

Resistance to Bt toxins in *Helicoverpa armigera*

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In the ongoing battle against *Helicoverpa armigera*, Bollgard II cotton varieties are about to replace Ingard varieties. Bollgard II produces two proteins from Bt (Cry1Ac and Cry2Ab) which are toxic to the moth's larvae (Ingard contains only Cry1Ac).

It is hoped that this double attack by Bollgard II will reduce opportunities for *H. armigera* to develop resistance to Bt cottons, and so prolong their 'life'.

H. armigera has proved itself to be very effective at evolving resistance to insecticides. There has always been a concern that it would also become resistant to the toxin in Ingard. Indeed, researchers at CSIRO have selected for a laboratory strain of the moth that is highly resistant to Cry1Ac. But, as yet, there is no evidence of resistance in the field.

In a CRDC funded project, we have now begun looking at the possibility of *H. armigera* becoming resistant to the toxins in Bollgard II. Early results from this research show that cotton growers need to remain vigilant, and that adherence to a Resistance Management Strategy (RMS) will be important in retaining the value of Bt cotton varieties.

The Transgenic and Insect Management Strategy Committee (TIMS) of the Australian Cotton Growers Research Association (ACGRA) has asked for the results of this preliminary research and planned future research to be made available to cotton growers. TIMS' purpose in doing this is to keep the industry informed on issues that may need to be reviewed prior to the final endorsement of the 2004–05 Resistance Management Plan (RMP) for Bollgard II. At present a draft 2004–05 RMP for Bollgard II has been supported by TIMS and submitted to the Australia Pesticides and Veterinary Medicines Authority (formerly the NRA) for approval.

What the research found



Resistant strain survival on a cotton variety that produces only Cry2Ab toxin.

A & B: Karen Olsen examines damage caused by *H. armigera* larvae from the field-derived Cry2Ab resistant strain.

C. Bioassay tray wells containing diet. The surface has been treated with a slurry of ground Cry2Ab cotton leaf. Seven days previously, one newly-emerged larva was placed in each well. Two surviving Cry2Ab resistant larvae have clearly eaten the cotton leaf material and grown to third instar. Susceptible larvae in the remaining cells have either died or failed to eat and remain at first instar.

One group of larvae, established from parents collected as eggs on a maize crop near Griffith, exhibited marked resistance to Cry2Ab. The results indicate a much higher frequency of resistant alleles (see 'Background' box) than expected, particularly as *H. armigera* populations have never been exposed to cotton-related Cry2Ab.

What it means

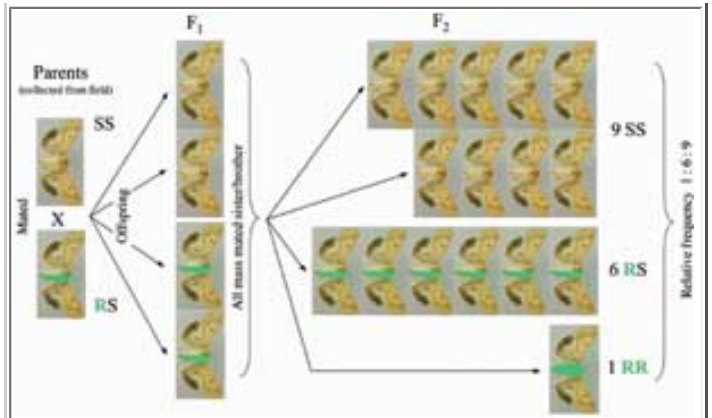
The expectation has been that Bollgard II, with its two toxins, will be much more resilient to the evolution of resistance than varieties like Ingard which carry only a single toxin. This would be true unless there is a gene that confers resistance to both Cry1Ac and Cry2Ab simultaneously.

This is considered unlikely, and there is no evidence of such cross-resistance to the two quite different toxins in other species of moths. But models indicate that the longevity of Bollgard II (the period before resistance evolves), is sensitive to the frequency of resistance alleles at the time it is introduced. The more common the resistance alleles, the shorter the time until resistance develops.

To date, the aim of resistance management in Bt cotton (Ingard) has been to maintain Cry1Ac resistance at low levels until two-gene cotton (such as Bollgard II) became available. This seems to have been successful, as there is no evidence of resistance to Cry1Ac in the field. But the background frequency of resistance alleles for the second toxin, Cry2Ab appears to be unexpectedly high, and it is possible this could impact on the longevity of Bollgard II.

While Cry2Ab resistance may be more common than expected, it must be emphasised that the level of threat, if any, to the longevity of Bollgard II posed by the resistance gene is not yet known. The resistant colony derived from field collected eggs and a quite independent colony selected by low doses of Cry2Ab in the laboratory (see below) are under intensive investigation right now to assess that risk.

Importantly, the research performed to date on insects from the field resistant colony (as yet, almost nothing is known about the laboratory selected colony) has shown that they show no



How resistance is detected?

Bt resistance is normally 'recessive'. That is, the moth must carry two copies of the mutant allele (green bodies) to be resistant. The moths half green and half brown are still susceptible to Bt.

Starting with field-collected eggs, the two-generation mating scheme demonstrated in the figure enables the production of some (1/16) of the 'grandchildren' carrying two resistance alleles (full green body) that are resistant.

Main Points

- Bollgard II cotton contains two Bt proteins (Cry1Ac and Cry2Ab) — both toxic to *Helicoverpa armigera*.
- Cry1Ac is the Bt protein in Ingard cotton varieties.
- After seven years of growing Ingard in Australia, there is no evidence of field resistance to Cry1Ac although a resistant laboratory culture has been developed.
- A group of larvae, from eggs collected on maize near Griffith, showed resistance to Cry2Ab.
- Current information indicates that Cry2Ab resistance may be unexpectedly common.
- Resistance to Cry2Ab has also been selected for in the laboratory.
- Work continues to see what impact this will have on the longevity of Bollgard II.

cross-resistance to Cry1Ac, implying that larvae carrying this resistance will still be susceptible to the Cry1Ac in Bollgard II. Equally importantly, the resistance appears to be largely recessive — that is it must be inherited from both parents before it is expressed, which will also hinder the development of resistance.

What could have caused it?

Forms of genes that confer resistance will be generated through naturally occurring mutations. Importantly, these are spontaneous and selection is not involved in the generation of mutations but is important later in causing such mutations to increase in frequency.

Insects with mutant versions of genes that confer resistance often suffer from poor fitness. This means they are unable to thrive or survive and so these gene versions (alleles) are likely to remain rare unless there is a selective force — such as a toxin expressed in a cotton plant which gives individuals carrying the mutant an advantage over non-mutant carrying individuals.

One explanation for the unexpectedly high frequency of alleles that confer resistance to Cry2Ab in *H. armigera*, is that there may be sufficient *Bacillus thuringiensis* (Bt) expressing this toxin in Australian soils to 'pre-select' populations of insects.

While superficially an attractive theory, there are problems with it. Soil-borne Bt in Australia frequently produce Cry1Ac, Cry2Ab or other Cry proteins. But Cry1Ac is by far the most commonly produced protein, so if the 'pre-selection' theory is true, why is resistance to Cry2Ab apparently more common than resistance to Cry1Ac?

An alternative theory is that the mutation rate at the gene or genes that confer resistance to Cry2Ab may be high, and/or, such mutations may not cause deleterious effects. Under high mutation rates and low fitness costs, mutant alleles would accumulate in the population.

The research — in more detail

When monitoring for resistance to Cry1Ac, the standard practice has been to 'challenge' larvae reared from field-collected eggs by feeding them

Background

Discriminating dose: A (single) dose of an insecticide that kills 99 per cent of all susceptible individuals of a particular species. Discriminating dose results are used to find out the frequency of resistant pests in the population.

Crystal (Cry) proteins are produced in *Bacillus thuringiensis* (Bt) by its Cry genes (over 100 genes in different strains) and these are toxic to the larvae of a number of destructive insect pests but non-toxic to humans.

Alleles are different forms of a gene. Each individual of a diploid species (for example humans, most plants and moths) inherit two copies of each gene, one from each parent. These copies may be the same or different. Many alleles may exist in a population for one gene. (such as hair colour in humans).

A recessive gene must be paired with one identical to it in order to influence a trait (such as blue eyes in humans).

on a diet containing a discriminating dose (see 'Background') of toxin. Any larvae which survive and grow on the diet could be resistant.

To date, the resistance monitoring program (presently carried out by CSIRO, but until the 2002–03 season by NSW Agriculture) has detected no evidence of increasing levels of resistance to Cry1Ac despite Ingard cottons being grown in Australia for seven years. So there appears to be no cause for concern that *H. armigera* is developing field resistance to Cry1Ac.

This favourable situation has been the objective of the RMS that has been in place since Ingard was first planted — including the deployment of refuges to 'dilute' resistant genes, the 30 per cent Ingard area cap and 'pupae busting' to reduce the population of possibly resistant *H. armigera* diapausing under Ingard.

The Bt monitoring program provides an overall assessment of changes in resistance levels in *H. armigera* populations throughout the industry and most cotton growing areas are included in the survey. But the program does not provide information on the frequency of resistance alleles, and this information is invaluable for improving models used to develop the RMS.

It is possible to obtain such information through the use of an 'F2 screen' that involves exposing the descendants (the human analogy would be the grand children) of two moths (one male, one female) to discriminating doses of toxin (Figure 1).

We were interested in looking at the potential for F2 screens to supplement discriminating dose assays as a more sensitive way of detecting resistance in its early stages. It was expected to be more sensitive as (in other species of moth and therefore probably in *H. armigera*) Bt resistance is normally 'recessive' (see 'Background').

However in the early stages of the evolution of resistance, such individuals are extremely rare. More common are individuals carrying one mutant and one normal form of the gene and these are likely to be still susceptible to Bt and therefore 'missed' in discriminating dose assays.

During the 2002–03 season we conducted a preliminary survey to evaluate this methodology

by testing the descendants of 33 single pairs of moths. In separate assays, groups of larvae were challenged with either Cry1Ac or Cry2Ab. Only minor, low-level resistance was detected for Cry1Ac but one pair of moths from Griffith produced descendants with unexpectedly high resistance to Cry2Ab.

As each parent of a 'pair' contributed two copies of each gene, the survey examined 33 (pairs) x 2 (female) x 2 (male) = 132 alleles. Thus the calculated frequency of the resistant form of the gene is 1/132 or 0.008. Resistance alleles were expected, but to find one among the first 132 examined was unexpected.

In related research, CSIRO's Dr Ray Akhurst and colleagues exposed a different colony of *H. armigera* established from eggs collected in various locations to low levels of Cry2Ab toxin. This colony now also exhibits resistance to Cry2Ab, implying that at least one of the individuals that were incorporated into the colony carried a resistance gene.

Ongoing research

Following discussions on the research reported above, TIMS has resolved that there is a need to learn more about background levels of resistance to Cry2Ab toxins in *H. armigera* populations and opportunities for survival of resistant genotypes on Bollgard II.

While the available information on the newly discovered Cry2Ab resistance (no cross-resistance and recessive nature) is particularly favourable, further research during the coming field season (2003– 2004) will allow a better evaluation of the threat posed by it.

We will be looking at several issues. Firstly, additional work will improve the accuracy of the assessment of the frequency of resistance genes. If an additional 100 or 200 F2 analyses are performed and no further resistance alleles are isolated, then there would be cause for celebration. On the other hand, isolation of further resistance genes would require additional research including a very careful re-evaluation of the resistance management model upon which the future management plan for Bollgard II is based.

Secondly, we will determine if resistant

individuals can survive on Bollgard II. The season-end decline of Cry1Ac may provide an opportunity for Cry2Ab resistant but Cry1Ac susceptible individuals to exploit. Lastly, the fitness of resistant individuals on non-Bt hosts needs to be examined.

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