BIOCHEMICAL RESISTANCE DETECTION IN *H. ARMIGERA*

R. V. Gunning

NSW Agriculture & Fisheries, Agricultural Research Centre, Tamworth.

Heliothis armigera is arguably the most important agricultural pest in Australia and insecticides are considered necessary for its control. Insecticide resistance with this insect threatens the viability of the Australian cotton growing industry. Resistance is one of the evolutionary consequences of pesticide use and is an environmental disaster that does not get much public attention, unlike the human health and environmental pollution aspects of insecticide use. However, the rate of resistance development can be reduced or controlled if insecticides are utilised ideally. "Ideal" insecticide use means insecticide use, guided by knowledge of the mode of action, mechanisms of resistance and resistance genetics of the insect targets.

Following the detection of pyrethroid resistance in *H. armigera* in early 1983, a resistance management strategy was implemented. Research has monitored developments and supported the strategy. Studies of resistance mechanisms and ecological genetics have provided the scientific input to direct and refine the strategy. Our understanding of the build up of resistance in populations is substantial, but not yet complete.

Last year I spent several months at Rothamsted Experimental Station in the U.K, as a Churchill Fellow, studying biochemical toxicology in *H. armigera*. I was inspired by the Insecticide Resistance Group's very practical application of basic research on biochemical resistance mechanisms, to develop sensitive and reliable methods for monitoroing resistance in field populations. I felt that in *H. armigera* too, that we will be able to use the power of biochemical and molecular genetic techniques not only to gain a fundamental understanding of the processes, but to exploit this knowledge to study problems in the field. In this paper, I want to share some of these very exciting developments with you.

THE *HELIOTHIS* RESISTANCE MANAGEMENT STRATEGY - WHAT RESEARCH SUPPORT IS NEEDED ?

Our *Heliothis* insecticide resistance management strategy is the largest program of its kind operating anywhere in the world. Continued research is needed to monitor developments and to provide the basis for modifications to the strategy and this is particularly important with pyrethroid and endosulfan resistance levels increasing. In the past, many efforts to combat resistance have been thrawted by lack of detail on its dynamics. The evaluation and improvement of the strategy requires:

1. **Effective tecniques in detecting resistance**, epecially in its early stages of development. Sensitive methods of detecting and monitoring resistance enhance the study of resistance by providing information about the population dynamics of insects as observed in the field. In order to verify that applied strategies for resistance management are effective, we must be able to detect resistant individuals at very low frequencies.

2. **Knowledge of insecticide resistance mechanisms and genetics.** Resistance is a problem that can be understood best from these perspectives.

The conventional detection of resistance is based on insecticide susceptibility tests. Bioassays are not designed to detect the underlying resistance mechanisms. Although the bioassay technique has beeen the best resistance detection approach available, it is far from perfect.

Bioassay limitations are:

- only one concentration can be tested on each insect.
- no information on resistance mechanisms is provided.
- resistance cannot be detected at low frequency using bioassay.
- results are not available till days, or even weeks after field sampling.
- results can be ambiguous when a population is heterogeneous.

BIOCHEMICAL RESISTANCE DETECTION

From fundamental resistance mechanism studies , simple biochemical assays for detecting resistance have been developed. It's an exciting spin-off from fundamental insecticide mechanism research in insects, based on biochemical, immunological and nucliec acid probe methods.

Biochemical resistance detection features:

- detection of resistance and biochemical mechanisms in single insects .
- Methods which are cheaper and simpler than conventional bioassays.
- resistance can be detected at very low freqencies.
- accurate, unambiguous and instant results.

METHODS

Biochemical detection of enzymes involved with resistance.

Resistance is often based on increased enzyme detoxification, or reduced sensitivity to enzyme inhibition by an insecticide. Simple biochemical enzyme assays have been very sucessful in resistance detection, when positively correlated with enzyme activity.

Immunoassays for enzymes associated with resistance.

Antibodies or monoclonal antibodies to resistance enzymes can be prepared and present new promising rapid methods for the detection of minute quantities of resistance enzymes. The trapped enzymes may or retain their activity, or otherwise their presence can be detected by enzyme linked immunosorbent assays (ELISA tests). These tests are cheap and simple and can be adapted for field work and have good specificity. The assays have been sucessfully been used to monitor for resistance in insects overseas.

Detection of resistance genes using nucliec acid probes.

With advances in molecular genetics and gene cloning, it is possible to detect the mutant alleles that confer resistance by using radiolabelled nucliec acid probes. The technology has been already employed in medical and toxicological research. Nucleic acid probes will facilitate the identification and isolation of rare proteins, thereby allowing for the production of ELISA tests for proteins that would otherwise remain obscure. Possible targets are the non-catalytic receptor proteins and *Kdr* ion channels that are impossible to isolate by conventional biochemistry. These expanding techniques should provide powerful tools for the the basic study of insecticide resistance and its genetic control and the detection of resistant insects.

BIOCHEMICAL RESISTANCE DETECTION IN *H. ARMIGERA*

The possibilities for these methods of resistance detection in *H. armigera* are very promising and will expand with more knowledge of resistance mechanisms.

Prospects of Biochemical Resistance Detection for *H. armigera*

- *Kdr* alleles in pyrethroid and DDT resistance
- possible esterase and monooxygenase metabolism of pyrethroids and carbamates
- the acetylcholine receptore target of organophosphorous and carbamates
- The GABA receptor and its associated chloride channel that may be the site of resistance to endosulfan

The Cotton Research Council has approved funding for basic research into the biochemical insecticide resistance mechanisms of *H. armigera*.. An important spin-off from our increased understanding of insecticide resistance in *H. armigera* will be the future development of biochemical resistance detection methods. Methods that are cheaper, faster, more accurate and better field adapted than the discriminating dose bioassays that we presently use to asess the effectiveness of the resistance management strategy.

CONCLUSIONS

Biochemistry is playing an increasing role in managing insecticide resistance, since it provides not only the data on the nature of the resistance mechanisms, essential in making rational decisions with control agents. As long as biochemical or immunological tests are backed by unquestionable evidence for the involvement in resistance of the biochemical process that is monitored, it provides a means of identifying the presence and nature of resistance mechanisms in large numbers of individual insects. This is of primary importance for developing strategies to manage resistance. Direct monitoring of the primary gene product causing resistance is the best means of measuring changes in gene frequencies in populations treated with insecticides.