CSIRO Division of Entomology

CSE41C -Trip Report for U.K.

August 1993

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Outcome

During this trip I was able to liaise successfully with the following colleagues concerned with resistance and its management: Drs Rick Roush (Cornell), Alan Devonshire (Rothamsted), Chris Curtis (London School of Hygiene and Tropical Medicine) and Alan McCaffery (Reading University). Outcomes of the discussions were:

(1) I received affirmation that the likely nature of the pyrethroid resistance recently detected in St George, was an esterase-mediated mechanism, as suggested by Dr Robin Gunning. I established contacts relevant to the new project on the esterase resistance in *Helicoverpa armigera*. Clones for the *para* gene for *kdr* resistance and for esterases were made available.

(2) I was given up-to-date information on pyrethroid resistance studies in *H. armigera* in India and Thailand, and *Heliothis virescens* in the USA. It appears that *kdr* type resistance is not common in Asian populations of resistant *H. armigera* as was first thought. In particular, the dominant form of pyrethroid resistance in India is metabolism Endosulfan resistance has been detected in Pakistan *H. armigera*.

(3) I discussed different aspects of resistance management to transgenic plants. As a result, I have become concerned about the directions being taken in models overseas that seem biased towards seed mixtures as the preferred control strategy. This bias arises more from social/political constraints in the USA cotton industry than because it necessarily is the only viable option to manage Bt plants. However, seed mixtures do seem to be the 'best' biological option in potato crops for the control of Colorado potato beetle because this insect is not very mobile.

Itinerary

Dates	Purpose	Talks/seminars
Friday, 13 August 1993	Travel to U.K.	
Saturday, 14 August	Arrive Birmingham	
Sunday-Friday, 15-20 August	17th International Congress of Genetics	Invited paper 'Evolutionary biology of insecticide resistance in the moth, *Helicoverpa armigera*'
Sunday, 22 August	Travel to Harpenden	
Monday-Wednesday, 23-25 August	Visit Rothamsted Agricultural Station, Harpenden	Seminar 'Resistance management for *Helicoverpa armigera* in Australia: problems, insights and changes'.
Wednesday, 25 August	Visit Dr Alan McCaffery, Reading University	
Thursday, 26 August	Visit Dr Chris Curtis, School of Hygiene and Tropical Medicine, London University	Seminar 'Insecticide resistance and its management'
Friday, 27 August	Visit Rothamsted Agricultural Station, Harpenden	
Sunday, 29 August	Depart London	
Tuesday, 31 August	Arrive Canberra	

Congress of Genetics

I was invited to give a talk in the symposium *Insecticide Resistance: Molecular and Population Genetics*. This symposium was part of the 17th International Congress of Genetics, Birmingham, U.K. The symposium was sponsored by Lawes Agricultural Trust as part of their 150th anniversary. The insecticide resistance symposium was in the last group of symposia held at the end of the week. It was attended by about 150 people. While the numbers were small, the audience was attentive and asked many questions of each of the papers, particularly at the end in general question time. During the conference I was able to make contact with Dr Rick Roush (Cornell) and Dr Nicole Pasteur (Montpellier) both of whom work on population genetics of insecticide resistance.

Visit to Laboratories in U.K.

Rothamsted.

I delivered a staff talk on resistance management in *H. armigera*.

The group headed by Alan Devonshire studies resistance and its management in a number of organisms. This group was of particular interest to me because of the breadth of their interest in resistance from molecules to populations.

Nerve insensitivity, kdr *and* superkdr *in houseflies*

Dr Martin Williamson described the work with Caroline Bell, Ian Denholm and Alan Devonshire on cloning of the *kdr* gene from houseflies. Using the *para* gene from *Drosophila* they cloned cDNA from houseflies. The *para* gene codes for the IVth domain and C terminal end of the sodium channel. They found 99% homology in sequence between their clone and *para*. They found RFLP using EcoR1 that differentiated between susceptible versus *kdr* and *super-kdr*.

Their crossing strategy has provided the basis for work undertaken in the Division by Stephen Trowell on endosulfan resistance.

Esterase-based resistance.

(a) Houseflies. Dr Williamson described the work with resistance in houseflies based on acetylcholine esterase (ACE). He described some exciting work done by the ICI (now Zeneca) group who were interested in graphical representations of the 3-D structure of proteins. ACE is a serine esterase. The protein folds to form a gorge lined with aromatic residues. This produces a strong negative charge. At the base of this 'negative' gorge is a triad of serine, glutamate and histidine that form the active site of the protein. The positive region of ACE is attracted into this gorge.

There are a number of hot-spots in the DNA sequence of ACE. Changes in the amino acid residues lead to a different patteru of resistance factors and cross-resistance. Work done with Didier Fournier (Entebbe) indicate that series of single point changes potentiate each other and increase cross-resistance.

Dr Williamson has cloned and sequenced the alleles from houseflies and has found that the mutations associated withe resistance in houseflies are in the same set of residues as found in OP resistant *Drosophila*. Additionally, he has found an additional mutation that gives resistance preferentially to malathion. These mutations are associated with aromatic areas in the 'gorge'.

These mutations change the shape of the gorge to make it too 'bulky' for the insecticide, but not ACE, to be able dock onto the triad.

Dr Graham Moores described work with a carboxy-esterase mediated resistance in houseflies. Flies with either ACE resistance or EST resistance had low levels of resistance (2-3 fold) compared with flies that had both (50-90 fold). Under field conditions, at low doses of OPs only the frequency of EST increased in response to selection. However at a dose 4 times the low dose both loci increased in frequency.

We also discussed Dr Robin Gunning's work on esterases in *Helicoverpa armigera* as a prelude to the work by my PhD student who commences in February 1994. He felt that the studies of Dr Gunning in *H. armigera* implicated satisfactorily the role of esterases in resistance to pyrethroids even though synergist work was less conclusive. He suggested an approach for Heliothis was to purify the protein band that Dr Gunning has detected on electrophoretic gels, add radio-labelled pyrethroids and look for hydrolysis.

(b) Myzus persicae. Dr Linda Field spoke again about her work on esterase-based resistance in this aphid. She had reviewed the studies on this resistance as part of the symposium at the Congress. The new work presented was on the inheritance studies of the esterase resistance. This mechanism is associated with an amplification with 2-16 copies of gene. Two alleles (at least) occur: E4 and FE4. A mutation in a stop codon has increased the length of the FE4 vs E4 by 10 amino acids. The E4 is closely linked to a translocation; the FE4 is not. It appears that the amplified gene is in a different place but the original gene remains in its location.

Dr Roger Blackman (BMNH) is doing crossing studies. In the U.K., a sexual phase does not occur although it does occur in the Mediterranean region. It was not possible to do all possible crosses because E4 homozygotes could not be made. However, F1 crosses suggested that the amplification was not segregating as a single gene. There was some indication of unequal crossing over within the amplified unit.

White Flies and Other Insects

Dr Ian Denholm and Matt Cahill (PhD student) are studying resistance management and ecology of *Bemisia* sp., white flies, that are a major problem in cotton production elsewhere in the world and in glasshouses. This insect is a potential threat to Australian agriculture.

Different biotypes have been identified: non-B and B biotypes. This identification is based on the presence of electrophoretic bands. The B-biotype was identified in the early 1980's. It has a different biology to the other biotypes, it is a wide host range (c.a. 600 species), greater fecundity, it carries a plant virus and it transmits other plant diseases. This biotype does not cross with non-B biotypes. It has broad spectrum resistance to pesticides.

Ecological studies use field simulators to gauge the effects of pesticide applications and parasitoid density on populations of *Bemisia*. Dr Denholm has been employed to assist in resistance monitoring of insecticide resistance in *H. armigera* in Pakistan

Dr Denholm also carries out general resistance program with European red mite, *Aphis gossypii.*

Pyrethroid Resistance

Dr Andrew Farnham discussed general studies using synergists. He indicated that failure of a synergist to eliminate enzyme activity did not indicate that a particular enzyme was not associated with resistance. In particular, the correct dose of synergist must be used to get effect. This was relevant to my future studies on esterase-mediated resistance in *H. armigera.*

Population Studies in Aphids using RAPDS

Dr Hugh Loxdale illustrated his work on the identification of species of aphids based on RAPDS and PCR techniques. These techniques provided good discrimination between species which otherwise showed no or little electrophoretic variation. The difficulty with RAPDS is that heterozygotes cannot be distinguished.

Dr Alan McCaffery, Reading University

Dr McCaffery was taking sabbatical leave with Zeneca Agrochemicals (formerly ICI Agrochemicals). He and colleagues have studied the mechanisms of resistance to pyrethroids in *Heliothis virescens* from the U.K. and in *H. armigera* from Thailand and India. He indicated that *kdr* was only a minor component of resistance in India; an equal component (with metabolism) in *H. armigera* from Thailand and a major component in *H. virescens*. He indicated that he is doing little work in this area at present because funding for these projects had been directed to the USA and India. However, at present he maintains two strains of *H. virescens* each which contains either metabolism or kdr based resistance. He has found *kdr* in different regions of the USA and has observed seasonal changes in frequency of this mechanism.

He has studied Indian resistance with Andy West (funded by NRI) and with Nigel Ames (ICRISAT). *Kdr* is not frequent in field populations. Cytochrome P450 based resistance is common. Resistance factors of order 30-50 fold.

He is trying currently to develop strains of *H. armigera* containing different mechanisms. He has a student commencing work on fitness of different *kdr phenotypes*.

London School of Hygiene and Tropical Medicine

I gave a short talk on resistance management in *H. armigera*.

Resistance Management in Mosquitoes. Dr Chris Curtis spoke on his work on resistance management for malaria carrying mosquito species. He has developed the use of insecticide impregnated nets for mosquito control and these have reached wide acceptance in Africa, Asia and China. He has a concern about resistance management, in particular, the move away from strategies using either mixtures or from a strategy in which an insecticide is used until resistance becomes a problem and then it is replaced temporarily or permanently. He argued that on theoretical grounds a pre-ordained rotation (as in *H. armigera* in Australia) was not always optimal, as was becoming dogma. Certainly, in practical terms the use of a single insecticide until if fails appears to be practical for mosquito control under some circumstances.

Dr Curtis also provided a preprint of his paper to be presented at the Rockerfeller conference on 'Biotechnology for Pest Management' October 1993. He discnsses possible means of driving genes into vector populations to make the populations harmless.

Esterase based resistance in Culex quinquefasciatus Dr Albert Ketterman, a post-doctoral fellow with Dr Janet Hemingway, discussed the groups work on esterase-based resistance in *Culex*. This resistance is similar to that reported in the same species from populations in the Mediterranean by Nicole Pasteur (Montpellier). Dr Ketterman's populations were collected from Sri Lanka. He finds two forms of esterase based resistance. A B2 band which is allelic with the B1 band reported by Pasteur. In addition, an A2 esterase is always associated with B2. A2 and B2 were tightly linked and cannot be separated. They have 97% homology at DNA level. Unlike Pasteur who observed amplification to involve 200 copies of the gene, Ketterman observed that B2 resistance only involved about 50 copies. Ashley Vaughan, a student working with Ketterman, is isolating genomic DNA from B2 esterase to look for amino acid differences.

Dr Ketterman gave me a number of preprints of his work on resistance in mosquitoes.