlled by vector resistance. In other instances, although virus control through vector resistance is only partially effective, the delays produced in virus epidemics and/or the decreased secondary spread provide significant economic benefits. In addition, several reports indicate the potential of plant types with relatively low levels of vector resistance (Jones, 1979; Lecoq *et al.*, 1979; Gunasinghe *et al.*, 1988) and further studies on the use of such plants in integrated control programmes involving chemical, cultural and biological methods may increase further the effectiveness of this material for virus control (Lecoq & Pitrat, 1983). In many crop species therefore, and sometimes in existing commercially acceptable material, there is variation of several kinds to be exploited.

SOME IMPORTANT CONSIDERATIONS

The extent to which virus spread is prevented depends on many complex interacting factors, such as the host range and vector relations of the virus, the mobility and breadth of host range of the vector, the type, effectiveness and durability of resistance to the vector and to the virus, and environmental factors (Kennedy, 1976; Jones, 1987). With such complexities, studies on vector resistance are often difficult and progress is usually dependent on using the collective expertise of plant breeders, virologists, zoologists and plant chemists.

Not all forms of vector resistance are equally effective in preventing virus spread and some forms may actually increase virus incidence (Kennedy, 1976). Furthermore, in some situations, the development of vector resistant material may not be the best method of virus control available, or may not be practical. Thus, with viruses that are spread by several vector species or with crops that are infected with several viruses having different vectors, breeding for effective resistance to all these vectors may be impractical unless the resistance mechanism is non-specific, e.g. plant hairs. Nevertheless, resistance to several vector species has been incorporated into single cultivars of some crop plants, e.g. wheat, rice.

SOME MECHANISMS INVOLVED IN RESISTANCE

Plants show a range of resistance mechanisms to virus vectors. Four types of resistance that interfere in different ways with normal plant/vector relations and that can influence virus infection have been distinguished (Jones, 1987). In addition to the primary effect of decreasing virus acquisition and inoculation, the expression of any of these types of resistance can also lead to premature vector dispersal, thereby influencing secondary spread.

1. Interference with host finding by vectors. Although in some instances vectors reach hosts simply by a random encounter, the well documented responses of insects to colour and other physical and chemical stimuli indicate that active mechanisms are involved in host recognition. Changes in the type or strength of these signals may decrease or eliminate alighting responses or feeding, thereby preventing acquisition or inoculation of virus. For example, host finding can be inhibited by changes in leaf colour, and by changes in the form of the crop canopy (see Jones, 1987).

2. Interference with the initial settling of vectors. Having located a host, chemical and physical stimuli from the plant are involved in the identification and establishment of suitable feeding sites. If the resistance is sufficiently strong to prevent probing altogether, no acquisition or inoculation of virus will occur, but if the vector is required to probe the plant before it is deterred from feeding, inoculation of non-persistent viruses is likely to occur. However, a delay in making the first probe, if sufficiently long, may exceed the time for which non-persistent viruses are retained by vectors, so lessening the probability of virus transmission. If the vector is sufficiently deterred from feeding after several brief exploratory probes, it may leave the crop. Where this happens, the spread of persistent and semi-persistent viruses might be expected to decrease, because no transmission is likely to occur in these brief probes and because secondary spread would be minimized. Plants have a range of physical (e.g. hairs, glandular hairs, thick cuticle) and chemical

(e.g. surface waxes and volatile compounds) attributes that can interfere with this initial settling phase before feeding begins (Lapointe & Tingey, 1984; Gunasinghe et al., 1988).

3. Interference with sustained feeding behaviour of vectors. When resistance in plants is due solely to antibiosis (an adverse effect on vector growth, reproduction and survival), the fact that vectors are more likely to remain on plants for lengthy periods would seemingly restrict the usefulness of this form of resistance in preventing virus spread. The most likely benefit might be a decrease in secondary spread through decreasing vector populations and possibly vector activity, but this would probably only affect the spread of persistently transmitted viruses. However, recent detailed studies on insect feeding behaviour have shown that, in several instances, antibiosis seems to operate by preventing ingestion or phloem-finding, and these effects seem to decrease the likelihood of acquisition and/or inoculation, both of persistent and of semi-persistent viruses (Auclair et al., 1982; Dahal et al., 1990).

4. Specific interference with vector transmission of virus. In a few instances, resistance of plants to inoculation of non-persistent viruses by aphids cannot be explained by any of the previous mechanisms of resistance. In some, but not all of these instances, the phenomenon is vector specific but not virus specific (Lecoq et al., 1979; Romanow et al., 1986).

SOME PROBLEM AREAS

The appearance of new vector biotypes as a result of growing resistant cultivars is a potential difficulty and has been the cause of major problems in some crops, e.g. leafhoppers and planthoppers on rice (Sogawa, 1982). However, there are also many examples where resistance to vectors has proved to be durable for many years. In general, biotypes occur most commonly in monophagous vector species and where resistance is determined by single genes, and seem less common in polyphagous vectors, and where resistance is controlled by several genes and/or is non-specific. Another possible problem is that resistance mechanisms to one vector may inadvertently increase susceptibility to colonisation by another vector or pest. Thus, breeding for pubescence in wheat to control the cereal leaf beetle and hessian fly increases infestation with the mite vector of wheat streak mosaic virus (Harvey & Martin, 1980).

Many problems exist for the breeder in the detection and exploitation of some of the mechanisms of resistance that promise to be useful. In particular, rapid screening methods are needed, especially for some of the less obvious mechanisms of vector resistance, and field scale testing methods are needed for evaluating the benefits of some forms of resistance that are not apparent in small scale tests. In addition, further studies are needed on integrated control methods to assess the usefulness of material containing the less effective forms of vector resistance.

SOME RECENT DEVELOPMENTS

New biochemical techniques for the rapid detection, identification and quantification of specific secondary plant metabolites, which are closely correlated with pest resistance in some plants, may prove useful for the

rapid and unequivocal selection of resistant plants and hybrids. At the basic level, such studies may aid research on the precise mechanism(s) underlying resistance. Genetical techniques now enable chromosome pieces to be transferred between species and even genera, and this has enabled vector mite resistance to be introduced in wheat and black currant. More recently, genetic engineering methods have succeeded in introducing specific genes for pest resistance into plants. There are therefore good reasons for optimism that, given the necessary resources and expertise, major strides can be made in studying and exploiting this natural means of virus control in several crop species.

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THE INTERACTION OF HOST PLANT RESISTANCE TO INSECTS WITH OTHER CONTROL MEASURES

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ABSTRACT

Insects on resistant plant varieties are usually smaller, slower breeding, more restless, more stressed and may contain higher levels of secondary plant substances than when on susceptible varieties. These characteristics are known to affect the susceptibility of insects to insecticides and the impact of biological control. There could also be interactions with other control measures such as the use of the confusion pheromone technique and antifeedants.

INTRODUCTION

Host plant resistance to insects as a sole control measure is not without its disadvantages (van Emden, 1987). There may well be a yield penalty resulting from the diversion of the plant's resources to resistance mechanisms, "problem trading" can occur when resistance to one pest is obtained at the cost of increasing susceptibility to another or to a pathogen, and there is always the possibility that pest biotypes able to overcome the resistance mechanism may arise. Using plant resistance in a pest management programme which utilises other suppression factors, such as some insecticidal and biological control, will alleviate these problems in the sense that partial rather than total resistance may be quite adequate when integrated with other control measures.

The interactions that can occur between host plant resistance and such other control measures are therefore of the utmost importance in modern pest control.

THE INTERACTION OF PLANT RESISTANCE AND INSECTICIDES

One general effect of antibiotic resistance is to reduce the body size of the insect pests. Toxicity of an insecticide to humans is a function of body weight (mg AI of toxicant per kg), as it is for insects (μg AI per mg). It is therefore to be expected that a lower concentration of insecticide than required for pests on a susceptible variety should give an equivalent kill on a variety that is antibiotic, and this phenomen was indeed shown by Selander *et al.* (1972) for *Myzus persicae* on chrysanthemums in the laboratory. The concentration of parathion needed to kill half the population (LC50) on a susceptible variety was double that needed on one that was partially resistant. Further work has shown that insect body weight differences do not, however, fully explain this effect. Attah (1984) compared the LC50 of malathion to *Metopolophium dirhodum* on the wheat varieties 'Maris Kinsman' (susceptible) and 'Emmer' (aphid weight reduced by

8 per cent) at the stem elongation stage. He found that the LC50 to aphids from 'Emmer' was only 44 per cent that from 'Maris Kinsman'. Even the dose corrected for aphid body weight (LD50) was still 39 per cent lower on 'Emmer', suggesting that aphids on the resistant variety were not only smaller, but also physiologically more sensitive to the insecticide. Similar conclusions have been drawn by Mohamad & van Emden (1989) with *Myzus persicae* on two Brussels sprout varieties. In field experiments, Raman (1977) found that the same dose of dimethoate as caused 75 per cent mortality of *Empoasca dolichi* on a susceptible cowpea variety resulted in 94 per cent mortality on one that was partially resistant.

Chewing insects can show a similar increase in susceptibility to insecticide on resistant plants (Abro & Wright, 1989). On a resistant cabbage cultivar ('Minicole F1') the pupal weight and fecundity of *Plutella xylostella* were both somewhat over 40 per cent lower than on the susceptible 'Offenham Flower of Spring'. The LD50s for abamectin and cypermethrin were respectively 78 and 25 per cent lower on the resistant than on the susceptible cultivar. Abro & Wright (1989) point out that other workers have shown similar results for Lepidoptera on soy bean and plant hoppers on rice; they also make the important point that there are two papers reporting resistant plants or their allelochemicals inducing resistance to insecticide. This latter point is clearly relevant to the production of resistant plants by transgenic methods, where it is much more likely than with traditional breeding methods that just a single and potent allelochemical will be transferred.

THE INTERACTION OF PLANT RESISTANCE AND INSECT NATURAL ENEMIES

Plant resistance relying on toxic allelochemicals may well be damaging to natural enemies. This topic has been reviewed by Herzog & Funderburk (1985), who list examples of alkaloids and other antibiotic chemicals proving toxic to parasitoids within hosts or otherwise reducing emergence from the prey. There are also many records of aphids feeding on plants with toxic allelochemicals proving toxic to ladybird, hover fly and lacewing predators (e.g Hodek, 1967; Philippe, 1972; Ruzicka, 1975). A somewhat different effect was reported by Orr & Boethel (1985); unparasitised *Pseudoplusia includens* were able to achieve 30 per cent survival on a highly antibiotic soy bean variety, but all caterpillars parasitised by *Copidosoma truncatellum* died before the parasitoids could emerge.

Another potentially disadvantageous consequence of plant resistance for biological control lies in the smaller size of hosts on resistant varieties, which could lead to reduced fecundity of natural enemies and, with parasitoids, a greater proportion of males being produced. This again suggests there is a disadvantage in seeking greater plant resistance in a pest management system than is necessary in the context of other restraints. Gowling (1989) found that adult weight of female *Aphidius rhopalosiphi* emerging from *M. dirhodum* was 34 per cent lower on the partially resistant wheat variety 'NG Avalon' than on the susceptible 'Armada' and that the number of mature eggs per female at emergence was reduced by 26 per cent. No significant effects on the parasitoid were evident when aphids were cultured on a variety ('Rapier') showing less resistance to aphids than 'NG Avalon'.

Much of the literature tends to emphasise these deleterious effects of plant resistance on insect natural enemies. However, specific examples of plant resistance can also have a beneficial interaction with biological control; perhaps the most unusual is the report that, on nectariless cotton varieties partially resistant to *Heliothis*, the ladybird *Cryptolaemus* becomes a valuable predator of the pest although it prefers the nectar from the leaf nectaries on normal cotton (Adjei-Maafo, 1980). Another specific example is given by Kareiva & Sahakian (1990). Population growth of pea aphids on a mutant 'leafless' genotype was lower than on the normal by less than 10 per cent. However, control of aphids by the ladybirds greatly magnified this difference, since many ladybirds fell off the normal variety through inability to mantain a grasp on the smooth, slippery leaves; by contrast, the beetles were observed clinging to the stems and tendrils on the 'leafless' variety.

There is also an important general beneficial interaction, especially where more horizontal plant resistance is concerned. This beneficial interaction was first proposed by van Emden & Wearing (1965). Experimental evidence has since accumulated from a number of studies on aphids; however, the interaction has wider application, including even to univoltine pests.

Evidence that economically acceptable biological control of *M. persicae* on chrysanthemums by *Aphidius matricariae* only occurred on partially aphid resistant varieties came from the accidental appearance of the parasitoid in a variety trial (Wyatt, 1970). Then Starks *et al.* (1972) showed in glasshouse experiments that control of *Schizaphis graminum* by *Lysiphlebus testaceipes* was similarly only achieved on resistant barley varieties. Since then Dodd (1973), Lykouressis (1982) and Gowling (1989) have provided further examples for parasitoids on cereals and brassicas (Fig.2).

van Emden (1987) simulated the interaction between plant resistance and biological control with an improved model, making three alternative assumptions about the nature of the interaction on two hypothetical varieties differing in resistance to the prey (Fig. 1). The intermediate assumption was the same per cent mortality of prey on both varieties as a result of biological control. A second assumption of reduced per cent mortality on the resistant variety was simulated by using the density dependence of the mortality built into the model. The third assumption of enhanced per cent mortality on the resistant variety was simulated with the rather extreme input of equal numerical mortality on the two varieties. Fig. 2 shows the proportional reduction in prey population on the resistant variety predicted by the third assumption in comparison with the susceptible for various degrees of plant resistance and various levels of biological control on the susceptible variety. The actual reduction is given from those experimental data referred to above from which it is possible to calculate the required inputs independently. Fig. 2 suggests that, particularly at low levels of plant resistance, a prediction based on equal numerical mortality on both varieties has been fulfilled.

So far, the following factors have been identified as contributing to this unlikely phenomenon:

1) There is an element of artifact. Fig. 1B shows that there is some magnification of apparent plant resistance with time as the prey generations progress, even in the absence of natural enemies.

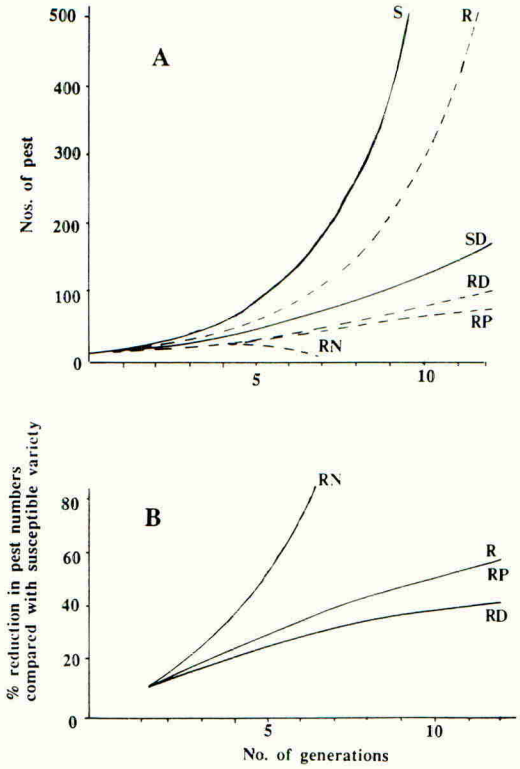

Fig. 1. The effect of biological control on the reduction of a pest population with time (B) on a partially resistant variety given various pest population growth curves (A).

S, Susceptible variety and R, partially resistant variety, both in the absence of biological control; SD, RD, the same varieties with biological control assumed to show density dependence; RP, resistant variety with same per cent mortality from biological control between generations as on the susceptible variety; RN, resistant variety with same numerical mortality between generations as on the susceptible variety.

2) The numerical response of natural enemies, in terms of their alightment on different varieties, may not be density dependent on differences in population levels of their prey. van Emden (1978) was the first to demonstrate the attraction of a plant odour (synomone) for an aphid parasitoid, with mustard oils in crucifers and *Diaretiella rapae*, a parasitoid of *B. brassicae*, involving both olfactometer and aphid-free plant experiments. The resulting interaction in this example was unfavourable; the partially resistant variety of Brussels sprout 'Early Half Tall' had low levels of mustard oil. In the glasshouse, a potentially slowly rising but only lightly parasitised aphid population on 'Early Half Tall' eventually overtook the heavily parasitised population on the susceptible variety 'Winter Harvest'.

Wickremasinghe (1989) has studied the discrimination of *A. rhopalosiphi* for different cultivars of wheat, using 'Timmo' as susceptible to *Rhopalosiphum padi* and 'Moghan 2' and 'Ommid' as two partially resistant Iranian cultivars. The variety on which the parasitoid had emerged was distinctly preferred. Thus she could produce parasitoids which preferred 'Timmo' to 'Ommid', or *vice versa*. Females emerged from 'Timmo' strongly preferred 'Timmo' to 'Moghan 2', but when the same choice was given to females emerged from 'Ommid', they failed to distinguish between the two cultivars.

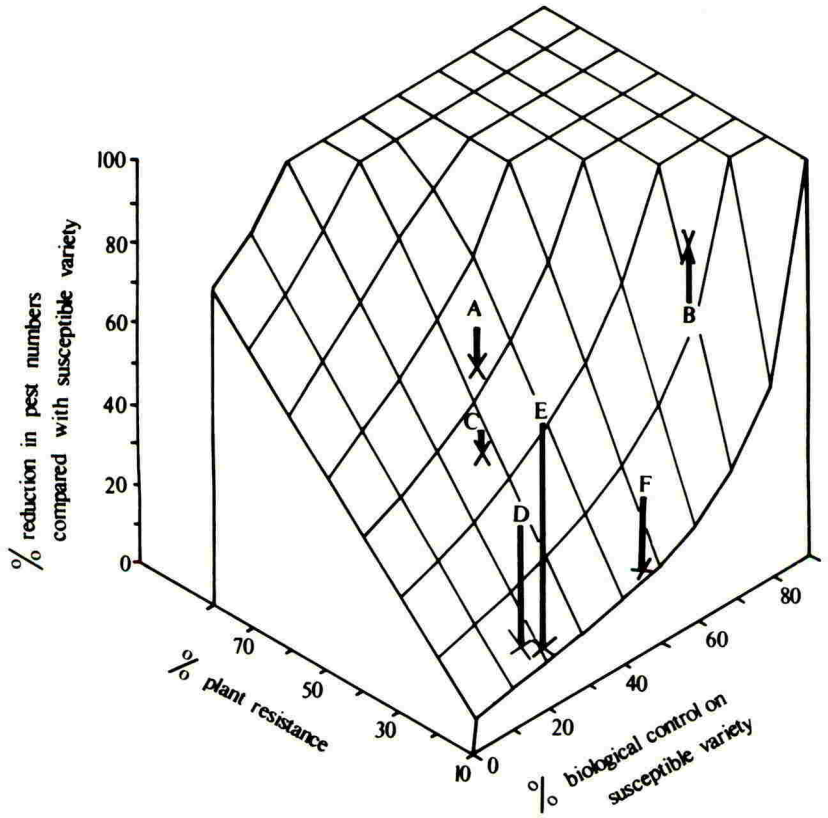

Fig. 2. Response surface of the reduction of a pest population on varieties with different levels of resistance (in comparison with a susceptible variety) with varying biological control mortalities on the latter and assuming the same numerical mortality on both varieties.

A, data of Starks *et al.* (1972) for *Schizaphis graminum* parasitised by *Lysiphlebus testaceipes*; B, data of Lykouressis (1982) for *Sitobion avenae* parasitised by *Aphelinus abdominalis*; C and F, data of Gowling (1989) for *Metopolophium dirhodum* parasitised by *Aphidius rhopalosiphi* on 'NG Avalon' and 'Rapier' respectively; D and E, data of Dodd (1973) and Gowling (1989) respectively for predation of *Brevicoryne brassicae* in the field.

3) Aphids on resistant varieties are more restless, and may fall off the plant with greater frequency when disturbed by parasitoids and predators than when on susceptible ones (Table 1). Using sticky cards for a week in the field, Gowling (1989) estimated that 26 per cent of *B. brassicae* fell from the Brussels sprout variety 'Bedford Winter Harvest' compared with only 17 per cent from the more susceptible 'Predora'; syrphid larva and parasitoids would have been the main natural enemies active on the plants.

4) Smaller aphids (as on resistant varieties) may be less able to avoid parasitisation and capture by predators (Dixon, 1985).

5) Aphids on resistant varieties are not only smaller, but also produce less honeydew, e.g. a mean of 0.0.172μl/day produced per *R. padi* on the resistant wheat cultivar 'Ommid' compared with 0.432μl/day on the susceptible 'Huntsman'. The efficiency of parasitoids is reduced by large amounts of honeydew, since the proportion of time used for antennal cleaning increases considerably (Wickremasinghe, 1989).

TABLE 1. Movement of *Metapolophium dirhodum* off susceptible ('Armada') and resistant ('Rapier') wheat cultivars during six days in the absence and presence of its parasitoid, *Aphidius rhopalosiphi* (data of Gowling, 1989).

	n	'Armada'	'Rapier'	P=
Parasitoid absent:				
Per cent aphids found off plants	80	14	15	ns
Per cent aphids regaining plants		55	49	ns
Parasitoid present:				
Per cent aphids found off plants	80	24	50	<0.05
Per cent aphids regaining plants		29	24	ns

THE INTERACTION OF PLANT RESISTANCE AND INSECT PATHOGENS

That aphids on resistant varieties are susceptible to insecticides to a greater extent than can be explained by their smaller size (see earlier) suggests some "stress" upon them which might also make them more susceptible to infection by pathogens. Moreover, the greater restlessness of aphids on resistant varieties might also increase their contact with pathogens. This latter effect has been reproduced by following fungus (*Entomophthora neoaphidis* and *Vertillicium lecanii*) sprays against aphids with an application of alarm pheromone to increase movement of the aphids (Mardell *et al.*, 1986). Herzog & Funderburk (1985) were indeed able to cite two examples of improved infection by pathogens of pests on resistant soy bean genotypes, *Bacillus thuringiensis* against *Heliothis zea* and *Nomouraea rileyi* against *Anticarsia gemmatalis*.

Since insect pathogens have some reputation for variability of success, and natural pathogen epidemics are usually associated with crowded insect populations (suggesting stress), the weakening of pests by plant resistance in relation to use of insect pathogens seems an area ripe for research.

THREE-WAY INTERACTION BETWEEN PLANT RESISTANCE, INSECTICIDES AND BIOLOGICAL CONTROL

The reduction in frequency of insecticide spraying on resistant cultivars, especially a delay in the first application, should be beneficial to biological control.

However, there is a potentially very useful interaction in the possibility of using reduced doses of insecticide on resistant cultivars (van Emden, 1987). Presumably because carnivores lack the highly effective enzyme systems evolved by herbivores for metabolising plant toxins (Plapp, 1981), the mortality response curve to insecticides of natural enemies is characteristically steeper than for their prey (Fig. 3A). This results in increasing selectivity of the insecticide in favour of the natural enemy as dose is reduced, no doubt the reason for the success of the early integrated control of *Therioaphis trifolii* on lucerne in California by application of reduced doses of insecticide.

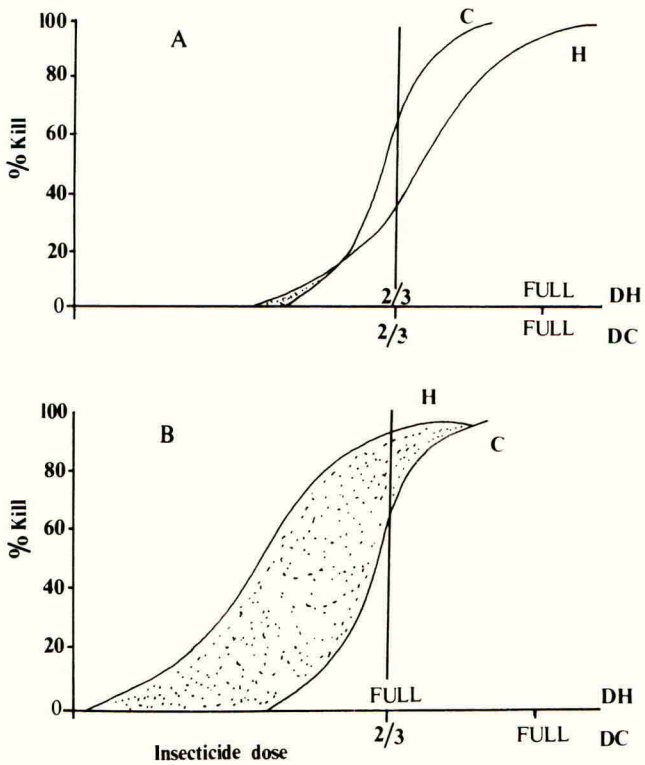

Fig. 3. Theoretical selectivity of pesticide application on a resistant (B) compared with on a susceptible (A) variety.

H, dose mortality curve of a herbivore; C, dose mortality curve of predator or parasitoid. DC, dose scale for carnivore; DH, dose scale for herbivore; selectivity "window" is stippled.

If it is possible to reduce insecticide dose on a resistant cultivar by even only one-third to maintain the kill of the pest obtained on a susceptible cultivar (see earlier), the potential for insecticide selectivity is dramatic (Fig. 3B) since kill of the natural enemy would now be much reduced by the lower dose.

INTERACTION OF PLANT RESISTANCE WITH SOME OTHER CONTROL MEASURES

Since some cultural practices (e.g. irrigation, fertiliser) can modify the physiology and rate of growth of plants, it is to be expected that they might enhance or decrease the expression of resistance of cultivars. Just as one extreme example, McMurtry (1962) showed that, although performance of *T. trifolii* on susceptible lucerne clones was unaffected by potassium and phosphorus deficiency, the former deficiency reduced resistance of resistant clones whereas the latter increased resistance. Where a resistance is due to the phenology of the cultivar, e.g. late flowering of faba bean genotypes and resistance to bruchids (Tahhan & van Emden, 1989), a change in sowing date can easily obviate the resistance.

One can also envisage interactions between plant resistance and the use of pheromones for mating disruption. One constraint on the efficiency of the method is high adult pest densities resulting in greater activity of males and more female-initiated sexual encounters (Rothschild, 1981); plant resistance would obviously be helpful in reducing this constraint.

Equally one can envisage that antixenotic resistance would enhance the effect of the application to plants of antifeedant chemicals.

CONCLUSIONS

Very valuable interactions can occur between plant resistance, chemical and biological control. By utilising such interactions, the dangers of resistance to pesticides and the breakdown of plant resistance are reduced. Such interactions seem most likely to be beneficial where the resistance to pests is only partial and not based on a single effective chemical constituent of the plant.

Modern gene transfer techniques are therefore likely often to disadvantage biological control if toxins accumulate in the prey. By creating a high selection pressure on the pest, they may also hasten the breakdown of the resistance and defeat the resistance of other varieties based on a similar character, but usually at a lower and variable level. If a gene producing a non plant-derived toxin is transferred, as e.g. the transfer of the gene for the *B. thuringiensis* endotoxin, then there must be real dangers of producing a high level of resistance in the pests to the natural bacterium.

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