ROTHAMSTED RESEARCH

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Host -parasite Interactions

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Host-Parasite Interactions

B.R.KERRY

Basic and strategic research on the control of parasites and the management of beneficial organisms has been refocused into five programmes. The main emphasis of these programmes is to identify and exploit targets for intervention in the control of invertebrate and microbial parasites through an understanding of chemical signalling, insect behaviour, host defence, pathogenesis and mechanisms of insensitivity to toxophores. Research on signalling processes affecting crop pest behaviour has been effectively applied to vectors of animal and human diseases. However, the priority for our research remains the removal of biotic constraints on crop productivity and quality caused by insects, nematodes and fungi. The challenge is to maintain the level of these crop outputs with protection methods that minimise impacts on biodiversity in farmland.

FUNGAL PATHOGENICITY AND TARGETS FOR INTERVENTION

Interactions at the host surface

The firm adhesion of fungal spores and germlings to the surface of host plants is crucially important at several different stages of the infection process. During dispersal, ungerminated spores must rapidly adhere to the aerial parts of plants to avoid displacement by wind and rain. Following spore germination, adhesion of germ-tubes is essential for sensing the host topographic signals that induce appressorium formation. Finally, appressoria must be firmly anchored to the plant surface in order to exert mechanical force during penetration. In many fungal pathogens, the adhesion of spores, germ-tubes and appressoria appears to be mediated by extracellular matrices (ECMs). Knowledge of the composition of these fungal 'glues' should lead to the development of novel chemicals that interfere with their secretion or polymerisation.

The downy mildew fungus, *Peronospora parasitica*, which causes important diseases on a wide range of Brassica crops, is used as a model system to study adhesion. The ECM of spores differs markedly in composition from that of germ-tubes and appressoria. For example, *N*-acetyl-β-D-galactosamine residues are present on spores, whereas I-D-mannose residues are located on germ-tubes and appressoria (Fig. 13). After dislodging the germlings from the substratum on which they were growing, fluorescent lectin labelling reveals tracks of ECM marking the previous positions of germ-tubes and appressoria (Fig.14). These results show that *P. parasitica* germlings secrete ECM onto substrata at the contact interface. Since this material remains firmly attached to the substratum it is likely to function in germling adhesion. Antibodies offer a much wider range of binding specificities than lectins and we are currently using phage antibody technology to identify further ECM components.

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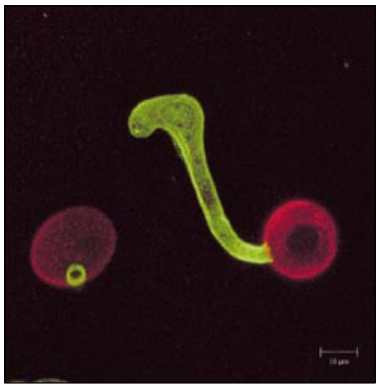


Fig. 13 *Confocal image of* Peronospora parasitica *germlings, showing soybean lectin labels N-acetyl-β-D-galactosamine on spores (red), while Snowdrop lectin labels I-D-mannose on germ-tubes and appressoria (green). Bar = 10 Tm*

Isolation of non-pathogenic mutants

Non-pathogenic mutants hold the key to understanding the function of genes that determine the pathogenic habit of many economically important plant pathogenic fungi, such as the *Septoria* wheat leaf-spot pathogen, *Mycosphaerella graminicola*. In addition, biochemical characterisation of the metabolic lesions leading to reduced pathogenicity provides valuable information for the discovery of novel target sites for chemical intervention in crop protection. A non-pathogenic mutant screen has been developed for *M. graminicola* which scores the ability of individual isolates to cause disease by assessing disease severity on the second leaf of wheat (cv. Riband) seedlings 28 days after inoculating the leaves with spores. Disease symptoms are scored on a severity scale of 1 to 5 (Fig.15). Out of 488 UV generated mutants screened, 35 exhibited significantly reduced pathogenicity compared to the wild-type pathogenic strain. Some of the non-pathogenic mutants recovered are auxotrophs and require supplements of specific metabolites in order to grow. Of three mutants characterised as auxotrophs, one was found to require leucine for growth, another required arginine or ornithine and the third appears to be defective in branch chain amino acid biosynthesis, requiring supplementation with leucine, isoleucine and valine.

An alternative approach to the generation of random mutations in unknown genes is to disrupt a gene encoding a product known or suspected to be important in the growth or pathogenicity of the fungus. Such genes represent potential targets for chemical intervention. Screening strategies for new antifungal products are often based on high throughput screening of compound libraries against specific enzymes. Thus, it is important that the target enzyme used in these assays is actually essential for the fungus to cause disease. The pathway of polyamine biosynthesis may be a potential target for anti-fungal compounds. The gene encoding ornithine decarboxylase (ODC), the first committed step of polyamine biosynthesis, has been cloned from *Stagonospora (Septoria) nodorum*. Pieces of DNA flanking the ODC coding region were used, along with a hygromycin resistance construct, to make a gene replacement cassette, which was transformed into the fungus. Of 150 transformants recovered on a polyamine containing medium, five required polyamines for growth and were thus putative gene replacement mutants. Southern analysis confirmed the gene replacement event in four of these mutants, with the other showing extensive rearrangement of the ODC locus. Subsequent pathogenicity tests on wheat showed that the ODC mutant strains were reduced in virulence thus confirming that ornithine decarboxylase activity is necessary for normal disease progression and has potential as a target for chemical intervention. This approach demonstrates the value of 'reverse genetics' to generate null mutants in specific genes of pathogenic fungi.

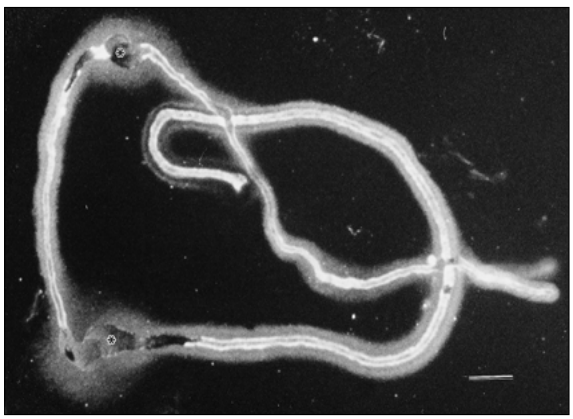
John Lucas - (IACR-Long Ashton)

CHEMICAL ECOLOGY

Carnivorous insects: nuisance pests and disease carriers

Since the early 1980s, the success of our work on the chemical ecology of arable crop pests has created an opportunity to study more diverse interactions. These include studies on carnivorous or haematophagous insects that act as sources of nuisance to ourselves and farm animals, and as vectors for a range of pathogens causing serious diseases throughout the world. Funding for

Fig. 14 *'Footprints' of ECM (labelled with Wheat germ lectin) mark the previous positions of germ-tubes and appressoria (asterisks) after their removal with a jet of water. Bar = 10 Tm*



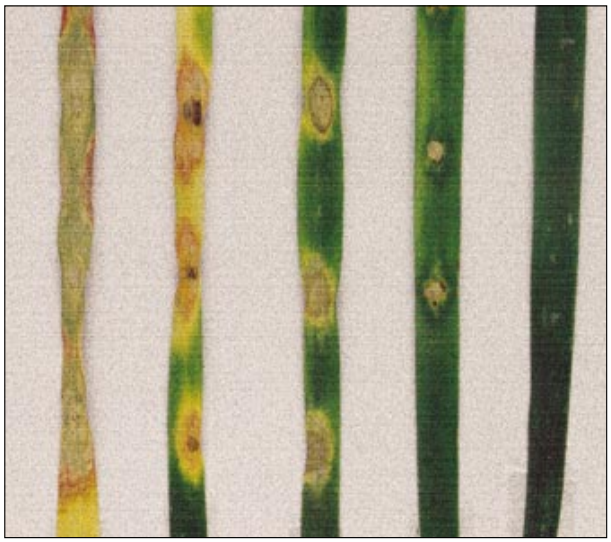


Fig.15 *Scoring the aggressiveness of* M. graminicola *mutants on a scale 1-5: 1. No symptoms/ light brown flecking beneath inoculation droplet; 2. Brown limited lesion restricted to the leaf tissue beneath inoculation droplet; 3. Lesions spreading from inoculation point surrounded by a halo of chlorotic tissue; 4. Large brown necrotic lesions and extensive chlorosis; 5. Spreading lesions and senescence*

these studies has been forthcoming from a range of agencies, including the Wellcome Trust, where collaboration with university departments has been involved, and the European Union, which has facilitated the establishment of new links with veterinary laboratories in the Netherlands and Denmark.

Work began with the first identification of a mosquito pheromone as the acetoxyhexadecanolide isomer (Fig.17.1), initially for *Culex quinquefasciatus* (Diptera: Culicidae) and then for other members of this genus. The pheromone is released from maturing eggs, normally laid on the surface of polluted water, and causes further oviposition of gravid females attracted by the pheromone. In collaboration with the University of Aberdeen, the synthetic pheromone was shown to be additively or synergistically active with components of polluted water, particularly amino acid metabolites including phenols and indoles. With support from Wageningen Agricultural University, The Netherlands, and the National Institute for Medical Research, Tanzania, an increase in oviposition was demonstrated by combining the pheromone with 3-methylindole (skatole). For resource-poor regions, synthesis of the mosquito oviposition pheromone is expensive. However, the summer cypress plant, *Kochia scoparia* (Chenopodiaceae) produces a synthetic precursor, Δ^5-palmitoleic acid, or (Z)-5-hexadecenoic acid (Fig.17.2). Professor Timothy Olagbemiro, working on sabbatical from Abubakar Tafawa Balewa University, Bauchi, Nigeria, has developed an efficient method for growing the plant, extracting the seed oil and converting this to the pheromone (Fig.16). Although the product is less pure, it has been shown by the University of Aberdeen to have similar activity to the mosquito-derived compound. The initial objective is to grow the plant in the UK and in Africa as a new industrial crop for production of the pheromone, but the long-term intention is to clone the gene for the Δ^5-desaturase from *K. scoparia* and express it in oilseed rape, allowing exploitation of the better agronomic performance of oilseed rape over *K. scoparia.*

The University of Keele has a long-standing collaboration with Brazil on sandflies, principally

Fig.16 *Summer cypress,* Kochia scoparia, *now being cultivated to produce Δ^5-palmitoleic acid, the precursor of the mosquito oviposition pheromone*

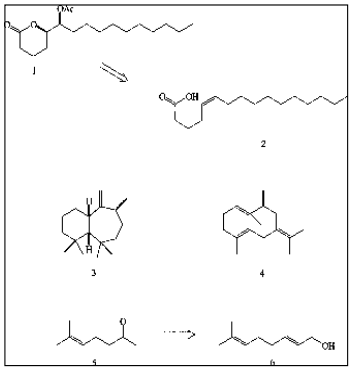


Fig. 17 *Structures of pheromones and other semiochemicals influencing the behaviour of mosquitoes, sandflies and flies*

Lutzomyia longipalpis (Diptera: Psychodidae). For this species, strains from different regions of Brazil produce chemically distinct pheromones. These insects have long been a priority for pest control because of their role in vectoring parasites causing the various forms of leishmaniasis. For practical development, an important aspect of the chemical ecology of this group of sandflies is a sex pheromone produced by males for attraction of the females, which carry the parasites. In collaboration with Keele, novel but tentative structures for pheromones of *L. longipalpis* from Jacobina and Lapinha regions were published. Although these proposals were based only on mass spectrometry results, recent work with the Science University of Tokyo, Japan, has, by synthesis, confirmed the original structures and demonstrated the stereochemistry for the Jacobina and Lapinha pheromones (Figs 17.3 and 17.4). Large-scale synthesis of these compounds for sandfly control is being developed in Brazil.

Phytophagous insects are known to locate suitable hosts by means of specific antennal responses to compounds typical of the host plant taxa. For a wide range of such insects, there are also specific olfactory neurons that respond to components of unsuitable hosts, thereby allowing the insects to avoid energy-wasting attempts to feed on, or colonise, plants upon which they cannot successfully develop. In the early 1990s, we advanced the hypothesis that this might also apply to carnivorous insects. Indeed, work with the Central Veterinary Institute, Lelystad, The Netherlands, and the Danish Pest Infestation Laboratory, Lyngby, Denmark, has demonstrated this to be the case for flies causing a nuisance in cattle and acting as vectors for cattle pathogens such as those causing summer mastitis. Volatile emissions from individual cattle that consistently have few flies were compared with those from animals within the same herd attracting a large fly load, and using coupled gas chromatography- electrophysiology on the fly antenna, a number of active compounds have been identified specific to the less attractive hosts. Already, this has allowed field trials of a push-pull strategy in which the main part of the herd is protected by a few highly attractive cattle. It is hoped to exploit this phenomenon further by active intervention using the synthetic non-host semiochemicals (Fig. 17.5), or by understanding the biosynthesis, which in the case of this compound is thought to be by oxidation of an isoprenoid precursor such as geraniol (Fig. 17.6).

John Pickett - (IACR-Rothamsted)

INSECT BEHAVIOUR

Parasitoid foraging behaviour

Parasitic wasps (parasitoids) are important natural enemies of insect pests which they locate by responding to behavioural cues, especially semiochemicals emitted by both the pests themselves and the plants on which they feed. Female aphid parasitoids show strong behavioural responses to chemical components of aphid sex pheromones (Fig. 18). When vials releasing synthetic aphid sex pheromones were positioned alongside aphid-infested plants placed in the field, parasitism of both cereal aphids *(Sitobion avenae)* and pea aphids *(Acyrthosiphon pisum)* was greatly increased. The foraging behaviours of the generalist aphid parasitoid *Praon volucre*, which appeared to fly directly to the source of the pheromone before starting to forage, differed from that of the more specialist *Aphidius* species, which landed on the vegetation and commenced foraging before reaching the pheromone source. Parasitism by the *Aphidius* species was increased significantly on plants placed between one and three metres away from the odour source, making these species potentially more useful than *P. volucre* in manipulation strategies. Such a strategy has been devised to promote early-season synchrony between parasitoids and aphid pests in crop fields by using pheromone lures to establish overwintering parasitoid reservoirs in field margins.

The responses by female parasitoids to aphid sex pheromones are probably innate, since individuals respond strongly without previous exposure to the pheromone. However, many behavioural responses to other semiochemical foraging cues are influenced or determined by post-emergence experience. Ovipositing aphid parasitoid females have a distinct preference for the host species on which they developed, even in species that have a wide host range. Host preference could potentially be expressed at two different stages;

Fig. 18 *Female aphid parasitoid (*Praon volucre*) attracted to a vial releasing synthetic aphid sex pheromone. This appears to be an innate response as parasitoids with no previous experience of sexual aphids are attracted*

when the parasitoid encounters a host and decides whether to attack or not (host recognition), or when it attacks a host and decides whether to release an egg or not (host acceptance). Alternatively, apparent host preference could be the consequence of the parasitoid surviving better during development inside one host compared to another (host suitability). Preference by aphid parasitoids was expressed principally at the host recognition stage and was therefore, largely a behavioural rather than a physiological phenomenon. In collaborative work with the University of Potenza (Italy), host recognition was influenced by semiochemical cues contained in the aphid cuticle, and sometimes by visual cues, principally colour. Plant-derived cues also play a role by reinforcing host recognition behaviour: parasitoids began to attack aphid hosts more quickly and attacked more hosts in a given time when the appropriate food plants were present. In collaboration with Professor H. F. van Emden from Reading University, host preferences of the generalist aphid parasitoid *Aphidius colemani* were changed by dissecting them from their mummy cases just prior to their emergence and allowing them to examine the case of an alternative host species. Contact with semiochemicals in the cuticle of the dead host, which forms the basis of the mummy case, appears to determine future preferences by newly emerged females (Fig. 19).

Fig.19 *Cereal aphids (*Rhopalosiphum padi*) showing three parasitised individuals (centre), two of which have died and become 'mummies' which contain the pupating parasitoids. The foraging behaviour of the adult parasitoids will be influenced by chemical information obtained from the skin of their dead host as they emerge*

Most aphid parasitoid species attack more than one host species and the availability of any one host can change dramatically both spatially and temporally. It is therefore important for parasitoids to be able to change their host preferences and their behavioural responses to host location and host recognition cues, to adapt to changing foraging opportunities. This is achieved as a result of successful foraging experiences. Wind tunnel studies of parasitoid responses to semiochemical foraging cues have demonstrated the important influence of experience on the type and strength of these behavioural responses. Inexperienced females of the aphid parasitoid *Aphidius ervi* respond to volatile semiochemicals emitted by broad bean plants *(Vicia faba)* infested with pea aphids *(Acyrthosiphon pisum)* but not to those emitted by uninfested plants. The feeding action of the aphid induced changes in the volatile profile of the plant to which the parasitoid has evolved a response. However, short experiences with pea aphids or host-related cues such as honeydew whilst foraging on bean plants not only heightened parasitoid responses to infested plants significantly but also induced responses to uninfested plants. This response to uninfested plants following foraging experience is thought to be elicited by plant volatiles other than those induced by aphid feeding which the parasitoid adopts as host location cues through a process of associative learning. Learning to respond to plant volatiles emitted by uninfested as well as host-infested plants appears to be counterproductive. However, it may serve to prolong foraging activity within a local habitat patch that has already provided successful encounters with hosts, which will be more efficient than expending energy resources dispersing in search of other patches which may or may not contain hosts, particularly when host densities are low.

Behavioural responses are determined or modified by experience, either immediately after adult emergence or during subsequent foraging and provide opportunities to improve the searching efficiency of mass-reared parasitoids by 'priming' them to target specific hosts before release in biological control programmes.

Wilf Powell - (IACR-Rothamsted)

Fig.20 Acyrthosiphon pisum *(pea aphid) infected with entomopathogenic fungus* Erynia neoaphidis

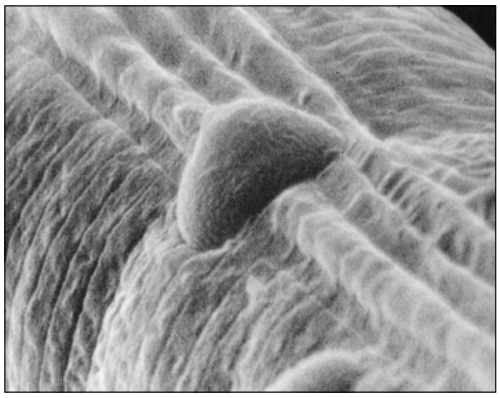


Fig.21 *Scanning electron micrograph of an endospore of* Pasteuria penetrans *adhering across the lateral line of the plant parasitic nematode* Meloidogyne incognita*: the spores have a diameter of 3.9 µm and adhere to any part of the cuticle of the infective larvae of the nematode*

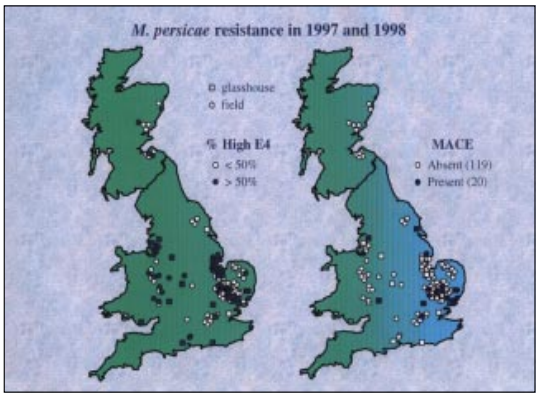


Fig.22 Myzus persicae *resistance in 1997 and 1998*

INVERTEBRATE PATHOLOGY

Parasites of aphids and nematodes

Erynia neoaphidis is a common fungal natural enemy of numerous aphid species in the UK (Fig. 20). It commonly causes spectacular epizootics, which may reduce aphid populations to zero at a local scale. However, these epizootics are unpredictable, often occurring too late to limit crop damage. To maximise the effect of this pathogen early in the season when insect control is required, the biotic and abiotic factors that affect its persistence, transmission and dispersal in the agro-ecosystem must be understood.

The ladybird, *Coccinella septempunctata*, may play a role in persistence, transmission and dispersal of *E. neoaphidis* within and between pea aphid, *Acyrthosiphon pisum*, colonies. Ladybirds were not susceptible to the fungus but did partially consume sporulating cadavers. However, transmission from either damaged or intact cadavers to healthy aphids was the same. Ladybirds contaminated with conidia during foraging passively carried them to other aphid colonies where they initiated infection, thereby contributing to dispersal of this pathogen between host populations. Furthermore, the presence of a foraging ladybird significantly increased fungus transmission within an aphid population. These positive interactions greatly outweigh the small reduction in persistence caused through feeding and indicate the potential for using these natural enemies together.

The obligate Gram-positive bacterial parasite, *Pasteuria penetrans*, has potential for the biomanagement of plant-parasitic nematodes, but the attachment of its infective spores differs greatly between populations of both host and parasite: in extreme cases spores from one isolate of the bacterium adhere to only one population of a nematode host (Fig. 21). Therefore, the selection of a compatible isolate is one of the main problems for the exploitation of this bacterium as a control agent for a specific nematode pest. One particular isolate of the bacterium used at IACR-Rothamsted was bulked up and distributed to collaborators in six countries in the tropics and sub-tropics, to test on different populations of root-knot nematodes to ascertain the level of biological variation. Spores of the bacterium were found to adhere to some populations of nematodes in each of the six countries. However, the numbers of spores that attached to the nematodes were much lower in some countries than in others. The nematode populations were characterised by isozyme patterns from single egg mass cultures but there was no relationship between nematode species and the number of spores attached. These results confirmed that one population of the bacterium would not be effective as a biological control agent in all countries. Results from attachment tests on a range of geographically distinct populations of root-knot nematodes in Ecuador showed that there were marked differences in the amount of spore attachment between those nematode populations tested in the Coastal Region, the Highland Region and the Orient. As compatibility between the different nematode populations and the spore of the bacterium was not random, other factors such as cropping history and/or soil factors may be important in the selection of nematodes that are compatible with the bacterium.

Varroa, viruses and honeybees

Chemical acaricides to control the parasitic mite, *Varroa jacobsoni*, are currently applied to honey bee colonies without detailed knowledge of mite population densities or damage thresholds. Successful strategies for the future which minimise chemical inputs and reduce colony losses will depend upon a better understanding of the relationship between mite populations, honey bee virus incidence and bee mortality.

Increases in the prevalence and mortality due to cloudy wing virus (CWV), deformed wing virus (DWV) and slow paralysis virus (SPV) have been observed in severely infested colonies. However, more detailed investigation of the epidemiology of these three viruses by ELISA of individual larvae, pupae, and live adult bees, indicated that whereas there was a strong correlation between the infection of brood with DWV, SPV and mite infestation, no similar association was evident for CWV. Transmission of CWV from adult bees to brood, probably via the brood food, may be more important than mite-mediated virus spread. Unlike the other two viruses, SPV is a rapidly fatal infection with a strong natural seasonality and it is

the most likely cause of colony collapse during the overwintering period. In collaboration with Dr S. J. Martin, Central Science Laboratory, York, information on virus epidemiology has been integrated into a model of bee and mite population development that will provide the basis for a simple decision strategy to more accurately target and time treatments.

Brian Kerry - (IACR-Rothamsted)

INSECTICIDE RESISTANCE AND MODE OF ACTION

Work on insecticide resistance involves the peach-potato aphid, *Myzus persicae,* one of the most serious pests of agriculture and horticulture in temperate countries, including the UK. Insecticides retain a central role in aphid control but the increasing incidence and severity of insecticide resistance undermine their efficacy. Until recently only one resistance mechanism had been identified in *M. persicae:* the overproduction of a carboxylesterase that sequesters or degrades insecticidal esters before they reach their target site in the nervous system. This mechanism was thought to confer strong resistance in the aphid to organophosphorus and pyrethroid insecticides and less resistance to carbamates.

However, a substantial component of the pyrethroid resistance is attributable to a modification to the target site of these insecticides. The point mutation responsible (leucine to phenylalanine) is in the neural voltage-sensitive sodium channel, at the same position in the aphid gene as that in the knockdown resistant *(kdr)* housefly where it was first identified. The work illustrates the potential for molecular biological studies to reveal hitherto unsuspected mechanisms of resistance in a species. This form of resistance is closely associated with the esterase E4-based mechanism and so the two have been co-selected by use of different insecticide classes. The apparent linkage between amplified E4 and *kdr* may arise from close chromosomal proximity of their genes or from the selection of both within a single clone, and has important implications for the choice of insecticides for controlling resistant aphids. In areas where the related esterase FE4 (which appears to be only weakly associated with *kdr*) predominates, there is a prospect of continuing to achieve good control with pyrethroids.

In the UK, *M. persicae* with strong E4-based resistance are less fit than susceptible aphids during the winter. It is still unclear whether this reduced fitness is a direct consequence of the E4 mechanism, or due to *kdr*, which generally occurs in association with high levels of E4 in this country.

Rapid PCR-based diagnostics to monitor the *kdr* mechanism are under development, with the aim of establishing the extent of its occurrence in the UK; the incidence of esterase-based resistance continues to be monitored. The proportion of aphids with high esterase levels (R_2 and R_3) has recently been larger than is typical of field crops in the UK. There is no obvious association with a particular crop or geographical area (Fig. 22), but the apparent incidence will have been influenced by collection of many samples from treated fields.

Insecticide-insensitive acetylcholinesterase (AChE), the target for organophosphorus and carbamate insecticides, was first detected in *M. persicae* in 1990, and is referred to as MACE (**M**odified **A**cetyl**C**holin**E**sterase). It confers strong resistance specifically to pirimicarb and triazamate. MACE aphids were first found in large numbers in the UK field during 1996 when numerous growers in south Lincolnshire encountered control failures with pirimicarb. Since then, the distribution of MACE has become much wider (Fig. 22). This localised establishment and spread of MACE aphids has compounded the practical problems posed by esterase-based resistance, because an important factor moderating its impact has hitherto been the lower resistance that esterase overproduction confers to pirimicarb. However, the combination of high esterase levels and MACE gives virtual immunity to pirimicarb, and current spray recommendations may therefore need to be revised.

The molecular basis of the insensitivity of the aphid enzyme is studied as part of an EU-funded programme on the structure and function of AChE. Biochemistry coupled with molecular modelling of the known crystal structure of

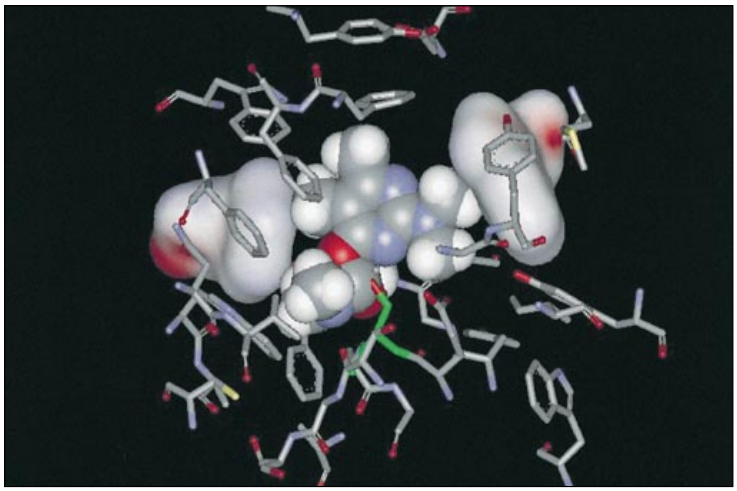


Fig. 23 *Model of a pirimicarb molecule docked in the catalytic centre of* Torpedo californica *acetylcholinesterase, with the carbonyl group of the insecticide 'bound' to the catalytic serine (shown green). Two other key residues, phenylalanine 330 (left) and tryptophan 84 (right) are highlighted as 'space-filling' rather than as the 'stick' representation used for other residues around the catalytic centre. Both N-dimethyl groups appear to pack closely against these and nearby aromatic residues, as does the 5-methyl group. Substitution of this methyl by isopropyl dramatically modulates the insensitivity shown by aphid acetylcholinesterase to primicarb (Courtesy of T. Lewis, Zeneca Agrochemicals)*

Torpedo californica AChE, has highlighted regions of the protein important for pirimicarb binding. Unlike the AChE insensitivity in many other species, that in *M. persicae* is very specific to the only two dimethylcarbamates in widespread commercial use, pirimicarb and triazamate. Analogues of these insecticides have been identified (through collaboration with Zeneca and Rohm & Haas) that show 10 to 100-fold lower insensitivity factors than the parent compounds, whilst still retaining good anticholinesterase potency. Molecular modelling of one such analogue, in which the methyl group on the 5-position of the pyrimidine ring of pirimicarb is replaced by an isopropyl group, indicates that this substituent would lie close to a phenylalanine (330) important in the catalytic mechanism of the enzyme (Fig. 12). Further refinement of the model awaits publication of the crystal structure of an insect AChE. This biochemical approach is complemented by molecular biological studies. Several mutations responsible for insensitivity of housefly AChE have been identified, and a corresponding sequence from *M. persicae* isolated, which is being analysed to identify any mutations associated with pirimicarb insensitivity. This research, together with that on the insect sodium channel (IACR Report for 1996, 20), illustrates the potential for the exploitation of mutations involved in insecticide target site resistance to gain a better insight of the functioning of these key proteins in the nervous system.

Alan Devonshire - (IACR-Rothamsted)