ECOLOGICAL GENETICS OF *HELIOTHIS*

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Ecological genetics is the study of genetic changes and how these are influenced by the biological parameters of a species. One genetic change of importance to the cotton industry is the evolution of insecticide resistance in *Heliothis armigera.* Biological parameters which are thought to determine the rate of evolution of resistance and its spread throughout the cropping areas include population size, migration, the species' ability to overwinter in cropping areas, the frequency of egg-lays and the abundance of non-cotton hosts. The results of these studies are of more than just theoretical interest; they have led to a number of recommendations about pest control. This paper will summarise a number of studies relevant to the ecological genetics of *H. armigera* and *H. punctigera.*

GENETIC VARIATION

H. armigera and *H. punctigera* differ genetically from one another (e.g. Daly and Gregg 1985). These differences will be exploited (by the Molecular Group in CSIRO Entomology) in the production of a field kit to distinguish between eggs and small larvae of the two species (Trowell and Daly, this volume). The kit will be similar to those used to detect insecticide residues in stored grains and used in medicine for the diagnostic testing of pregnancy, and infectious diseases such as AIDS. The kit will allow a grower or consultant to determine within a few hours the proportion of each species that is present in their crop. An appropriate insecticide may then be chosen to ensure maximum efficacy e.g. pyrethroids could be used with confidence in Stage 2 if most individuals present were *H. punctigera.*

Within each species, populations of *Heliothis armigera* and *H. punctigera* from around Australia appear to be genetically similar for a variety of traits such as resistance status, food preference and enzyme variation (Gunning *et al.* 1984; Daly and Gregg 1985; Firempong and Zalucki 1990). There is no evidence of major geographic divisions within either species, although populations from north-western Australia do differ in gene frequency from those in the eastern States (Daly and Gregg 1985). All these observations provide additional evidence that both species are migratory. Thus, characteristics of populations acquired in one region (such as resistance to a particular insecticide) should spread throughout the species range within a few years.

GENETIC BASIS OF RESISTANCE

The genetic basis of resistance defines the number of genes involved in resistance. This enables us to identify the different kinds (=genotypes) of resistant individuals that are found in field populations. Such information is essential in order to monitor resistance accurately and to determine the factors which lead to changes in resistance frequencies.

Resistance in field populations is usually conferred by a single gene, or a small number of genes, each of which has a major effect on the insect's ability to survive a commercial dose of insecticide. Insects, like most higher organisms, are diploid, that is they have two complete sets of genes, one set inherited from each parent. Thus, resistant insects may either carry one copy of the resistant gene (=heterozygous individual) or two copies (=homozygous resistant individual). If heterozygous individuals are almost as resistant as homozygous resistant individuals (the gene is said to be dominant) then resistance evolves in field populations much faster, and is a problem at lower resistance frequencies, than if the heterozygous individuals are more similar to susceptible individuals (the gene is recessive).

When pyrethroid resistance was first detected in 1983, it was observed that resistant insects differed from susceptible ones: they had reduced nerve sensitivity, pyrethroids penetrated the cuticle more slowly and their mixed function oxidase enzymes differed in some unknown way. Dr Robin Gunning (NSW Agriculture and Fisheries, Tamworth) observed that nerve insensitivity was the major form of resistance present in the strains of insects collected from the 1983 field failures in the Emerald district. This mechanism conferred a high degree of resistance in homozgyous individuals and showed cross-resistance to DDT. We isolated this gene from other resistance genes in insects collected from the field in 1985. Much to our surprise, our bioassays indicated that nerve insensitivity was associated with a single recessive gene, which by itself (i.e. in the absence of any other resistance genes) did not confer any resistance to the insect. Dr Gunning (pers. comm.) confirmed this observation by directly testing the nerve sensitivity of resistant insects. Furthermore, she reports that during 1985 this mechanism became much less common in field collections. At present, resistance in field populations is due to the presence of the mixed function oxidase (MFO) resistance. Thus, since resistance was first detected in Emerald seven years ago, we have seen a change in the frequency and characteristics of a major resistance mechanism.

MFO enzymes form part of the insect's natural defense against toxins in its foodplants and against insecticides e.g one MFO is involved in the first stage of breakdown of the pyrethroids in the insect. The action of the MFOs can be eliminated by the addition of piperonyl butoxide (pbo), a chemical which is sometimes added to commercial mixtures of pyrethroids (although not up to this last cropping season for *Heliothis* control).

The *mfo* gene responsible for this resistance mechanism is dominant in its expression and confers about 20-50 fold resistance in both heterozygotes and homozygotes. It is the frequency of this gene which is observed to fluctuate cyclically from year-to-year by the resistance monitoring program (Forrester 1989). The gene is very stable in its expression and does not appear to confer any major disadvantage to the insects in the absence of insecticide.

What has been most unexpected is that the *mfo* gene involved in resistance in the Australian *H. armigera* affects the penetration of the pyrethroids (Dr R.V. Gunning, pers. comm., A.H. unpublished data) and not their metabolism by the gut MFOs, as is the case in other examples of pyrethroid resistance in insects (Little *et al.* 1989). One of us (A.H.) has studied the

MFOs of both resistant and susceptible insects *in vitro* (the enzymes are isolated from insects in a test tube) and can find no difference between the strains in their ability to breakdown pyrethroids. This result is consistent with those of Dr Robin Gunning who studied metabolism *in vivo* (in live, whole insects) and observed differences in the uptake of pyrethroids between the strains but no difference in metabolism. Future studies by both Dr Hobbs and Dr Gunning will attempt to determine just how the mixed function oxidases are involved in pyrethroid resistance in Australian *H. armigera*.

LIFE-STAGE AND RESISTANCE

Genetic studies investigate the different life-stages during which resistance is expressed. In the laboratory, metabolic resistance can be detected in larvae of all sizes from neonates to large larvae, although larger larvae, in general, can tolerate much higher doses of insecticide than can smaller larvae, whether they are resistant or susceptible.

In freshly emerged adults, resistant individuals are 200 fold more tolerant of insecticide than are susceptible ones. However, the tolerance of resistant adults declines with age so that by 6-days old, there is little difference in resistance status between resistant and susceptible individuals. Piperonyl butoxide effectively eliminates metabolic resistance in adults and larvae.

FIELD STUDIES OF RESISTANCE

Genetic studies of resistance in field populations investigate the factors responsible for changes in gene frequency. Monitoring programs follow the changes that are occurring but by themselves cannot reveal why the changes occur. The frequency of pyrethroid resistance has fluctuated cyclically over the past seven years (Forrester 1989). Frequencies start each cropping season at relatively low levels, rise in frequency during stage 2 when pyrethroids are used, peak in the beginning of stage 3 and then decline slightly. At the beginning of the next season, resistance frequencies have again fallen. Superimposed onto this annual pattern, there has been a general increase in the proportion of resistant individuals observed in stage 2 and 3 of each year.

Our field studies reveal the multitude of factors responsible for these changes. Commercial applications of pyrethroids can discriminate between resistant and susceptible larvae and adults under a number of conditions (Daly *et al.* 1988, Daly and Fisk unpublished) given below. Thus, the use of pyrethroids causes resistance frequencies to rise.

(1) **Age of larvae in crop.** Resistant larvae between 4- and 12-days old can survive freshly applied applications of pyrethroids.

(2) **Days since spray application.** Pyrethroids are less efficacious for control of younger resistant larvae (< 4-d old) as the concentration of insecticide on the leaves is reduced by weathering or by plant growth. Even resistant neonates can survive field exposure to pyrethroids if it is more than ten days since spray application of a pyrethroid.

(3) **Age of Adults.** Resistant adults that are freshly emerged can also survive field application rates (Daly and Fisk unpublished). However, it is likely that older resistant adults will be sensitive to field rates of application.

More recent genetic studies have focused on the importance of autumn, winter and spring populations of *H. armigera,* that is, those populations that are not sprayed with pyrethroids (in association with Dr Gary Fitt, CSIRO Entomology, Narrabri). Pyrethroids are used in January/February when *H. armigera* populations are rapidly expanding in the cotton growing areas. The high frequency of resistance found in *H. armigera* populations on cotton in early autumn (in the next generation of *H. armigera*) indicates that there is little immigration from unsprayed crops and native host plants which could dilute the resistant populations. It is these populations under cotton crops which have high resistance frequencies (>50%) that give rise to the majority of the overwintering pupae in the Namoi Valley (Fitt and Daly 1990; Daly and Fitt 1990). Thus, the timing of stage 2 in mid-summer results in high resistance frequencies in overwintering populations.

Preliminary evidence suggests that resistant pupae are just as adapted to overwintering in diapause as susceptible ones. The emergence of these overwintering pupae in late spring probably accounts for the presence of some populations with high resistance frequencies in spring. However, most spring populations of *H. armigera* have relatively low frequencies of resistance (< 35%). The origin of these populations is not completely clear but trapping data suggests that they are immigrants (Fitt and Daly 1990). We do not yet understand the natural factors that can alter the relative contributions of local overwintering pupae versus spring immigrants to the spring populations of *H. armigera*. However, growers can assist nature by cultivating crop stubble to destroy overwintering pupal populations (Fitt and Forrester 1988).

RECOMMENDATIONS ABOUT PYRETHROID USE

Ecological genetic studies have given us insight into why pyrethroid resistance frequencies have been fluctuating during the last seven years. The results give us confidence that the Strategy implemented for the pesticide control of summer pests has extended the usefulness of the pyrethroids for *Heliothis* control. The rational use of pesticides does have its rewards!

In addition, genetic studies have had an impact in a number of areas of practical pest control:

1. Results indicate that it should be feasible to produce an *Heliothis* ID kit.
2. It is recommended that pyrethroids should not be used in stage 2 when medium-sized larvae are present.
3. Results predict that pyrethroids should be effective against resistant adults > 6-day old and very small larvae present at the time of application.
4. Piperonyl butoxide should be an effective synergist enhancing the efficacy of the pyrethroids.
5. Cultivation of overwintering pupae should help to reduce resistance frequencies in spring populations.

FUTURE GENETIC STUDIES

Future studies will be focused on three topics. The first will be to determine the origin of the spring immigrants and their resistance status. The second area will be to understand how metabolic resistance functions in the Australian *H. armigera.* The third area will be to investigate endosulfan resistance, in particular, the factors which determine its frequencies in cotton growing areas.

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