SESSION 1
CHEMICALS IN CROP PROTECTION

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Volatility, vapour pressure and vapour activity

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Fungicide resistance risk management

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INTRODUCTION

Fungicides are important tools in crop production systems for the control of most plant diseases, and this will remain so for the foreseeable future. Given the extent of private and public research investment to bring novel fungicides to the market, and their key role in ensuring profitable production of food and fibre crops, it is of concern that performance can decline through the development of resistance in target fungi. Although resistance arises through selection of rare mutations in pathogen populations, armed with sufficient knowledge about the resistance mechanisms, good monitoring procedures, and access to products with different modes of action, it should be possible to manage resistance effectively. Indeed, to date no fungicide group has been lost solely because of resistance. However, assessment of resistance risk for new compounds during their development is a difficult concept, and certainly not yet an exact science. In this short paper we briefly consider factors which influence resistance risk, and how this risk might be managed. A more detailed review of resistance management has recently been presented by Brent (1995).

GENETICS AND BIOCHEMICAL MECHANISMS OF RESISTANCE

Resistance is a genetically inherited trait that results from one or more mutations in the target fungus. In cases where resistance becomes a practical problem, mutations usually cause an alteration to the biochemical target site, so that the fungicide either binds less effectively, or not at all. These major gene mutations cause high levels of resistance, and although the vast majority will be associated with some fitness penalty, or even lethality, a few may not. Mutations may be in nuclear genes or, as in the case of the recently introduced strobilurin fungicides, in mitochondrially encoded genes.

The exact nature of the amino acid changes are only known for benzimidazole, DMI and strobilurin fungicides (Davidse & Ishii, 1995; Delye *et al.*, 1998; Koeller, 1999). Not surprisingly perhaps, given the conserved nature of these fungicide targets, it is usually the same mutation that causes resistance to each particular fungicide group. The consequence of these conserved target site changes is that cross resistance will extend to all fungicides with the same mode of action, and testing for cross-resistance to existing compounds is an important first step in risk evaluation. The conserved nature of target site changes also means that once resistance is identified, perhaps through a mutation screening programme, with modern PCR based diagnostics, point mutations can be searched for in field

populations of other pathogens, and detected at frequencies well below what can easily be achieved through bioassay.

But not all resistance is caused by single gene changes. Instead many mechanisms may contribute to resistance in an additive way, albeit overlaying a target site change. Although a point mutation in the target sterol 14 alpha demethylase gene (CYP51) is linked with resistance to DMI fungicides, other mechanisms particularly affecting uptake (Sanglard *et al.*,1998) or efflux (de Waard, 1997) also contribute to performance difficulties.

Where multiple changes are involved resistance builds up more gradually, and can be reversed when selection is withdrawn. Bioassays are the only practical way to monitor these changes, and although underlying cross-resistance patterns may follow modes of action, resistance factors may differ substantially between different analogues. For instance, resistance to triadimenol soon became a practical problem in the control of barley leaf blotch, caused by *Rhynchosporium secalis*, but resistance factors for other DMIs were lower and these remained largely effective (Kendall *et al.*, 1993).

COMPONENTS OF RESISTANCE MANAGEMENT.

Fungicide resistance first became a practical problem over thirty years ago, following the introduction of benomyl, the 2-aminopyrimidine powdery mildew fungicide, ethirimol, and the rice blast fungicide kasugamycin. This prompted interest in ways to manage fungicide use to minimise the economic consequences of the problem, and to date this has been successful and disease control has not been lost for key diseases. To a large extent this has been due to success in discovering new fungicides with novel modes of action, but experience has shown that resistance management must involve other actions.

These include:

- Risk assessment
- Baseline sensitivity measurements
- Devising strategies to minimise risk
- Evaluation of anti-resistance strategies

Risk assessment

Risk assessment has recently been reviewed in some detail by Brent & Hollomon, (1998). It must be an ongoing programme which starts early in the development phase of a new product, and continues into commercial use. The responses to fungicides in different pathogens, and in different parts of the world, may modify risk assessments.

Despite a single site of action, difficulties surrounding production of resistant mutants in plant pathogens led to a preliminary assessment of moderate risk for strobilurin fungicides. The appearance of significant practical resistance in powdery (Chin *et al.*, 2000) and downy mildews (Ishii *et* al., 1999)) in some parts of the world within the first two years of widespread use, caused the resistance risk for strobilurins to be revised upwards from moderate to high for these pathogens.

Inherent disease-associated factors also influence resistance risk. Pathogens with short generation times and extensive sporulation can cause explosive epidemics requiring frequent fungicide applications and, consequently, greater resistance risk than pathogens with just one generation a year (e.g. smuts). Treatment of isolated epidemics in glass-houses or plastic tunnels increases resistance risk, since dilution by wild-type sensitive individuals is kept to a minimum.

However, even an assessment of high risk does not mean that the problem can not be effectively managed. The degree of risk merely implies the type and severity of management strategies required to manage the risk.

Baseline sensitivity

Even before introduction of a new fungicide some variation in sensitivity will exist in natural wild-type populations. Defining the range of variation in baseline sensitivity provides a valuable benchmark against which later monitoring exercises can be compared, and shifts in sensitivity towards resistance evaluated. Developing appropriate monitoring methods provides a valuable resource to evaluate anti-resistance strategies during the commercial life of a fungicide. The importance of establishing good base-line sensitivity distributions is now recognised in the registration process, at least in the European Union, where data on sensitivity testing must be included in the registration submission.

Further if resistance is already detectable in baseline populations, these initial frequencies may also provide an indication of the potential rates at which selection for resistance could occur.

Devising strategies to minimise resistance

Not to use a fungicide is a sure, but impractical way, to avoid the development of resistance. Usually anti-resistance strategies restrict the number of treatments with "at-risk" fungicides in any season, by reducing the numbers of cycles of selection to which the pathogen is exposed. Even so, treatments are generally applied when the pathogen is most active during the early phase of epidemics, and when the pathogen population is vulnerable to selection. So risk may not always be reduced directly in proportion to the reduction in treatments.

The effect of dose rate on selection may also modify resistance risk, but the relationship between dose and selection is complex. In practice, strategies based on full, recommended dose rates are difficult to implement since growers reduce rates to save costs yet obtain adequate disease control if disease levels are not too high.

There is some evidence from modelling studies that reducing dose rate lessens the risk of single gene resistance (Brent, 1995) but the potential loss of control and resultant increases in population size could increase the probability of selection for resistance. FRAC groups have recently recognised the importance to stress 'effective' rates in their recommendations (e.g. FRAC-STAR, in www.gcpf.org). In the case of resistance involving several genes, reduced dose could increase the risk because it allows resistance to develop in a step-wise manner.

Certainly, repeated treatments with just one, persistent, "at risk" fungicide over a large geographic area should be avoided. Agronomic factors which minimise disease, and steps that restrict treatments when non-damaging levels of disease are present, are also valuable components of anti-resistance strategies. But the cornerstone of anti-resistance strategies is

the use of fungicide mixtures, or alternations, where the partner fungicides have different modes of action. This is a simple concept for growers to grasp, although it is, of course, based on combinations, the components of which select different mechanisms of resistance.

Triazoles and morpholines belong to different cross-resistance groups of fungicides, and alternations of the two groups have for example been largely successful in extending the life of both for the control of sigatoka disease of bananas. Mixtures of benzimidazoles and phenylcarbamates (Leroux *et al.*, 1994), which both target β tubulin yet which can select different resistance alleles; and more recently cyprodinil and fludioxonil (Forster & Staub, 1996) has been deployed as an anti-resistance strategies against grey mould (*Botrytis cinerea)*, especially in grapes

The choice of alternations or mixtures is dependent on the specific requirements of control strategies for each pathosystem. In general however, alternations are more dependent for their effectiveness on a loss of fitness of the corresponding resistant types than are mixtures. Without a loss in fitness of the resistant genotype to one fungicide (and where partner fungicides of a negative cross-resistant group are not available) there would be no reversion to sensitivity to that fungicide, when the alternating partner fungicide is being used. In the case of mixtures other factors like the reduced frequency of the combined resistance, and their potential effects on fitness may play a role.

Of course an even more challenging strategy for the pathogen would be to alternate the use of mixtures with other products. Finally mixtures provide the insurance against product failure in the case of resistance developing against one of the components.

Evaluation of anti-resistance strategies

Once anti-resistance strategies are in place it is important to check their effectiveness. This can be done by monitoring performance, but commercial crops seldom have untreated areas so that critical evaluation of fungicide performance can be difficult. Poor disease control can be due to many factors including wrong dose rate, poor application timing or spray coverage, product deterioration, or simply using the wrong fungicide for the target pathogen.

But where the fungicide-disease combination is a high risk one, monitoring sensitivity and reference to base-line sensitivity data allows signs of poor performance to be properly assessed against any shift in sensitivity. Monitoring can also be done in trials which test the same strategy over a long period of time. Monitoring programmes based on bioassay, and especially for obligate pathogens such as powdery mildews and scabs, are resource intensive and costly, so that there is increasing interest in molecular diagnostic techniques where these are available to detect common resistance alleles.

Resistance to strobilurin fungicides, which appears linked to a single point mutation in the target cytochrome bc-1 gene in a number of pathogens, and where resistance aleles can be detected by molecular techniques at frequencies down to 1:10,000 sensitive alleles (Fraaije, personal communication 2,000), could provide a good test of the value of molecular diagnostics in monitoring programmes.

CONCLUSIONS

Resistance threatens to weaken our ability to control many important pests, weeds and diseases. For plant pathologists it is a relatively new problem, but co-operative research representing private and public interests, has successfully managed the problem, and avoided serious losses in control. In addition to new chemistries, this has been achieved through improved understanding of the mechanisms of resistance, and the inherent factors associated with particular diseases and fungicides which can contribute to resistance.

Although risk assessment and management are imprecise sciences, enough is known to devise practical schemes to minimise the risk of resistance. Adding to this knowledge base is an ongoing process which can only improve the ability to manage the resistance problem.

The discovery of resistance to a new fungicide does not spell its demise. It only provides insights into how the fungicide may be used in a sustainable manner in the future.

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Insecticide resistance and its implications for the field performance of pesticides

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ABSTRACT

Predicting risks posed by resistance to the field performance of insecticides is a complex task. The likelihood of resistance appearing in the first place depends of numerous genetic, ecological and operational factors whose combined effects can sometimes be rationalised in hindsight but remain difficult to anticipate in advance. Once resistance has appeared, its impact on control efficacy depends on both the potency and frequency of resistance mechanisms, whose accumulation in pest populations has particularly serious implications for crop protection. Although prevention of resistance is the most desirable goal, counter-selection for susceptible individuals in the absence of insecticides can contribute substantially to mitigating resistance problems.

INTRODUCTION

Predicting the field performance of crop protection agents is often fraught with difficulty since a large number of factors, both biotic and abiotic, can intervene to compromise expectations based on laboratory evaluations or field trials conducted under carefully prescribed conditions. In addition to variation attributable to extrinsic causes such as weather, operator inconsistency, differences in product quality etc, there may be variation within pest populations with a potential to affect control efficacy. This can be largely phenotypic, e.g. when responses depend on age, size or life-stage and vary within the life span of an individual, or can reflect genetic polymorphisms whereby individuals differ in their ability to avoid, take-up, detoxify or excrete pesticides, or in the sensitivity of their target-site enzymes or receptors. If such differences are sufficient to enhance survival or reproduction under field conditions, pests possessing resistance genes may be selectively favoured and increase gradually in frequency. The field performance of a pesticide should then deteriorate accordingly, often leading to more frequent applications or the need to switch to a non cross-resisted product.

Attempts to anticipate, and circumvent, the impact of resistance on control efficacy should ideally address three conceptually different but inter-related questions:

i) Is resistance likely to develop and pose a potential threat?
ii) If it does appear, what are the practical implications likely to be?
iii) What can be done to delay its appearance, or at least to reduce its deleterious effects on field performance?

In this paper we review briefly these three aspects with particular reference to insecticides and examples involving aphids and whiteflies, two of the world's most important groups of Homopteran insect pests.

WILL RESISTANCE ARISE?

Given the breadth and diversity of resistance mechanisms reported to date, it can confidently be assumed that no insecticide, however novel or unconventional in its effect, is immune to the appearance of resistant genes. The probability of these achieving detectable frequencies depends instead on a suite of ecological and genetic factors, and how these interact with pesticide use patterns (reviewed by McKenzie, 1996; Denholm *et al.*, 1998). As a consequence, the same product can face very different risks with different pest species, and even with the same species in different cropping systems.

The prevalence of resistance to established insecticide groups - organophosphates (OPs), carbamates and pyrethroids - among aphid pests of UK agriculture and horticulture provides a case in point. The three species that have historically proved most problematical are the damson-hop aphid, *Phorodon humuli*, the cotton-melon aphid, *Aphis gossypii*, and the peach-potato aphid, *Myzus persicae*. Their capacity to evolve resistance can, to an extent, be rationalised in hindsight in terms of differing life-histories and the crop systems they inhabit. During the summer, *P. humuli* is virtually restricted to wild and cultivated hops, on the latter of which insecticides remain the predominant means of aphid control. In major hop-growing regions, the net effect has been to select for strong resistance to a succession of compounds, with wild hops proving wholly ineffective as an untreated reservoir for retarding its development (Lewis & Madge, 1984). *A. gossypii* is restricted to glasshouses in the UK, where selection pressures are generally far more intense than in the open field (Denholm *et al.*, 1998). *M. persicae* is also a glasshouse pest, but in addition infests a range of field crops including potatoes, brassicas, lettuce and sugar beet, all treated with insecticides for aphid control. The overall effect of exposing *M. persicae* to the same range of chemicals on glasshouse and field crops has been to select for a number of mechanisms that collectively threaten all available insecticides except the more novel agents imidacloprid and pymetrozine (Field *et al.*, 1997; Foster *et al.*, in press).

At the other extreme, there are key aphid pests with no history of resistance whatsoever. Two good examples are the cereal aphids, *Sitobium avenae* and *Rhopalosiphum padi* which, despite a long history of control with insecticides, have retained susceptibility in laboratory tests (Furk *et al.*, 1983), and no reductions in field performance have been reported. Compared with *P. humuli*, *A. gossypii* and *M. persicae*, these species appear to present a contrasting situation in which migration between treated crops and extensive areas of wild grass hosts has precluded any directional increase in the frequency of resistance genes.

However, any comfort that can be drawn from these two apparently explicable extremes is challenged by other comparisons that are less readily reconciled. The currant-lettuce aphid, *Nasonovia ribisnigri*, is virtually restricted to lettuce as a secondary host, but is only confirmed to have developed resistance within the last three years (Barber *et al.*, 1999). In many respects the bionomics of *N. ribisnigri* are very similar to those of *P. humuli*, since both are largely restricted to a single species of heavily-sprayed crop grown in disjunct localities in the UK. It is therefore puzzling why resistance appeared in one species at least

two decades earlier than in the other. Similarly, the ecology of the potato aphid, *Macrosiphum euphorbiae* has much in common with that of *M. persicae*. Both inhabit glasshouses and a range of field crops and yet resistance in the former, first confirmed in 1998 (IACR-Rothamsted, unpublished data) appears to have taken at least 30 years longer to evolve. Factors determining whether and when resistance will arise can clearly be subtle and merit further study utilising new molecular and theoretical approaches for investigating the population biology of pests and predicting the practical and evolutionary consequences.

PRACTICAL IMPLICATIONS

Once resistance has arisen and been detected, a second challenge is to predict its likely implications for the performance of insecticides applied correctly under field conditions. It is self-evident that a resistance gene already selected to detectable frequencies must confer some increased prospect of survival or it wouldn't have been selected in the first place. However, it is wrong to equate any significant increase in tolerance in laboratory bioassays with markedly decreased control efficacy in the field. Effects on field performance depend both on the potency of resistance conferred by particular mechanisms, and on their frequency in treated populations. Even highly potent mechanisms may be tolerable if present at sufficiently low frequencies, whereas mechanisms of low potency could possibly occur at substantially higher frequencies without demonstrably impairing field performance.

Clearly, the most damaging combination of all is a highly potent mechanism present at a very high frequency. This is exemplified well by bioassay data on the response of samples of the glasshouse whitefly, *Trialeurodes vaporariorum*, collected from UK glasshouses to an insect growth regulator, buprofezin (Gorman *et al.*, 1998, and unpublished data). In leaf-dip assays against whitefly nymphs, fully susceptible insects gave an LC_{50} of *c.* 0.01 ppm a.i., whereas others showed distinct plateaus in the concentration-response relationship with differing proportions of insects surviving concentrations as high as 5000 ppm a.i. (Figure 1). Some samples (e.g. UK-3 and UK-4 in Figure 1) consisted almost entirely of individuals with this extremely resistant phenotype. This finding supported grower's claims of loss of control with buprofezin in glasshouses, a conclusion reinforced by exposing susceptible and resistant strains of *T. vaporariorum* to treatment with buprofezin at the recommended rate in 'field simulator' cages. These cages (described by Rowland *et al.*, 1991), which enable pest populations to be established, increase in size, distribute themselves on host plants, and be treated in a manner similar to that in the field, have proved ideal tools for investigating relationships between resistance and field performance under controlled conditions in the laboratory, and for monitoring resistance development (e.g. Cahill *et al.*, 1996). In the present example, a single treatment with buprofezin suppressed very effectively the emergence of the following generation of adults of the susceptible strain, but elicited no effect at all against resistant insects (Figure 2).

Relating resistance data to anecdotal or confirmed information on field performance is not always so clear-cut. Bioassays against eggs of the cotton whitefly, *Bemisia tabaci*, showed samples from a rose greenhouse and a cotton field to exhibit *c.* 500-fold resistance to a juvenile hormone mimic, pyriproxyfen, compared to the response of a laboratory susceptible strain (Figure 3). In keeping with this result, field simulator experiments exposing susceptible and resistant strains of *B. tabaci* on cotton to a single treatment with pyriproxyfen demonstrated a very effective reduction in the subsequent build-up of the

susceptible strain but little or no effect on the resistant one (Figure 4). However, there are reports from Israel of cotton growers continuing to achieve acceptable control of *B. tabaci* with pyriproxyfen despite a widespread increase in the frequency of resistance in that country (Horowitz *et al.*, 1999). This conflict may in part reflect the dual mode of action of pyriproxyfen, which as well as being an effective ovicide (by direct or transovarial action), also elicits mortality of nymphs. In side-by-side bioassays, resistance in Israeli strains was more strongly expressed in terms of egg hatch (as shown in Figure 3), than nymphal or pupal survival (Horowitz & Ishaaya, 1996; Horowitz *et al.*, 1999). Thus, it is possible that application of pyriproxyfen against populations containing a more varied age structure than those in the experiment reported above would result in improved control and a better basis for predicting field performance.

The above examples relate to single forms of resistance affecting very specific compounds. Implications of the sequential selection of multiple resistance mechanisms for the field performance of insecticides are probably best demonstrated by work on the aphid *Myzus persicae*. The three mechanisms affecting current aphicides that have been characterised in UK and European populations are as follows:

i) Overproduction of one of two closely-related carboxylesterases (E4 and FE4) that sequester or degrade insecticidal esters - OPs in particular. This results from the structural amplication of esterase genes (Field *et al*, 1997), and can lead to progressively increasing levels of resistance that for convenience are classified as S (susceptible), R_1 (moderately resistant), R_2 (strongly resistant) or R_3 (very strongly resistant) (Devonshire *et al.*, 1998).

ii) Target-site resistance to pyrethroids, termed knockdown resistance or kdr, resulting from changes to a voltage-gated sodium channel protein in nerve membranes (Martinez-Torres *et al.*, 1999).

iii) Target-site resistance to the dimethyl carbamates pirimicarb and triazamate, conferred by structural alterations to the enzyme acetylcholinesterase and termed MACE (Modified AcetylCholinEsterase) (Moores *et al*., 1994).

The effect of aphids accumulating these mechanisms on the efficacy of different control options is being explored by exposing genetically well-characterised clones of *M. persicae* to a range of pesticides in field simulators (Foster & Devonshire, 1999, and unpublished data). Figure 5 shows a subset of these data for four clones tested on Chinese cabbage and potatoes with pirimicarb and the pyrethroid lambda-cyhalothrin. The move from R_1 to R_3 carboxylesterase resistance had little impact on the performance of these insecticides but would have had a substantial effect had an OP (e.g. dimethoate) also been included. The addition of kdr led to loss of control with the pyrethroid, and the further addition of MACE severely compromised the performance of pirimicarb. Thus, the progressive accumulation of resistance mechanisms, each with their distinct cross-resistance characteristics, led to a progressive erosion in the performance of a range of aphicides. This accords with results of experiments monitoring the response of *M. persicae* to insecticides in population cages in the field (e.g. Dewar *et al.*, 1998).

MITIGATING THE RISKS

The diversity of tactics proposed or implemented for mitigating resistance risks have been reviewed elsewhere (e.g. Roush, 1989; Denholm & Rowland, 1992) and are beyond the scope of this paper. However, one often overlooked phenomenon with important consequences for the sustainability of field performance is the potential for resistance genes to decline in frequency in the absence of insecticides due to counter-selection against resistant individuals. Work on *M. persicae* has disclosed a number of apparent fitness costs associated with different resistance mechanisms, one of the most important being the reduced ability of aphids with highly overproduced carboxylesterases to survive harsh climatic conditions during winter (Figure 6). It is unclear at present whether this is attributable to the esterase mechanism *per se* or to other traits (e.g. kdr resistance) that are tightly linked to amplified esterase genes as a consequence of parthenogenetic reproduction (Foster *et al.*, in press). Whatever the cause, these fitness costs appear not only to have prevented a consistent increase in the frequency of resistant phenotypes from year to year, but during extended periods of low aphid abundance (e.g. 1997-1999) have also promoted a consistent reduction in the frequency of resistance (Figure 7). Although secondary to the desirability of preventing the appearance of resistance in the first place, such factors are clearly also an important consideration when predicting the likely impact of resistance on the field performance of pesticides.

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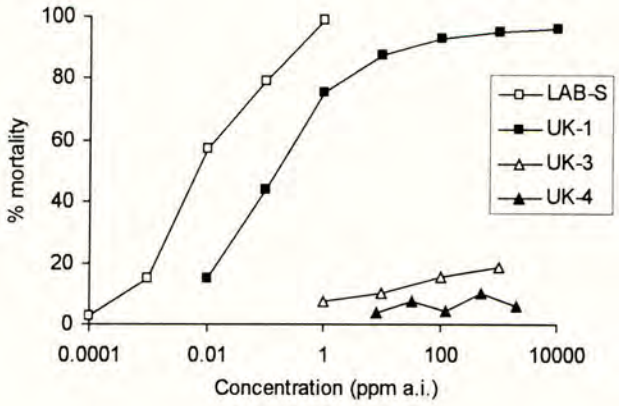

Figure 1. Concentration-response relationships for four strains of the glasshouse whitefly, *Trialeurodes vaporariorum*, tested in a leaf-dip bioassay with the insect growth regulator, buprofezin. Buprofezin was applied to second-instar nymphs and mortality assessed at the fourth-instar stage, c. 12 days later. LAB-S is a fully susceptible reference strain. UK-1, UK-3 and UK-4 are field strains collected between 1997 and 1999 from glasshouses in the UK.

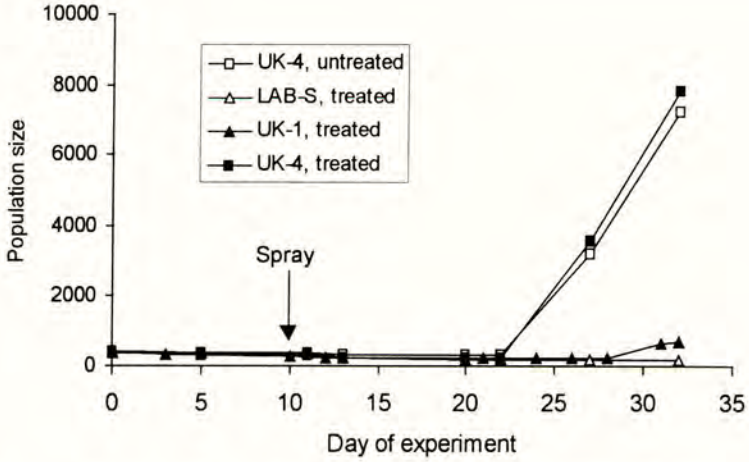

Figure 2. Changes in the number of adults of three strains of the glasshouse whitefly, *Trialeurodes vaporariorum*, following treatment in field simulators with the insect growth regulator, buprofezin. Buprofezin was applied on day 10 as a foliar spray at a concentration of 75 ppm a.i., corresponding to the recommended field rate. LAB-S is a fully susceptible reference strain. UK-1 and UK-4 are field strains collected between 1997 and 1999 from glasshouses in the UK.

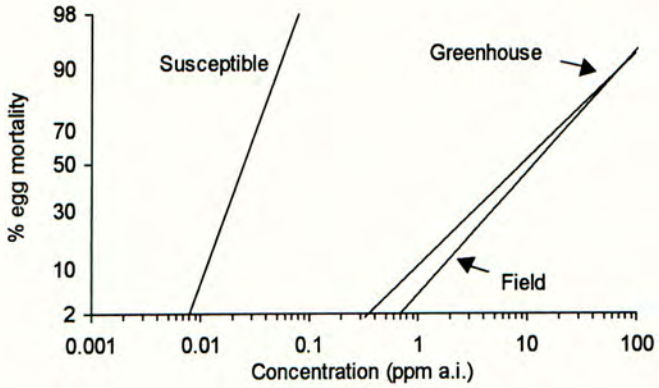

Figure 3. Probit lines describing the response of a susceptible and two resistant strains of the cotton whitefly, *Bemisia tabaci*, to the insect growth regulator, pyriproxyfen, in a leaf-dip bioassay. Mortality was assessed as the hatching failure of eggs laid by females exposed to pyriproxyfen deposits on cotton leaves.

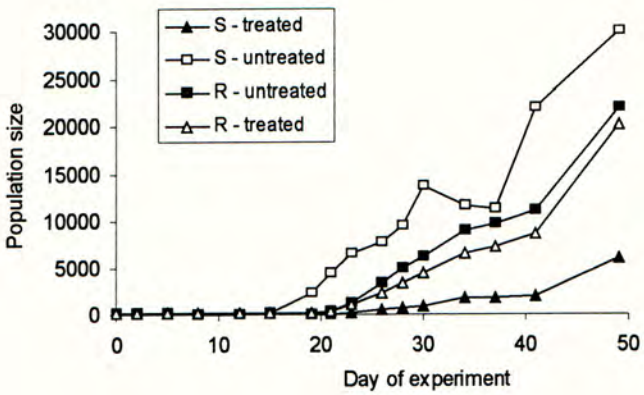

Figure 4. Changes in the number of adults of susceptible (S) and resistant (R) strains of the cotton whitefly, *Bemisia tabaci*, on cotton plants in field simulators left untreated or treated with pyriproxyfen on day 0, immediately before the initial release of adult whiteflies. Pyriproxyfen was applied at a concentration of 40 ppm a.i., corresponding to the recommended field rate.

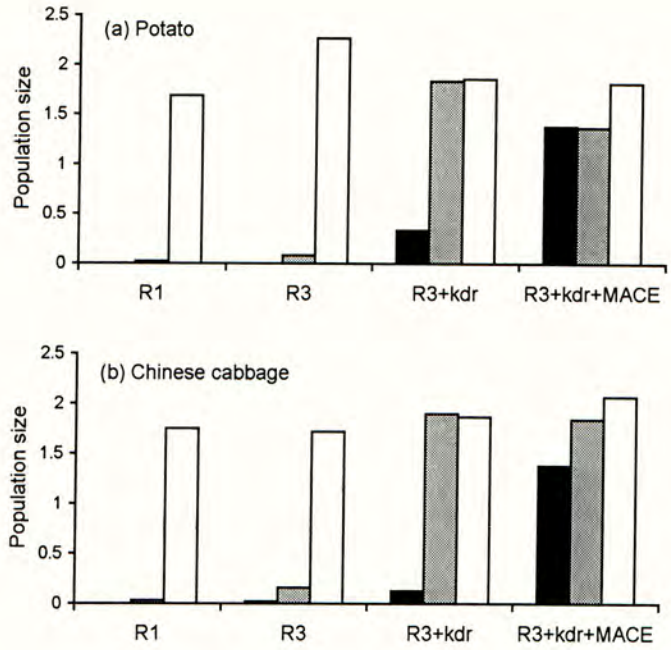

Figure 5. Response of four clones of the peach-potato aphid, *Myzus persicae*, to two insecticides applied at manufacturer's recommended rates in field simulator cages. The vertical axis represents the mean number of aphids/plant present three days after spraying expressed as a proportion of numbers present immediately before spraying. Black bars = pirimicarb, grey bars = lambda-cyhalothrin, white bars = untreated controls.

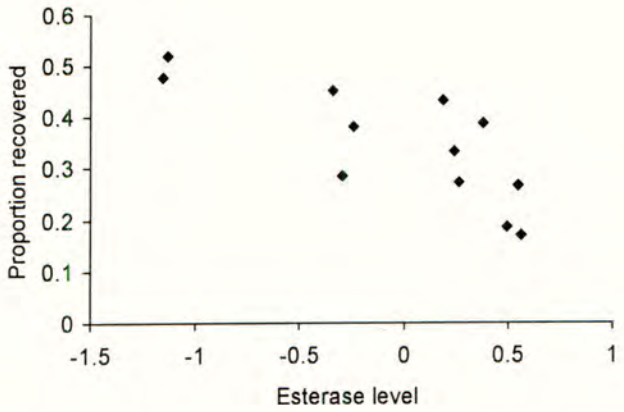

Figure 6. Relationship between the proportion of individuals of 12 UK clones of the peach-potato aphid, *Myzus persicae*, released onto oilseed rape plants in the field in February 1994 that were recovered 21 days later, and their level of esterase-based resistance. Esterase levels are expressed as Log_{10} E4 esterase activity measured using an immunoassay. The inverse relationship is highly significant (logistic linear regression coefficient = -0.621, df = 32, P = 0.002).

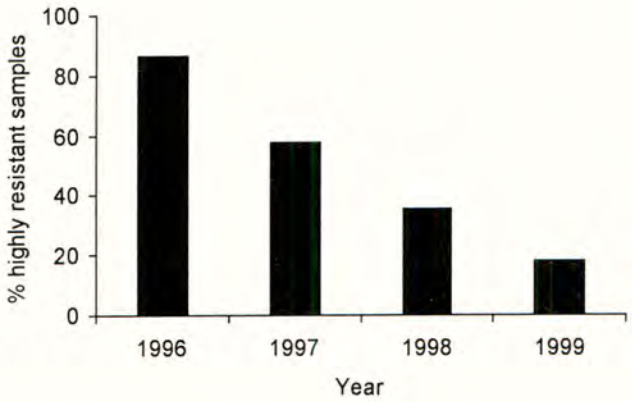

Figure 7. Decline in the frequency of samples of the peach-potato aphid, *Myzus persicae*, obtained by IACR-Rothamsted between 1996 and 1999 that contained 50% or more individuals possessing R_2 or R_3 levels of carboxylesterase-based resistance to insecticides.

Predicting pesticide and adjuvant field performance by physical/chemical characteristics and glasshouse studies

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ABSTRACT

Predicting the field performance of a pesticide/adjuvant mixture based upon physical/chemical characteristics and/or glasshouse studies would improve precision of specific pesticide/adjuvant recommendations. However, a number of factors are involved and all must be taken into consideration when attempting to predict field performance. These include species susceptibility to the pesticide, pesticide formulation, and spray volume. Numerous research studies have indicated that, for some pesticides and pesticide classes, good correlation exists between adjuvant physical/chemical characteristics and field performance of the pesticide. However, other research studies have indicated that good correlation does not exist between adjuvant physical/chemical characteristics and field performance due to factors which cannot be accurately predicted by laboratory methods, such as spray deposition within the canopy.

INTRODUCTION

Recommendation of pesticide and adjuvant combinations is dependent upon several factors, such as target pest, prevailing environmental conditions, and spray application parameters. The development of these combinations under actual field conditions can be a lengthy and expensive process, as typically trials can be conducted only once per growing season. It would be ideal to be able to predict the field performance of pesticide/adjuvant combinations using data developed more rapidly, such as the physical/chemical properties of the adjuvant or results from glasshouse research.

Adjuvants enhance pesticide activity by a number of processes. Adjuvants can influence pesticide activity by enhancing the ability of pesticide sprays to adhere to target surfaces or to alter deposition patterns, coverage of the target surfaces, penetration into the pest, and translocation therein. Several test methods have been developed and adopted to characterize the adjuvants that can help investigators attempt to predict adjuvant performance. In addition, glasshouse research can be conducted initially to determine if these laboratory evaluations can be confirmed. It would be ideal if these research procedures could be utilized to develop pesticide/adjuvant combinations for field use. This paper summarizes the use of physical/chemical properties of adjuvants and glasshouse evaluation to predict performance of various pesticide/adjuvant combinations under field conditions.

GLASSHOUSE VERSUS FIELD EVALUATION

Pesticides are often more active under glasshouse conditions than under field conditions (Eberlein *et al.*, 1988; Horowitz & Ishaaya, 1994; Lich *et al.*, 1997; Longley & Jepson, 1996; Minton *et al.*, 1989). Thus, reduced rates of the pesticides are frequently used to evaluate enhancement of pesticide activity. Under glasshouse conditions, this can lead to differing results from trials conducted under field conditions, especially when adjuvants are being evaluated because of factors such as pesticide formulants and pesticide solubility.

It is known that pest species vary in their tolerance to a given pesticide. Depending on the relative susceptibility of an individual species to that pesticide, substantially reduced rates may be required to obtain differences in adjuvant enhancement of that pesticide. For example, Green and Green (1993) used a very low rate of rimsulfuron when evaluating adjuvant effects on *Setaria faberii*. However, Nalewaja *et al.*, (1995, 1999) used nicosulfuron rates over 15 g ai/ha when evaluating adjuvant effects on *Digitaria sanguinalis*. *S. faberii* responds quite similarly to nicosulfuron and rimsulfuron, requiring approximately the same herbicide dosage (Mekki and Leroux 1994). The higher use rate when evaluating nicosulfuron was critical in the development of the adjuvant Quad-7, which uses increased spray solution pH to improve nicosulfuron solubility. If *S. faberii* had been used as a test species, the solubility factor would probably not have been identified due to the low dose of nicosulfuron required to kill this weed under glasshouse conditions.

A second factor influencing glasshouse evaluation of adjuvants are the formulants and inerts in the pesticide formulation. For example, glyphosate is sold as a variety of formulations, which vary in the amount of active ingredient and surfactant system present. In glasshouse research, glyphosate rates used can be much lower than those used for field research (Lich *et al.*, 1997). If the formulation of glyphosate being evaluated contains surfactant, then surfactant concentration in the spray solution will also be reduced. This may result in tank mix adjuvant enhancement of glyphosate efficacy under glasshouse conditions. However, under field conditions, if the recommended dose of glyphosate formulated product is applied, the concentration of built-in surfactant is sufficient for most situations and addition of tank mix adjuvant has little affect. It is also known that negative interactions can occur between organosilicone-based surfactants and other adjuvants (Policello & Murphy, 1993). When reduced rates of formulated pesticide are used in the glasshouse, these negative interactions may not occur with reduced concentrations of formulants. However, at higher pesticide use rates under field conditions, these negative interactions might be observed.

Other factors influencing glasshouse evaluation of adjuvants include application timing and spray quality. Uniformly grown plants are frequently used in glasshouse evaluation of pesticides to minimize variability. However, under field conditions, the plants will not be uniform, increasing variability in response to the pesticide-adjuvant mixture. Also, distilled or deionized water is often used in glasshouse evaluation of pesticides. However, water quality used for field evaluations may be of lower quality and may affect the efficacy of a number of pesticides (Fagerness & Penner, 1998; McMullan, 1994; Nalewaja *et al.*, 1989; O'Sullivan *et al.*, 1981).

Finally, environmental factors, in addition to temperature, moisture stress, etc., will influence pesticide efficacy, resulting in differences between glasshouse and field evaluation of pesticide-adjuvant mixtures. A number of pesticides, particularly cyclohexanediones, are susceptible to degradation by ultraviolet light (Barnby *et al.*, 1989; Matysiak & Nalewaja,

1999; McMullan, 1994). This negative effect is typically removed under glasshouse evaluation due to the filtering effect of the glasshouse covering. For some pesticides, soil activity is an important factor influencing pesticidal efficacy (Baird *et al.*, 1989; Lamoureaux & Rusness, 1995). Soil effects of pesticides are often negated in glasshouse evaluation as the soil is covered with some type of absorbent. Greater soil activity in the field may mask adjuvant enhancement of above ground activity of the pesticide.

PREDICTING PESTICIDE EFFICACY UTILIZING PHYSICAL/CHEMICAL PROPERTIES

Chemical/Physical Properties

Static and dynamic surface tension, contact angle and spread factor can be utilized to predict the ability of an adjuvant to affect spray coverage and wetting properties using methods developed by the American Society for Testing and Materials (Methods E 2-0044-99 and D1331-89). In a study designed to compare the effects of several adjuvants, HM7912, an experimental crop oil concentrate, had the highest surface tension and contact angle, while several of the other adjuvants had low to very low surface tension and contact angle values (Table 1). It would be expected then that experimental adjuvants such as HM8802-A, HM9110, and HM8902 would be effective with insecticides and fungicides, as these pest control products require thorough coverage to be effective. Alternatively, HM7912 would be expected to be effective with herbicides, where penetration into the leaf is critical, as research has shown that oil-based adjuvants are often superior to surfactants in enhancement of herbicide absorption (Becket *et al.*, 1992; Nalewaja and Skrzypczak 1986; Thompson *et al.*, 1996).

Table 1. Physical properties of adjuvants utilized in research trials.

Adjuvant	Adjuvant type[1]	Rate % v/v	Static surface tension, mN/m	Contact angle	Spread factor
None		0.0	72.0	94	1.0
HM7912	COC	1.0	35.3	59	2.0
HM8802-A	MSO-Osi Blend	0.5	27.6	37	3.0
HM9110	NIS	0.25	30.0	47	2.0
HM8902	Osi Blend	0.125	22.7	0	5.5
HM9121-A	TAE	0.5	35.3	55	2.5
Silwet L-77	Osi	0.125	22.6	0	8.0

[1]Abbreviations: COC=crop oil concentrate, MSO=methylated seed oil, NIS=nonionic surfactant, Osi=organosilicone surfactant, TAE=tallow amine ethoxylate

Herbicides

Organosilicone-based surfactants have superior wetting and coverage characteristics compared to other adjuvant types (Table 1). The "superspreading" characteristics of these adjuvants can promote stomatal infiltration of the herbicide and can result in extreme

rainfastness (Buick *et al.*, 1993; Stevens *et al.*, 1991). Although coverage can be important for herbicide efficacy, the extreme spreading resulting from organosilicone-based surfactants added to the spray mixture can result in decreased efficacy. In addition, the extreme spreading may result in rapid drying of the herbicide deposit. For example, HM8902 was less effective than HM9110, a conventional surfactant, in enhancing glyphosate efficacy on *Ipomoea lacuonosa* and soybean and chlorimuron efficacy on *Amaranthus retroflexus* and *Kochia scoparia*, depending upon the spray volume (Table 2).

Table 2. Adjuvant enhancement of herbicide efficacy under field conditions.

Herbicide	Species	Spray volume litre/ha	Alone	HM9110	HM8902
				% control	
Glyphosate	*Ipomoea lacunosa*	94	52	68	63
Glyphosate	*I. lacunosa*	188	47	67	55
Glyphosate	soybean	94	30	88	83
Glyphosate	soybean	188	33	78	30
Chlorimuron	*Amaranthus retroflexus*	94	50	75	58
Chlorimuron	*A. retroflexus*	188	73	88	88
Chlorimuron	*Kochia scoparia*	94	50	55	40
Chlorimuron	*K. scoparia*	188	42	82	88
Prometryn	*Convolvulus arvensis*	94	80	77	68
Prometryn	*C. arvensis*	188	73	40	47

The high degree of wetting afforded by organosilicone-based surfactants can result in decreased enhancement of herbicide efficacy as spray volume is increased (Table 2). For example, glyphosate efficacy decreased 8% and 53% on *I. lacunosa* and soybean, respectively, as spray volume increased from 94 to 188 litre/ha when HM8902 was used as the spray adjuvant. Conversely, glyphosate efficacy did not change or decreased only 10% on these same two species when HM9110 was included as the adjuvant to the same spray volumes. For chlorimuron, however, efficacy increased on both *A. retroflexus* and *K. scoparia* when spray volume increased from 94 to 188 litre/ha and HM8902 was included as the adjuvant. These results indicate that it is very difficult to predict consistently the enhancement of herbicide efficacy based upon the degree of wetting afforded by an adjuvant.

Research has demonstrated in many situations that methylated seed oil-based adjuvants are more effective than petroleum oil-based adjuvants or nonionic surfactants (Hart, 1997; Nalewaja *et al.*, 1990; Nelson *et al.*, 1998). The methylated seed oil-based adjuvant HM8802-A was more effective than HM7912, a petroleum oil-based adjuvant, with both clethodim and sethoxydim (Table 3). Both clethodim and sethoxydim are systemic herbicides where herbicide uptake is more important than spray coverage. However, for the contact herbicide bentazon, HM7912 was more effective than HM8802-A. At the higher spray volume, the superior wetting properties of HM8802-A compared to HM7912 (Table 1) probably resulted in some runoff and loss of herbicide. These results show that predicting field performance of adjuvant-herbicide combinations is dependent upon the wetting properties of the adjuvant tank mix and the spray volume used to apply the herbicide.

From Table 1, it is evident that HM9121A generally has less wetting ability than conventional nonionic surfactants, such as HM9110. It would be expected that HM9121A would enhance herbicide efficacy less than a conventional nonionic surfactant.

Table 3. Herbicide efficacy as influenced by adjuvant under field conditions.

Herbicide	Weed species	Spray volume litre/ha	Alone	HM7912 1 % v/v	HM8802-A 0.5 % v/v
				% control	
Clethodim	*Sorghum halepense*	94	3	54	61
Sethoxydim	*Digitaria sanguinalis*	94	38	75	79
Bentazon	*Cyperus esculentus*	188	64	89	84
Bentazon	*Erigeron canadensis*	188	--	92	81

However, in several replicated field trials, HM9121A enhanced glyphosate efficacy to a much greater extent than conventional nonionic surfactants (Table 4). HM9121A greatly increased long-term (3 months after treatment) control of *Elytrigia repens* compared to nonionic surfactant. Previous research has shown that adjuvants of the class of HM9121A enhance glyphosate efficacy by promoting absorption of glyphosate across the plasmalemma (de Ruiter & Meinen, 1996; Reichers *et al.*, 1994).

Table 4. Enhancement of glyphosate[1] efficacy by adjuvants under field conditions.

Adjuvant	Rate, % v/v	*Elytrigia repens* 1 MAT[2]	*Elytrigia repens* 3 MAT	*Apocynum cannibinum* 1 MAT	*Kochia scoparia* 1 MAT
		% control			
None	0	10	25	30	40
NIS[3]	0.2	88	59	70	45
HM9121A	0.5	91	89	80	77

[1]Glyphosate formulation without adjuvant; [2]Months after treatment; [3]Nonionic surfactant

This increased movement of glyphosate could result in increased translocation of the herbicide and would account for the increased long-term control of *E. repens* when HM9121A was added to the spray mixture with glyphosate. The ability of HM9121A to increase glyphosate translocation can not be predicted based upon physical data for such things as wetting or spreading due to the mechanism by which HM9121A enhances glyphosate efficacy.

Insecticides / Acaracides

Adjuvants are not typically recommended for use with insecticides. However, almost all EPA registered insecticide labels recommend thorough uniform coverage of all plant parts for maximum insecticide impact. Several factors can influence insecticidal activity, such as coverage, deposition or distribution through the plant canopy. Adjuvants with superior

wetting or sticking properties added to the spray mixture with the insecticide may enhance insect control. HM8902, an organosilicone-based surfactant, greatly improved mite control with three different miticides (Table 5). Acarids typically inhabit the underside of leaves. Although nonionic surfactants such as HM9110 can reduce surface tension and droplet spread compared to water alone (Table 1), these wetting properties were not sufficient to improve acaracide efficacy (Table 5). However, the predicted coverage afforded by HM8902 did relate to increased efficacy.

Table 5. Adjuvant enhancement of acaracide efficacy under field conditions.

Acaracide	Alone	HM9110	HM8902
		% control	
Clofentezine	44	41	8
Abamectin	27	13	7
Fenbutatin-oxide	78	57	13

Pyrausta nubilalis is an important insect pest of field corn in the United States. All adjuvants increased cypermethrin control of *P. nubilalis* compared to cypermethrin applied alone (Table 6). Both HM8802-A and HM8902 gave greater enhancment of insecticidal activity than did HM9110. *P. nubilalis* larvae are located in the tightly wrapped leaves of the whorl. The ability of the spray solution to reach these areas is critical for enhancing control of this pest. HM8802-A, HM8902, and HM9110 all improve the wetting and spreading characteristics of water (Table 1). However, HM8802-A and HM8902 have lower contact angle values and greater spread factors than HM9110, which relate to the increased enhancement when these two adjuvants were used (Table 6).

Table 6. The influence of adjuvant on *Pyrausta nubilalis* control in field corn with cypermethrin applied at 0.112 kg ai/ha.

Adjuvant	Rate % v/v	Cavities per stalk	Cavity length mm	Percent control
None	0	0.98	36.6	42
HM8802-A	0.5	0.38	13.0	69
HM8902	0.125	0.40	10.9	64
HM9110	0.25	0.73	24.1	59
Untreated		1.23	37.1	50

Interestingly, HM8902, which has superior wetting characteristics compared to HM8802-A did not significantly decrease *P. nubilalis* damage to field corn compared to HM8802-A. Previous research has indicated that HM8802-A is highly effective in enhancing deposition of the spray solution (Redding *et al.*, 1998). It is difficult to predict the ability of an adjuvant to enhance spray deposition on a crop and this type of data needs to be determined for individual species.

Fungicides

At the label rate, prochloraz plus cyproconazole provided good to excellent control of septoria in winter wheat on leaves 1 and 2 (Table 7), but at the reduced rate of prochloraz plus cyproconazole, control of septoria was unacceptable. However, HM8802-A increased the control of septoria at the reduced rate of the fungicide being equal to that of the full rate of the fungicide applied alone. In contrast, the film-forming adjuvant NuFilm actually reduced the control of septoria by the fungicide compared to the fungicide applied alone.

Table 7. The influence of adjuvant on *Septoria species* control by prochloraz plus cyproconazole in wheat under field conditions.

Prochloraz plus cyproconazole rate[1]	Adjuvant	Rate % v/v	% Septoria control Leaf 1	% Septoria control Leaf 2
1X	None	0	100	70
0.5X	None	0	90	30
0.5X	HM8802-A	0.5	95	70
0.5X	NuFilm	0.12	70	20
0	None	0	20	0

[1]For prochloraz plus cyproconazole rate, 1X=label rate, 0.5X=one-half label rate

Adjuvant effects on *Botrytis cenerea* control by iprodione in grapes were also evaluated. Both HM8802-A and HM7912 extended ipriodione control of bunch rot and reduced the severity of the disease compared to iprodione applied alone (Table 8). HM8802-A gave greater enhancment of fungicidal activity than did HM7912. HM8802-A has better spreading characteristics than HM7912, which may account for its greater enhancement of fungicidal activity.

Table 8. The influence of adjuvant on *Botrytis cinerea* control by iprodione at 840 g ai/ha in grapes.

Adjuvant	Rate % v/v	Clusters, % infected 9-16-93	Clusters, % infected 10-12-93	% Severity 9-16-93	% Severity 10-12-93
None	0	1.8	4.3	2.5	3.5
HM7912	1	1.3	4.3	1.3	3.0
HM8802-A	0.5	0.5	1.8	1.3	2.5
HM8902	0.125	1.8	1.8	2.5	3.8
Untreated		5.0	13.8	5.0	8.8

Plant Growth Regulators

There has been little research published on the influence of adjuvants on plant growth regulators. In research trials conducted for Helena Chemical Company, results indicated that HM8802-A at 0.5% v/v improved suppression of regrowth in defoliated cotton compared to defoliants alone or with HM7912, a crop oil concentrate adjuvant (Table 9).

Table 9. Cotton regrowth as influenced by defoliant and adjuvant.

Defoliant	Alone	HM7912	HM8802-A
	% regrowth		
Ethephon	15	10	5
Combination[1]	53	20	15

[1]Average of results for ethephon, thidiazuron, and tribufos

HM8802-A has superior wetting and coverage properties compared to HM7912 (Table 1), which could account for the greater suppression of cotton regrowth. Recent research has indicated that HM8802-A increased coverage of spinosad, an insecticide, in cotton compared to HM7912 (Redding *et al.*, 1998). The improved deposition of defoliant/regrowth inhibitor when HM8802-A was added to the spray mixture compared to no adjuvant or HM7912 could also account for the increased regrowth suppression. Currently, there is no laboratory methodology available to evaluate adjuvant effects on pesticide deposition. Thus, using only the physical data in Table 1 to predict the enhancement of plant growth regulator activity by HM8802-A would not have predicted.

DISCUSSION

Adjuvants can enhance pesticide efficacy by improving deposition, coverage, wetting, penetration and to a limited extent translocation of the active ingredient. The physical properties of an adjuvant, such as effect on surface tension, contact angle, and droplet spread, can be used to predict how well an adjuvant may influence processes such as coverage and wetting.

Research presented here and from the literature suggests that using physical properties of an adjuvant to predict its effect on pesticide performance is often difficult as the interaction is pesticide specific. For example, glyphosate efficacy was reduced when HM8902 was used as the tank mix adjuvant, particularly at higher compared to lower spray volumes (Table 2). However, the same adjuvant greatly enhanced fenbutatin-oxide efficacy on acarids (Table 5), where direct contact of the pesticide with the acarids is important. An individual adjuvant is not equally effective with herbicides with different modes of action. For example, at high spray volume, HM8902 increased the efficacy of chlorimuron but reduced the performance of glyphosate (Table 2).

An important field factor which influences pesticide efficacy is the distribution and deposition of pesticide through the crop canopy. This can be affected by adjuvants (Farris

1991; Farris and Hirrel 1989). Thus, HM8802-A increased the activity of prochloraz plus cyproconazole for the control of septoria in wheat compared to the fungicide applied alone or tank mixed with a sticker-based adjuvant (NuFilm). It is likely that the beneficial effects of HM8802-A result from increased pesticide deposition within the crop canopy.

Perhaps the greatest factor influencing or limiting the ability to predict pesticide and adjuvant field performance based upon physical/chemical characteristics and/or glasshouse studies is the large difference in spray application equipment and practices. Currently, in the United States, there are well over 150 different types of application equipment and the performance of these depends on the operator. In addition, each pest complex may have pests which have not been previously evaluated for their response to adjuvant/pesticide mixtures. The physical/chemical properties of an adjuvant as well as glasshouse evaluations allow the researcher to generalize about adjuvant enhancement of pesticide efficacy. Precise prediction of field performance is not possible. The reasons for this include: the differences in pesticide application properties; the size and surface conditions of over 250 different crops and even more weeds, insects, diseases, and other pest; the combination and permutations of the numbers of pesticides, nutritional, additives, carrier, and other application inputs; the tremendous variability in environmental conditions and other variables.

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Microencapsulation of Lambda-Cyhalothrin for crop protection - the Zeon technology

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ABSTRACT

The Zeon technology for microencapsulation of Lambda-cyhalothrin insecticide was developed at Zeneca's Western Research Center. By use of isocyanate interfacial polymerization chemistry and Zeneca's novel protective colloids and emulsifiers system, a simple process was developed for high active ingredient loading microencapsulation. As a result of this technology, toxicity in nearly all categories was reduced compared with the EC formulation. The same technology can be used for other active ingredients to extend residual control, reduce toxicity, reduce phytotoxicity and retard volatility.

INTRODUCTION

The concept of encapsulation and/or entrapment of agrochemical active ingredients for release rate control, toxicity reduction, product performance, presentation, etc. dates back to the 1940's and 1950's (Bungenberg de Jong, 1949 and Leeds, 1957). Through technology advancement, the encapsulated particle size became smaller and smaller. Finally and with micron and submicron sizes, the term microencapsulation was born.

Microencapsulation provides benefits to many industries. These include control of toxicity, release rate and soil movement, rainfastness in agro-chemicals; release rate, storage stability absorption enhancement in pharmaceuticals; homogeneity and processing in paints/inks; filming, forming and surface properties in dyes and textiles and surface treatment and packaging in electronics. The concept of microencapsulation is to replace the surface or bulk properties of the active ingredients by another material which will eventually represent the surface/bulk of the active ingredients in the products. This material encapsulates the active ingredient to form an enclosed capsule. A daily life example of an encapsulated product is a pharmaceutical gel capsule which is frequently used to prevent vomiting in oral administration due to the odour or taste of the active ingredient.

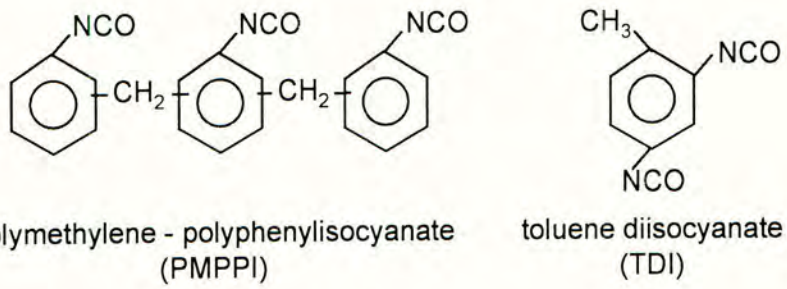
There are several routes to microencapsulate active ingredients (Scher *et al.*, 1999). Among, these, surface polymerization (Morgan & Kvolek, 1959) is suitable for pesticide formulations because it allows high active ingredient loading which is economically essential. With this concept in mind, Zeneca (then Stauffer Chemical Company) launched a research program in the early 1970's to investigate microencapsulation technology which eventually led to the development of the Zeon technology along with many other microencapsulation processes (Scher, 1973; 1977 a and b; 1981; Scher & Rodson 1990; 1992; Chen *et al.*, 1997;). A series of premium products resulted from these processes.

Zeon Technology is a state-of-art technology with inputs from chemistry, physics, and engineering. It is based on an isocyanate surface polymerization chemistry to form polyurea microcapsules (Scher & Rodson, 1990). This technique is mainly for hydrophobic pesticide active ingredients, such as EPTC, flurochloridone, acetochlor, fonofos, tefluthrin and lambda-cyhalothrin. Zeon technology includes several steps, the most crucial of which are the selection of protective colloids and emulsifiers and process condition control. Through the combination of these parameters, the size of the microcapsules can be readily controlled from 2 to 15 μm. The degree of cross-linking, which governs the permeation (release) rate is controlled through varying the molecular ratio of the monomer to crosslinker.

This technology enables the preparation of high loading Lambda-cyhalothrin formulations with reduced toxicity, including acute oral LD_{50}, dermal LD_{50}, inhalation, skin and eye irritation, and paraesthesia (commonly caused by pyrethroid insecticides).

INTERFACIAL POLYMERIZATION AND THE ZEON TECHNOLOGY

Zeon technology adopts the isocyanate polymerization chemistry (Vandegaer, 1971) to form the capsule wall. Polymethylene-polyphenylisocyanate (PMPPI) serves as the monomer (See Figure 1) and toluenediisocyanate (TDI) as the cross linking agent.



polymethylene - polyphenylisocyanate (PMPPI)

toluene diisocyanate (TDI)

Figure 1. Molecular structure of PMPPI and TDI.

The polymerization reaction is initiated by dispersing the oil phase in an aqueous phase. The reaction can be accelerated by heating the system to an elevated temperature. The reaction mechanism is as follows. When isocyanate monomers are exposed to water at the oil-water interface, they are hydrolyzed for form amines (see Figure 2) which, in turn, react with nearby unhydrolyzed monomers to form the polyurea. If the organic phase is the dispersed phase e.g. oil droplets, the polyurea will form a wall encapsulating the oil droplet. With this reaction mechanism, one is able to design O/W emulsion of various droplet size distributions to control total surface area of the disperse phase, which, in the case of agrochemical applications, directly relates to bioefficacy.

As described above, a O/W emulsion is needed prior to performing the interfacial polymerization reaction. In order to disperse the organic phase with proper droplet size

distribution and active ingredient loading, a suitable protective colloid system is required to prevent inter-droplet polymerization from occurring. In addition, an effective emulsifier system is required to disperse the oil droplets to a desired size distribution. This technical challenge is the heart of the Zeon technology.

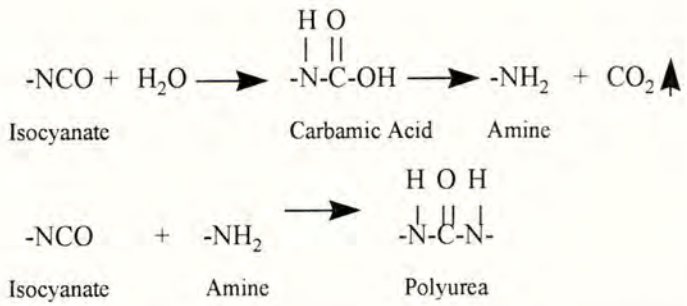


Figure 2. The reaction mechanism of isocyanate at the oil/water interface.

A normal colloidal system of hard core (or shell) exhibits a hard sphere potential when there is no surface charge. In this case, the maximum packing volume fraction is 0.53 for a simple cubic crystalline structure and 0.68 for a random packing structure. However, there are many systems exhibiting much lower maximum packing volume fraction, due to surface roughness which imposes inter-particle interactions through various degree of entanglement (or overlap). Therefore, one expects a "safe" upper limit volume fraction of the disperse phase for the interfacial polymerization to be somewhere around 0.4 to 0.5. This takes into account the volume fraction of the disperse phase (the oil phase and the protective colloids) and the dynamic activity (entropic effect) of the droplets during polymerization at an elevated temperature. In order to maximize this volume fraction, one needs to optimize the protective colloids and the emulsifiers for each different organic phase material. While developing the Zeon technology, numerous pair of protective colloids and emulsifiers were tested to increase the volume fraction of the disperse phase. Success is judged by how much inter-particle polymerization would occur. This is reflected in change of the particle size distribution after polymerization.

The protected colloid and the emulsifier are selected to establish two potentials between two particles. One is the short ranged "soft" potential from direct droplet-droplet contact and the other is a long ranged interaction between protective colloids of two droplets. The inter-droplet soft potential would change, as polymerization proceeds, from an oil-like soft potential to a polyurea-polyurea hard sphere potential when the reaction is completed. On the other hand, the protective colloids should ideally remain on or near the surface during and after polymerization, so the inter-droplet potential remains unchanged. Through numerous tests, the Zeon disperse system was found. It is able to disperse approximately 0.5 volume fraction of the oil phase with good control of the particle size distribution.

THE ZEON PROCESS

The Zeon process starts with preparation of two separate solutions. The organic phase contains the active ingredients, PMPPI and TDI (with a ratio to control wall quality). The aqueous phase contains the protective colloids and emulsifiers. The next step is to mix these two phases with appropriate agitation to control particle size of the emulsion droplets in the range of 2-15 microns. This is a crucial step. It needs to control the particle size distribution within the desired range while preventing inter-particle polymerization from occurring. The final step is to raise the temperature of the emulsion to 50 °C to initiate the interfacial polymerization reaction. The reaction usually takes approximately 3 hours to complete. After encapsulation, the system is allowed to cool before post reaction formulation with buffering agents, suspending agents, and biocides (Figure 3).

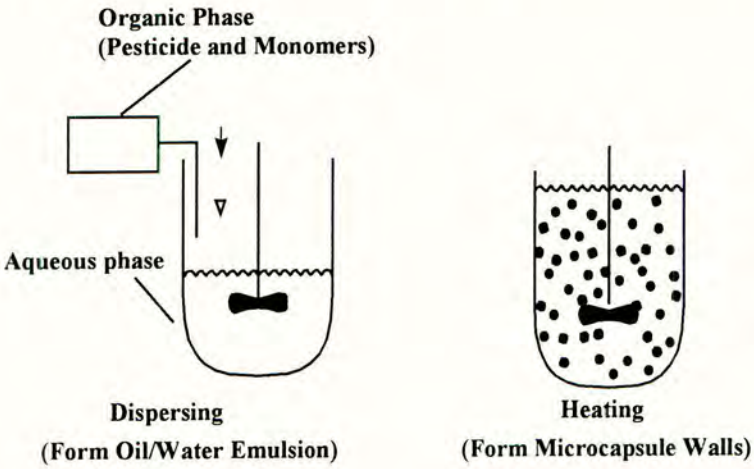


Figure 3. Emulsification and interfacial polymerization processes.

CHARACTERIZATION

Particle Size Distribution

Particle size distribution should be measured at three stages: 1. during emulsification to optimize the agitation, 2. after emulsification and before raising the temperature for the polymerization reaction and 3. after polymerization. A successful process should have nearly identical particle size distributions before and after polymerization (Figure 4).

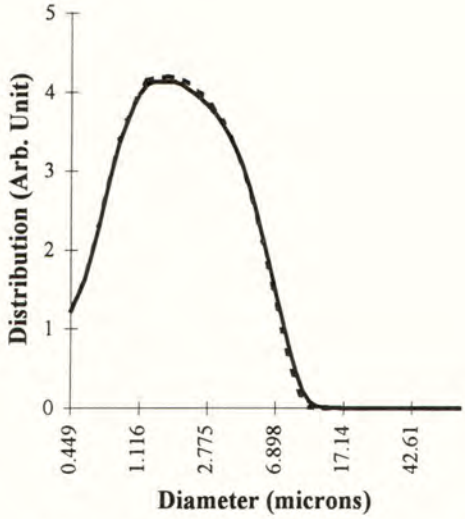


Figure 4. Particle size distribution before (dashed line) and after (solid line) polymerization of a 25% Lambda-cyhalothrin CS formulation.

Microcapsule Wall

The wall property and morphology are governed by several closely related parameters, namely, the PMPPI to TDI ratio, particle size distribution, and temperature. Thus, one needs to systematically evaluate the effect of each parameter, so that an appropriate processing conditions can be designed. In general, the thickness of the wall should be decided first. This is largely controlled by the PMPPI to TDI ratio. The other relevant parameters are pH and temperature. These two parameters were optimized via testing.

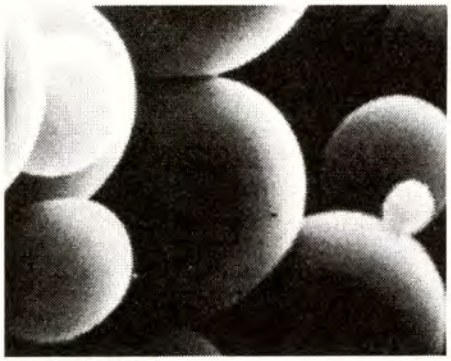


Figure 5. Spherical and polydisperse capsules with smooth surfaces.
The average particle is ~ 10 μm in this case.

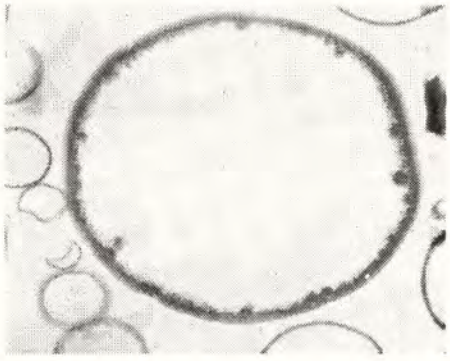


Figure 6. Transmission electron microscopy shows wall density decreasing toward the hydrophobic core.

The other parameter is the agitation during polymerization. Figure 5 and 6 show the quality, thickness and density distribution of a typical capsule wall.

General Physical Properties

Table 1 shows the physical properties of a Zeon technology prepared 25 CS formulation. The average particle size is approximately 2.6 μm, small enough to provide larger surface area for bioefficacy enhancement. The actual particle size is from approximately 1 to 15 μm. The post polymerization formulation results in a viscosity of 90 centipoise which is low enough for handling. As for stability and storage temperature, the freezing point was found to be -2°C.

Table 1. Physical properties of Lambda-cyhalothrin 25% CS

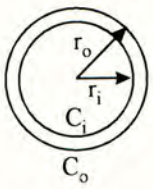
Property	**Zeon 25 CS**
g a.i./litre	249.27
wt % a.i.	22.8
density (g/litre)	1.1
pH	5.0
average particle size (microns)	2.6
Viscosity (centipose) at 100 sec-1 shear rate	90
freeze point (°C)	-2.0°C

Release Rate Control

Using Zeon technology, it is possible to control the wall thickness, density, and morphology, which in turn will control the permeation rate. A typical diffusion process across a homogeneous capsule membrane can be expressed as

$$\frac{dM}{dt} = \text{Release Rate} = \frac{(4p\, r_o r_i)\, P\, (C_i - C_o)}{r_o - r_i}$$

M = Active ingredient
r = radius
P = KD = Permeability
K = Solubility Coefficient
D = Diffusion Coefficient
C = Concentration



The above equation clearly shows that one can control the permeation rate, and thus the release rate via several material and processing parameters. For example, an effective way to

increase the release rate is to reduce wall thickness (r_o-r_i) or increase the total surface area through reducing average particle size.

Toxicity

Table 2. The toxicity data for lambda-cyhalothin 25 CS formulation compared with an EC formulation.

Category	Karate Zeon	EC
Oral LD50	245 M	101 F
(mg/kg)	180 F (II)	64 M (II)
Dermal LD50 (mg/kg)	>2000 M F (III)	>2000 M F (III)
Inhalation LC50 (mg/L)	3.72 M	0.315 M
	3.12 F (III)	0.175 F (II)
Eye Irritation	Mild Irritant (III)	Moderate Irritant (II)
Skin Irritation	Mild Irritant (III)	Extreme Irritant (I)
Skin Sensitivity	Mild Sensitizer	Mild Sensitizer

The toxicity reduction from encapsulation is substantial for acute oral and particularly the inhalation category, eye and skin irritation.

DISCUSSION

In developing Zeon technology, three stages were defined with their technical issues addressed systematically. The first stage is the choice of protective colloids and emulsifier for ample control of particle size and active ingredient loading. Selection is made to maintain sufficient repulsive potential between the oil droplet to prevent cross polymerization which leads to gelation. In addition, one needs to determine the PMPPI to TDI ratio for permeation rate control. This is the most essential stage and is considered the heart of the Zeon technology.

The second stage is to determine appropriate polymerization conditions. During the manufacturing process, it is essential to control the polymerization temperature, pH and reaction time, in order to minimize the cost and maximize the polymerization reaction efficiency, and to some degree control the wall morphology. A high temperature usually results in faster reaction kinetics, thus, faster generation of carbon dioxide which may cause manufacturing difficulty. Furthermore, the wall morphology is sensitive to the polymerization kinetics.

The last stage is the post polymerization formulation, including addition of the suspending agents, biocides, and pH adjustment. This stage mostly controls the stability and physical properties of the neat formulation and tank mixing compatibility.

CONCLUSION

Lambda cyhalothrin is a potent active ingredient, but in common with other pyrethroid insecticides its toxicity to non-target organisms is a concern. Microencapsulation enables utilization of its potency while reducing its unwanted toxic effects. However, to obtain a reliable effective product several steps in the microencapsulation process must be closely controlled including emulsification, particle size, wall thickness, density, reaction temperature, pH and viscosity. These parameters were optimised particularly the selection of protective colloids and emulsifiers. The resulting Zeon technology enabled an economic and simple manufacturing process with excellent control of particle size distribution. The product has a release rate equivalent to the EC formulation, but significantly lower toxicity to non-target organisms and equivalent field performance.

ACKNOWLEDGEMENTS

Many of my colleagues at Zeneca's (formerly Stauffer Chemical Company) Western Research Center contributed to the development of Zeon technology and in particular I would like to thank Herb Scher who introduced me to the world of microencapsulation.

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Aspects of the variability in pesticide-soil interactions

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ABSTRACT

One of the main environmental concerns with pesticides is their potential to affect soil or water quality which is controlled primarily by their persistence and mobility in the soil. A number of mathematical models have been developed to simulate or predict persistence and mobility of residues in the field. Major input parameters required are the physical and chemical characteristics of the soil plus appropriate weather data. The main driving forces for most of these models, however, are data concerning the degradation rate and sorption partition coefficient of the chemical, and accurate estimation of these parameters is essential. Variability in degradation rate and sorption between sites is expected because of major differences in soil properties, but there is increasing evidence of variation in these parameters on a relatively small spatial scale in fields where the soil appears to be uniform. This paper gives a brief overview of the observed variations in sorption and degradation data for some specific compounds, and illustrates how variation in input parameters can be accounted for in a probability assessment of leaching risk.

INTRODUCTION

An essential component of the data package for pesticide registration is information concerning persistence and movement of residues in the soil. The data set usually comprises sorption parameters measured in the laboratory on a number of soils with contrasting properties, and degradation data derived from laboratory incubations with controlled moisture and temperature regimes. In addition, persistence data from practical field use situations are also presented, as are data for residue distributions in the soil profile at different times after application. The laboratory and field degradation data are used to make an assessment of likely persistence problems, and the laboratory degradation and sorption measurements are used in conjunction with the field observations to assess the likelihood of significant movement in the soil, and the potential for contamination of surface or groundwater resources. As an aid to these assessments, a number of mathematical models have been developed, and correct parameterisation of these models is essential in order to obtain robust simulations or predictions. As discussed by Laskowski (1999), there has been a marked shift in the evaluation of environmental chemistry data, with an increased awareness of the importance of environmental variability. There has been a move away from risk assessment based on analysis of "average" or "worst case" scenarios, to what Laskowski refers to as "all case" assessments.

The purpose of this paper is to give a brief summary of the variations that can occur in some parameters that describe pesticide/soil interactions, and to indicate how the significance of this variation can be assessed when using pesticide fate and behaviour models. It is not the intention to give a comprehensive review of all the available data, but more to illustrate specific points with appropriate examples.

SOIL SORPTION

Distribution coefficients and soil properties

Most of the older, more established soil-applied pesticides are non-polar organic molecules and their sorption to soil is influenced primarily by soil organic matter content. For this reason, adsorption distribution coefficients are often equalised for soil organic matter content (K_{om}), or more usually, for soil organic carbon content (K_{oc}). Although such values can give a good approximation to the average situation, K_{oc} can vary quite significantly between soils as was well illustrated in the data base of Wauchope *et al.* (1992). They located 14 separate estimates of K_{oc} in the literature for the herbicide linuron, for example, and these varied from 93 to 863 litre/kg. For metalaxyl, the reported values range from 7 to 160; for atrazine from 38 to 170, and for alachlor from 33 to 742. Given that the ionisation status of none of these compounds should change significantly in the normal pH range of agricultural topsoils, this degree of variation is somewhat greater than might be expected. Reasons for the differences may include the use of very low and very high organic matter content soils, a significant contribution from mineral surfaces in some low organic matter systems, and pH effects if extremes of soil acidity or alkalinity are also considered. Some of the more recently introduced groups of pesticides (particularly herbicides) are of variable charge, and their sorption is strongly influenced by soil pH. Particular examples are the sulfonylurea herbicides which are non-ionised at lower soil pH, and are relatively strongly adsorbed under these conditions, with K_{oc}-values often in the range from 200-500. At higher soil pH (>5-6), they dissociate and become anionic, and adsorption is weak and up to two orders of magnitude lower. Highly complex relationships are possible when pH-dependent variable charge soils are involved (Kookana *et al.*, 1998).

Kinetics

Standard methodology for measuring pesticide adsorption by soils is to shake a small amount of sieved, air-dried soil with an aqueous solution of the test compound. The change in the concentration of pesticide in solution is used to calculate the amount adsorbed by the soil. Under these conditions, equilibrium is usually established within 2 to 4 hours, and the adsorption distribution coefficients measured for specific pesticide-soil combinations can be highly reproducible. Natural soils in the field do not comprise 2-mm mesh sieved uniform air-dry soil, but they are made up of variable-sized aggregates which during significant leaching events will usually be at moisture contents close to field capacity. Under these conditions sorption occurs more slowly, and diffusion to the internal matrix of the aggregate can be a rate limiting step (Johnson *et al.*, 1999; Walker *et al.*, 1999). With rapid water flow during leaching events in highly structured clay soils, for example, contact times between the aggregated soil and the solution will be short, and sorption equilibrium will not be established. In extreme situations this can lead to very rapid downward transport of pesticide to field drainage systems (Johnson *et al.*, 1996; Brown *et al.*, 1999).

DEGRADATION IN SOIL

The rate at which a pesticide degrades is influenced by the chemical properties of the soil such as organic matter content, pH and nutrient status, and is also influenced by environmental conditions that control soil temperature and soil moisture content. Variability in degradation rate between different soils is expected because of the variability in soil

properties, and numerous studies have provided evidence for field-to-field variation in the degradation rates of herbicides (Walker & Brown, 1983; Allen & Walker, 1987; Pussemier *et al.*, 1997), insecticides (Gerstl, 1984; Parkin & Shelton, 1992) and fungicides (Walker, 1987). In several of these examples (Walker, 1987; Parkin & Shelton, 1992; Pussemier *et al.*, 1997), the most rapid rates of degradation were associated with soils to which the study pesticide had been applied regularly, indicating that the phenomenon of enhanced biodegradation had occurred (Racke & Coats, 1990).

The effects of soil on pesticide degradation rate are difficult to predict. Soil affects rates of loss indirectly by controlling the availability of a pesticide for degradation, and directly by controlling the degradation process itself. Variation in any specific soil property can affect either or both of these mechanisms, often in opposing directions. For example, as discussed above, an increase in soil organic matter content usually increases the extent of adsorption, thus reducing availability for degradation in the soil solution. However, soil microbial biomass and activity are often greater in more organic soils which may promote more rapid biodegradation. Walker (1994) gave examples from the literature where the sorption parameter appeared to dominate and increasing soil organic matter content was accompanied by reduced rates of biodegradation. Examples were also provided where the microbial parameter was dominant, and increased rates of degradation in more organic soils were reported. In a similar way, positive and negative correlations between degradation rate and soil pH have also been recorded. Effects of pH depend upon the relative importance of acid or alkaline hydrolysis, the influence of pH on ionisation and hence availability for degradation, and pH effects on microbiological activity of soils.

The above examples all referred to data involving soils from contrasting sites. Recent research, however, has indicated that there can be considerable variation in degradation rate within a single field where the soil appears to be uniform (Oliver *et al.*, 1999; Walker *et al.*, 2000). In the experiments of Walker *et al.* (2000), thirty samples of soil were taken at 50-m intersections on a grid pattern over an area of 250 x 200 m within a single field with nominally uniform soil characteristics. Incubations of isoproturon under standard conditions (15°C; - 33 kPa soil water potential) indicated considerable variation in degradation rate of the herbicide, with the time to 50% loss (DT_{50}) varying from 6.5 to 30 d. The kinetics of degradation also varied between the sub-samples of soil. In many of them, there was an exponential decline in isoproturon residues; in others, exponential loss was followed by more rapid rates of decline; in a few soil samples, rapid rates of loss began shortly after the start of the incubations. These differences in kinetics suggest differences in the reactions of the soil microflora to the presence of isoproturon. A progressive, exponential rate of degradation represents co-metabolic activity, where the herbicide is degraded as a consequence of metabolism of other organic substrates, but the pesticide is not used as an energy source (Torstensson, 1980). Rapid decline after an initial slow rate of loss, suggests that components of the microflora may have adapted their metabolism in order to utilise the compound as an energy source, or that an active pesticide-degrading microflora has proliferated in response to the presence of the chemical (Bergström & Stenström, 1998). Rapid degradation soon after application indicates that a population of micro-organisms with the ability to metabolize the compound is present in the soil initially (Torstensson, 1980). Soils showing rapid biodegradation were generally of higher pH and contained more available potassium than those showing slower degradation rates. They also had a larger microbial biomass and greater metabolic diversity as determined by substrate utilisation patterns on Biolog GN plates. The field had received occasional applications of isoproturon over a period of 20 years, and it seems probable that a fraction of the soil microbial

community had adapted its metabolism to degrade the herbicide but only at micro-sites where soil characteristics were particularly favourable. A fuller discussion of the process of enhanced biodegradation of pesticides in soils and its implications for pesticide efficacy can be found in the paper by Suett (1994).

MODELLING APPROACHES

There is little doubt that modelling is an economic way of assessing pesticide behaviour under field conditions (Boesten, 2000); it is cheaper and faster than field experimentation, and can identify the most important soil/pesticide properties that should be measured in the laboratory. Mathematical models can be used at increasing levels of complexity starting with screening models that use relatively simple input parameters to make generalised predictions. At the next level are computer simulation models that take account of the interactions between the various processes that control environment behaviour, and finally there are more comprehensive systems that operate at the larger scale and usually link with Geographic Information Systems (Hollis & Brown, 1993; Hollis *et al.*, 1993).

An example of a screening model that has proved particularly useful was described by Gustafson (1989). The basis of this model is that chemicals are more likely to leach to groundwater if they are both weakly adsorbed and persistent in soil. Gustafson examined the results from well water surveys in the USA, and listed those pesticides for which analyses were positive and those for which analyses were negative. He then estimated average K_{oc} values and field half-lives from the literature, and constructed a diagram of the type shown in Figure 1. The groundwater contaminants are shown by the closed symbols; the pesticides analysed for but not found are shown by the open symbols. There was a grouping of the groundwater contaminants towards the left hand portion of the diagram which lead to the development of a GUS (Groundwater Ubiquity Score) screening index:

$$GUS = \log(DT50) * [4 - \log(K_{oc})] \quad \ldots\ldots\ldots 1$$

Gustafson further suggested that a GUS value in excess of 2.8 would indicate a high potential for groundwater contamination, whereas a value below 1.8 would indicate a low potential for contamination. Clearly, the intrinsic variability in estimates of DT50 and K_{oc} discussed above limit this approach to some extent, but it does give a first-stage assessment of potential leachability.

The uses and limitations of current fate and behaviour simulation models have been reviewed recently by Boesten (2000). Processes such as volatilisation from soil-injected or soil-incorporated volatile pesticides can be simulated with some precision. Persistence can also be predicted if site specific parameters to describe degradation rates are available. Soil mobility is also predictable with mechanistic models such as LEACHP (Hutson & Waganet, 1992) or PESTLA (Boesten & van der Linden, 1991) when assessing leaching of the bulk of the applied dose. However, the EU drinking water limit of 0.1µg/litre implies leaching of less than 0.1% of a dose of 1 kg/ha, and the validation status of the models is poor at these very low levels.

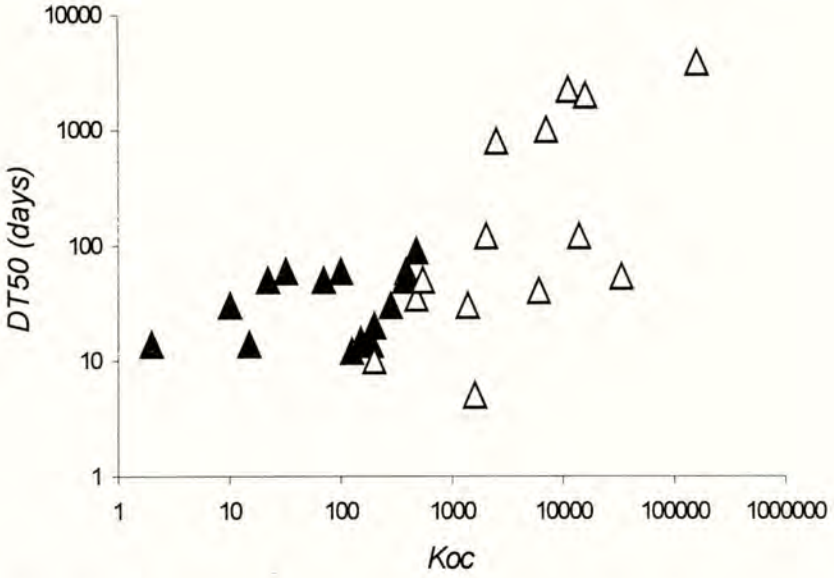

Figure 1. Pesticide leaching potential in relation to K_{oc} and half-life (after Gustafson, 1989). Groundwater contaminants analysed for and found (▲) or not found (Δ).

PARAMETER ESTIMATION

Another aspect of variability that has recently come to light, is the way in which individual researchers interpret experimental data to derive sorption and degradation parameters for use in model calculations. This was highlighted by Brown *et al.*, (1996) who gave the same data set concerning sorption and degradation of an experimental fungicide to five research groups, all of whom had considerable experience of fate and behaviour modelling. They used the data set to derive appropriate input parameters for the models LEACHP, VARLEACH and PRZM2 and then used the models to simulate soil mobility data obtained in a field experiment with the test compound. Differences between modellers in terms of simulated output with a specific model were as great as differences between models, and were also as great as the average deviation from the observed soil residue data. Similar results were obtained in a more comprehensive model testing exercise carried out under the auspices of the EU COST66 action on "Environmental fate of pesticides". In this exercise, 20 modellers tested a number of pesticide leaching models against standard data sets (Vanclooster *et al.*, 2000). The outcome again provided large differences in simulated results between modellers, even when working with the same model. The overall conclusion was that the effect of the modeller was more important than the choice of model, and that interpretation of simple laboratory data was open to considerable subjectivity on the part of individual researchers.

PROBABILITY MODELLING

As discussed above, it has been common practice to take single values of K_{oc} and half life (or DT50) to characterise the sorptive and degradative properties of a specific compound

when parameterising pesticide leaching models. The results of Walker *et al.* (2000) demonstrated that even within a single field, the effective DT50 for isoproturon could vary by a factor of about four (6.8 to 30.1 days) between soil samples. In the same experiments, sorption of the herbicide was also measured in 30 individual soil samples and the estimated sorption distribution coefficient (K_d) varied from 0.99 to 1.44. There was no correlation between degradation and sorption. The effects of the variation in adsorption and degradation on predicted leaching of the herbicide have been evaluated using the LEACHP model (Hutson & Waganet, 1992). The DT50 data were converted to apparent first-order rate constants which gave a mean of 0.0406 day^{-1} with a standard deviation of 0.0160 day^{-1}. Similarly the sorption distribution coefficients converted to a mean K_{oc} of 90 litre/kg with a standard deviation of 19.9 litre/kg. Runs of the LEACHP model were made using weather data for the 11 year period from January 1980 to December 1990 assuming isoproturon application at 2.5 kg/ha on November 1st of 10 consecutive years. Combinations of degradation rate (0.0246, 0.0406, 0.0566 day^{-1}) with K_{oc} (70, 90 and 110) were used as input parameters to give a total of 90 model runs. The data recorded from the model output included the average leachate concentration at 50 cm depth over a 12-month simulation, the mass of chemical leached below 50 cm, and total soil residues at different times after application. The probability assessments of some of the output data are shown in Figures 2, 3 and 4. The 50th percentile (median) leachate concentration was 3.5 µg/litre, whereas there was a 1% probability of a leachate concentration greater than 32 µg/litre (Figure 2). The frequency distribution of the computed mass leached (Figure 3) gives a further illustration that, in general, only trace amounts (<0.5% of the applied dose) will leach below 50 cm depth, but that over 3% may move below 50 cm with particular weather patterns in combination with low adsorption and high half-life. The frequency distribution of predicted soil residues at harvest (10 months after application) also indicates that residue decline in small areas may be significantly slower than in the bulk of the field. The probability assessments put "worst-case" estimates into a true perspective, but also highlight factors that may require more detailed experimental evaluation. A fuller discussion of probability modelling and its potential uses was given by Laskowski *et al.* (1990) and Laskowski (1999).

GEOSTATISTICS

Another way to evaluate the importance of spatially variable parameters is to use geostatical techniques. The basic concept in geostatistics is spatial continuity which means that variables (such as soil properties) are spatially correlated. In effect this means that soil properties of locations close together are more similar than those of more distant locations. The central tool of geostatistics is the variogram which provides a summary of the way in which the variance of a property changes as the distance and direction separating any two points varies. A recent study has used the principles of geostatistics to estimate the spatial variation in leaching of the herbicides atrazine and isoproturon (Oliver *et al.*, 1999). The main observation from the experiments with atrazine was that both the rate of degradation and the extent of adsorption were directly correlated with soil organic carbon content. Measurements of organic carbon content were made in soil from 208 sampling points within a field. The geostatistical technique of kriging was used to estimate the values at unsampled locations and thus build up a complete map of the within field patterns of organic matter content. A similar but much smaller scale study was made of the variation in mean pore water velocity and dispersion coefficient in intact soil columns, so that the overall variability

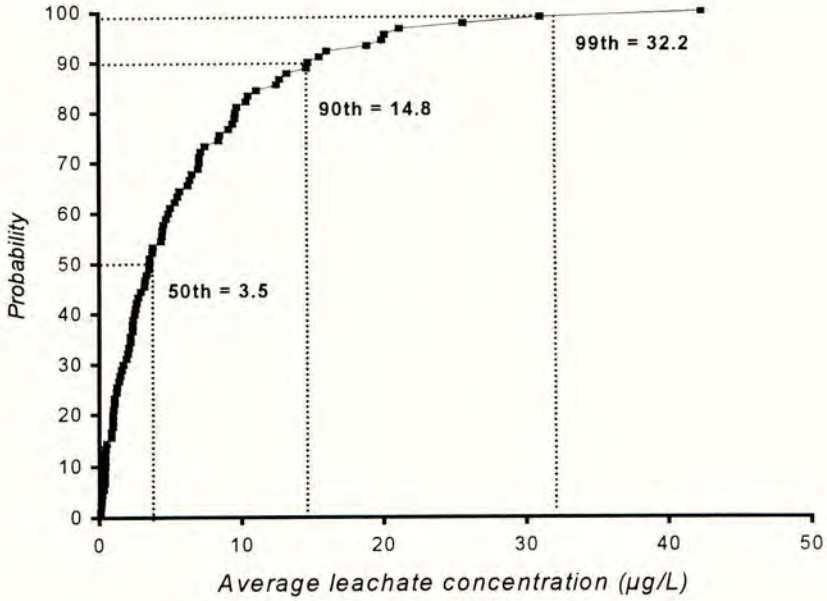

Figure 2. Cumulative probability distribution of average leachate concentration of isoproturon from 90 runs of the LEACHP model.

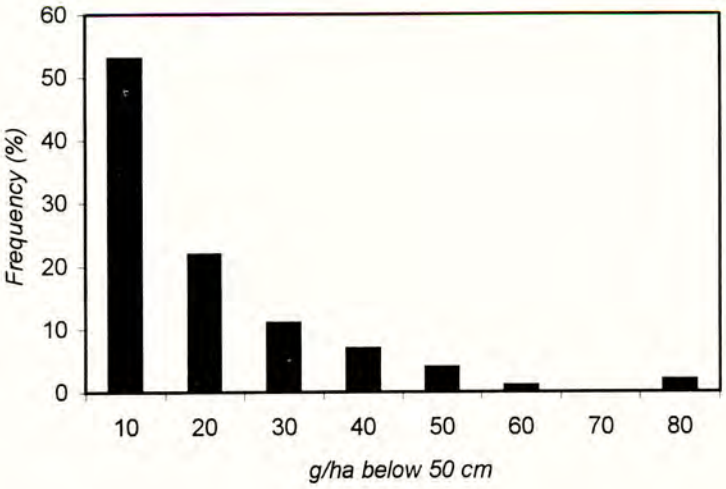

Figure 3. Results from 90 LEACHP simulations of isoproturon flux at 50 cm as a frequency distribution

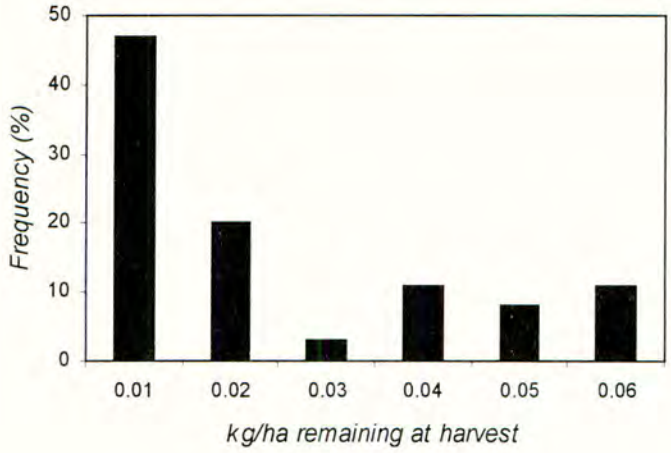

Figure 4. Results from 90 LEACHP simulations of isoproturon residues in soil (10 months after application) as a frequency distribution.

in hydraulic characteristics and in pesticide sorption and degradation parameters could be established. Input of appropriate parameters to the LEACHP model predicted that significant losses of atrazine below 1 m depth would have occurred from just under 10% of the whole field, and that the contribution to leaching losses from the remaining 90% of the field was negligible. These results are consistent with those presented in Figure 2 from the alternative probability modelling approach which also indicate that small areas of a field can provide disproportionately large leaching losses.

CONCLUSIONS

The main processes that influence the potential of pesticides to affect soil or water quality are sorption and degradation. Both are strongly influenced by soil characteristics. Even when soil properties appear to be relatively uniform as, for example, in a single field or across a single soil series, there can still be significant spatial variability in soil/pesticide interactions. Appropriate statistical techniques are available to take account of this variation when assessments are made of potential behaviour at the field scale.

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