DNA PROBES FOR KEY INSECTICIDE RESISTANCE GENES – 1. ENDOSULFAN RESISTANCE IN AUSTRALIAN *H. ARMIGERA*

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This is a new project that aims to develop diagnostic molecular probes for the most threatening insecticide resistance mechanisms in Australian *H. armigera*.

INDUSTRY BACKGROUND

Insecticide resistance amongst *H. armigera* is one of the three main threats to the short-term sustainability of cotton production, the others being insufficient water supply and increased regulatory restrictions. Insecticide resistance is also probably the threat over which the industry has the greatest control. Currently, use of endosulfan and pyrethroids is threatened by insecticide resistance. Whilst other chemicals or Bt can be substituted, these are significantly more expensive. Chemical companies acknowledge that they currently have no alternatives which can fully take the place of pyrethroids. Furthermore simply substituting one insecticide for another is not a long-term solution. The cotton industry should conserve all available control agents including endosulfan, pyrethroids, Bt, genetically engineered cotton or viral insecticides. The best response to increasing resistance levels is to implement a well-designed and well-informed resistance management strategy (RMS), the aim of which should be to minimise selection pressure and its adverse affects.

The cotton industry needs at least three elements in an optimal RMS. The first is as wide a range of control measures and insecticides, with different modes of action, as possible. The industry is supporting the development of engineered cotton

plants and viral insecticides so that a wide range of environmentally acceptable control agents will be available. The second element is the ability to distinguish susceptible from resistant insects. At least to a first approximation, this capacity is provided by the LepTon™ Test Kit (see Trowell *et al.* elsewhere in this volume). Thirdly, optimum management of resistance under changing selection pressure requires accurate information on the population dynamics of the polymorphic resistance genes involved. At the moment it is impossible to obtain this information.

Current resistance monitoring has to rely on discriminating dose bioassays which are relatively slow and costly and provide limited information on mechanisms of resistance, the number of alleles present, their severity, or their frequency. The research described here is part of a project aimed at developing methods to allow this crucial information to be obtained for three of the commercially important resistance mechanisms in Australian cotton cropping systems.

SCIENTIFIC BACKGROUND

Endosulfan resistance is common in Australian *H. armigera* (Forrester and Bird, 1992) and its genetics have been examined in some detail (Daly and Fisk, 1991; Daly, 1992). We know that endosulfan resistance is conferred by a sex-linked gene and that it is semi-dominant in males (Daly and Fisk, 1991).

Endosulfan is a cyclodiene type insecticide having the same mode of action as other members of its class (such as dieldrin) although without their typically prolonged environmental persistence. The target site is a protein in the nervous-system of the insect known as the "GABA-gated chloride channel". Resistance to cyclodienes is the most widely reported type of insecticide resistance known, with over 300 instances noted in the literature (ffrench-Constant *et al.*, 1991). The molecular

details of resistance to one cyclodiene (dieldrin) have now been worked out for a few species of insects, including some flies of the genus *Drosophila* (ffrench-Constant *et al.*, 1991; ffrench-Constant *et al.*, 1993), a mosquito (Thompson *et al.*, 1993), a cockroach and a flour-beetle (Thompson *et al.*, 1993). In each case the resistant insect has a single changed amino acid at a particular location within the target site.

This was the starting point for our study. In order to develop a molecular tag for the endosulfan-resistant allele or alleles, we needed first to identify the putative mutation that confers resistance in Australian *H. armigera*. Because only one type of mutation has previously been reported, we started our study by looking for this same mutation in *H. armigera*.

RESULTS AND DISCUSSION

We used the polymerase chain reaction (PCR) with degenerate primers designed to amplify genes homologous to *Rdl* from other insect species. This enabled us to amplify and sequence a small portion of the *Rdl* homologue (*Rdl*Ha) from endosulfan susceptible and two independent resistant strains of *H. armigera*. This revealed two surprises:

Susceptible *H. armigera* (4)	Leu	Asn	Arg	Asn	Ala	Thr	Pro	Ala	Arg	Val	**Gln**	Leu	Gly	Val	Thr	Thr
Resistant *H. armigera* (5)	Leu	Asn	Arg	Asn	Ala	Thr	Pro	Ala	Arg	Val	**Gln**	Leu	Gly	Val	Thr	Thr
Susceptible *D. melanogaster*	Leu	Asn	Arg	Asn	Ala	Thr	Pro	Ala	Arg	Val	**Ala**	Leu	Gly	Val	Thr	Thr
Resistant *D. melanogaster*	Leu	Asn	Arg	Asn	Ala	Thr	Pro	Ala	Arg	Val	**Ser**	Leu	Gly	Val	Thr	Thr

Fig. 1. Amino acid sequences of part of the *Rdl*Ha and *Rdl* genes. The numbers of individuals sequenced are indicated in brackets (The Drosophila sequences are taken from ffrench-Constant *et al.*, 1993).

First, the deduced amino acid sequence was identical to that found in other insects except at the critical residue where the mutation that confers resistance occurs. At this residue we found the amino acid glutamine. In susceptible individuals from

other insect species this residue has always been found to be an alanine (Thompson *et al.*, 1993). Glutamine is a very different amino acid to alanine, with a much larger and more polar side chain, so this is an unexpected result. Second, we found that this amino acid is the same in susceptible and resistant insects whereas in the other insects examined to date there is always a *different* amino acid (Thompson *et al.*, 1993).

These data imply there may be significant differences between the version of the GABA-gated chloride channel found in *H. armigera* (a Lepidopteran) and that found in other insects. They certainly add weight to the argument that the mutable amino acid plays a very important role in the function of the protein. More to the point they indicate that endosulfan resistance in *H. armigera* represents a second kind of cyclodiene resistance mechanism.

This could be as small a difference as a mutation elsewhere in the same gene. We have therefore cloned a much larger region that includes the *Rdl*Ha gene and we are examining it for other changes. We have also identified one silent polymorphism in the region of the gene we sequenced. However we have determined that this polymorphism is not linked to resistance. Alternatively the difference could be a radically different mutation in a different gene. Working in collaboration with Dr. David Heckel (Clemson University, South Carolina) we have obtained additional preliminary indications that the mutation is located outside the *Rdl*Ha region.

These findings have important implications for the future direction of the project. They indicate that endosulfan resistance in Australian *H. armigera* is probably different, perhaps very different, from other cases of cyclodiene resistance. This means that we cannot rely on results obtained with other insects (or at least non-

lepidopterans). We would therefore need to perform a much more extensive study to establish the basis of resistance before being able to develop markers.

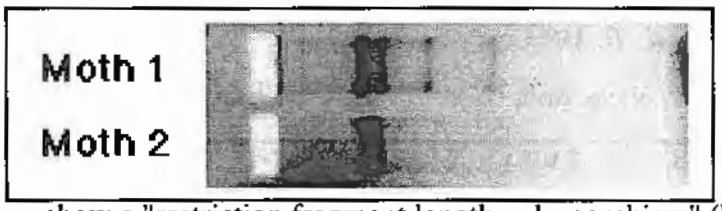

Fig. 2. Here we show a "restriction fragment length polymorphism" (RFLP) in the *Rdl*-homologous region of *H. armigera*. RFLPs can be used to determine resistance status in individual moths. However we have shown that this particular RFLP is not linked to resistance.

This research avenue could be worthwhile because of the development by Rhône-Poulenc of fipronil, an insecticide for sucking insects that has the same target site as endosulfan (Moffat, 1993) . Fipronil is seen as being important for control of sucking pests after the introduction of engineered cotton but cross-resistance to endosulfan is an issue. For example, existing cyclodiene-resistant strains of housefly have significant cross-resistance to this new compound (Bloomquist, 1993; Cole *et al.*, 1993). On the other hand the longer term nature of this research would incline us to focus our attention on developing molecular markers for pyrethroid resistance, not because this will be less complex but because resistance to this chemical group is currently more economically significant in the field.

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