SUMMARY

a) BACKGROUND TO THE PROJECT

Insecticide resistance in *Helicoverpa armigera* is a major threat to the economic production of cotton in Australia. So far, the effects of resistance in the cotton industry have been ameliorated by a resistance management strategy and the introduction of commercial BT transgenic cotton. However, the marginal performance in the field of transgenic cotton and large areas of non-transgenic cotton, has resulted in an increased reliance on conventional chemicals and ever increasing resistance and environmental problems associated with insecticide and conventional insecticides are likely to remain an important component of future *Helicoverpa* control strategies. Avoidance or minimisation of resistance can only be achieved by effective resistance monitoring and understanding underlying resistance mechanisms.

While new *Helicoverpa* spp. control chemicals continue to be slowly introduced into the resistance management strategy, the risk of the development of resistance to new insecticides, due to overuse, is extreme. It is necessary to keep the older insecticides, which are the mainstay for *Helicoverpa* control, working. Thus, it is essential that research to overcome resistance to older insecticides (pyrethroids, carbamates and organophosphates) is continued so that their efficacy is maintained or even improved against resistant *H. armigera*

b) PROJECT OBJECTIVES

- To monitor pyrethroid, endosulfan, organophosphate and carhamate resistance in *H. armigera* and *H. punctigera.* from all cotton areas and to refine resistance management strategies.
- Determine the genetic basis of carbamate in *H. armigera* and *H. punctigera* and organophosphate resistance in *H. armigera*
- To develop and promote use of rapid biochemical techniques for the detection of organophosphate and carbamate resistance in the field.
- To evaluate new insecticides for *Helicoverpa* control, establish baseline susceptibility data and investigate their inherent resistance potential.

All objectives of this project have been achieved.

c) RESULTS

(i) *Helicoverpa* resistance monitoring

Helicoverpa spp. eggs or larvae were collected during the cotton season, from each of the major cotton growing areas in NSW, Queensland and WA. *Helicoverpa armigera* were assayed for resistance to pyrethroids, endosulfan, carbamates (thiodicarb, methomyl), organophosphates (methyl parathion, profenofos and chlorpyrifos), amitraz, chlorfenapyr and spinosad. *Helicoverpa punctigera* were bioassayed with fenvalerate, endosulfan and methomyl. Results are disseminated to the cotton industry via the internet.

(ii) Selection for *H. armigera* carbamate resistance by ovicidal rates of methomyl

Ovicidal use of methomyl against *Helicoverpa* spp. The results clearly demonstrated that insecticide resistance in expressed in the black egg stage of *H. armigera.* Both larvicidal and ovicidal rates of methomyl selected for resistance in black eggs quite strongly. While it is not clear how laboratory data relates to the field, we must not assume that ovicidal use of methomyl has no selective effect on eggs.

(iii) Genetic basis of *H. armigera* carbamate resistance

Carbamate resistance in *H. armigera* , is, as a result of, an altered target site acetylcholinesterase, which is insensitive to inhibition by methomyl and thiodicarb. Genetic experiments, indicate that the resistance mechanism is caused by a single, incompletely dominant, autosomal gene . There are three genotypes homozygotes (RR) heterozygotes (RS), and susceptible (SS). Resistance is incompletely expressed in the heterozygotes giving rise to a lower resistance factor than homozygotes. The frequency of homozygotes (RR) in field populations are at a much lower frequency than predicted by population genetics . This indicate that there may be a lack of fitness of homozygotes in the field and could be very advantageous for resistance management.

(iv) Carbamate resistance in *H. punctigera*

H. punctigera that are carbamate resistant are widespread in the field. The resistance factor is approximately 12 fold. The resistance mechanism is an altered target site acetylcholinesterase esterase, insensitive to inhibition

by methomyl and thiodicarh. Genetic experiments indicated that resistance is a result of a single, incompletely dominant gene which is carried on the female sex determining chromosome.

(v) Organophosphate resistance in *H. armigera*

Selection of field *H. armigera* with profenofos achieved high levels of resistance to both profenofos (92 fold) and methyl parathion (52 fold) compared to the susceptihle strain, however, strains were susceptible to chlorpyrifos. The resistance mechanism is an altered target site acetylcholinesterase esterase, insensitive to inhibition by the metabolites of methyl parathion and profenofos., but is still is susceptible to chlorpyrifos. Genetic experiments are incomplete, however, there are three genotypes homozygotes (RR) heterozygotes (RS), and susceptible (SS). Resistance is incompletely expressed in the heterozygotes giving rise to a lower resistance factor than homozygotes. Homozygous larvae have a severe fitness deficit.

(vi) Rapid Biochemical resistance detection

Studies have shown that pyrethroid, carbamate and organophosphate in Australian *H. armigera* resistance are as a result of biochemical resistance mechanisms. Pyrethroid resistance in Australian *H. armigera* is caused by a massive overproduction of esterase enzymes which detoxify pyrethroids by sequestration and hydrolysis. Esterase activity is correlated to resistance factor. Carbamate and organophosphate resistance in *H. armigera* are as a result of two different types of insensitive acetylcholine esterase (AChE). A result of resistance mechanism studies, has been the development of rapid biochemical methods for the detection of pyrethroid, carbamate resistance in Australian *H. armigera.* The methods are based on assays for resistance enzymes. Such methods can be utilised for laboratory based resistance detection, and field based, resistance detection kits have been produced and tested.

(vii) New control chemicals for *Helicoverpa*

Baseline data has been accumu;lated and pre-emptive resistance mechanism studies have been undertaken for new *H. armigera* control chemicals. *H. armigera* are showing some resistance to chlorfenapyr and a resistance mechanism has been identified.

(viii) Pyrethroid synergism - biochemical studies

The basis of pyrethroid synergism by piperonyl butoxide (PBO) and propargite (Comite®) is inhibition of the esterase enzymes which metabolise pyrethroids. Both synergists are only partial enzyme inhibitors so that use will select for populations which are more highly resistant to pyrethroids. Some organophosphates (such as ethion), on the other hand, are excellent esterase inhibitors and pyrethroid synergists.

(ix) Pyrethroid/esterase binding in *H. armigera*

Bioassay results show that all pyrethroids did not act identically on resistant *H. armigera* . Flucythrinate, fenvalerate and es-fenvalerate, were the least effective while permethrin, deltamethrin, bifenthrin, zeta-cypermethrin and alpha-cypermethrin were more toxic to resistant *H. armigera*,. Inhibition studies showed that this differences appears due to a differing ability of pyrethroids to bind to esterase enzymes. An examination of the structures of the pyrethroids, indicates marked differences in structure in those which interacted more readily with *H. armigera* esterases and those which bound less readily. The former group, are pyrethroids which contain a halogenated benzyl group. While in the latter group of pyrethroids (the benzyl ring was replaced by a dihalogenated alipbatic entity.

(x) Bifenthrin resistance in *H. armigera*

Until recently, pyrethroid resistant *H. armigera* were effectively susceptible to bifenthrin, but there been a considerable increase in the frequency of *H. armigera* larvae are resistant to bifenthrin over the last 3 years. Resistance is of a low order (~10 fold) and results from the evolution of additional esterase isoenzymes, which can bind to bifenthrin more efficiently. The resistance mechanism appears specific to bifenthrin.

(xi) Pyrethroid resistance in *H. punctigera*

Pyrethroid resistance can occur in *H. punctigera* and is a consequence of metabolism by esterase isoenzymes.