

An update on Bt resistance in *Helicoverpa*

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Cotton varieties that produce insecticidal Cry toxins are valuable tools for controlling *Helicoverpa armigera*. By reducing the application of conventional insecticides this technology also delivers important environmental benefits. To enjoy this tool in the long term we must be pro-active in managing resistance to the Bt toxins within transgenic cotton.

With the introduction of Ingard in 1996, the Cotton Research and Development Corporation started a program to monitor incipient resistance by *Helicoverpa* to Cry1Ac (the toxin present in Ingard). In 2002, the program was expanded to include assays to detect the initial development of resistance by *Helicoverpa* to Cry2Ab (the toxin that is paired with Cry1Ac in Bollgard II).

Since 2002, CSIRO has used a method called an F2 screen to detect resistance genes (see Figure 1). This screen assays the 'grandchildren' of single mated pairs of insects collected from the field to identify rare resistance alleles.

A major advantage of this screen is that it can detect heterozygous (RS) individuals that contain only one copy of a resistance allele where the resistance is completely recessive. The CSIRO F2 screens for Bt resistance in

the 2006–07 season were completed in July 2007. We screened 532 alleles from *H. armigera* against Cry1Ac and Cry2Ab.

Alleles conferring resistance to Cry1Ac remain rare

In 2006–07 CSIRO isolated no cases in *H. armigera* of alleles conferring resistance to Cry1Ac. In fact, there have been no cases of resistance found in 2212 tests since the program began. This suggests that the frequency of alleles conferring resistance to Cry1Ac remains low after 10 years exposure to cotton containing this toxin (initially Ingard and now Bollgard II).

Additionally, Cry1A toxins are the most dominant in the spray formulation DiPel and therefore use of this conventional insecticide would also have presented selection pressure for resistance.

Alleles conferring resistance to Cry2Ab are more common than expected

In 2006–07 CSIRO isolated five cases in *H. armigera* of alleles conferring resistance to Cry2Ab. This validates our initial findings for *H. armigera* that indicated an elevated baseline frequency of alleles conferring resistance to Cry2Ab.

While we found this frequency unexpectedly high, research from around the

world on resistance to Bt toxins is accumulating and indicates that such a baseline frequency is not unique. There have now been 12 tests indicating alleles with resistance to Cry2Ab from a total of 2216 since the program began. These alleles have been isolated from insects collected throughout the main cotton growing areas rather than from a specific location.

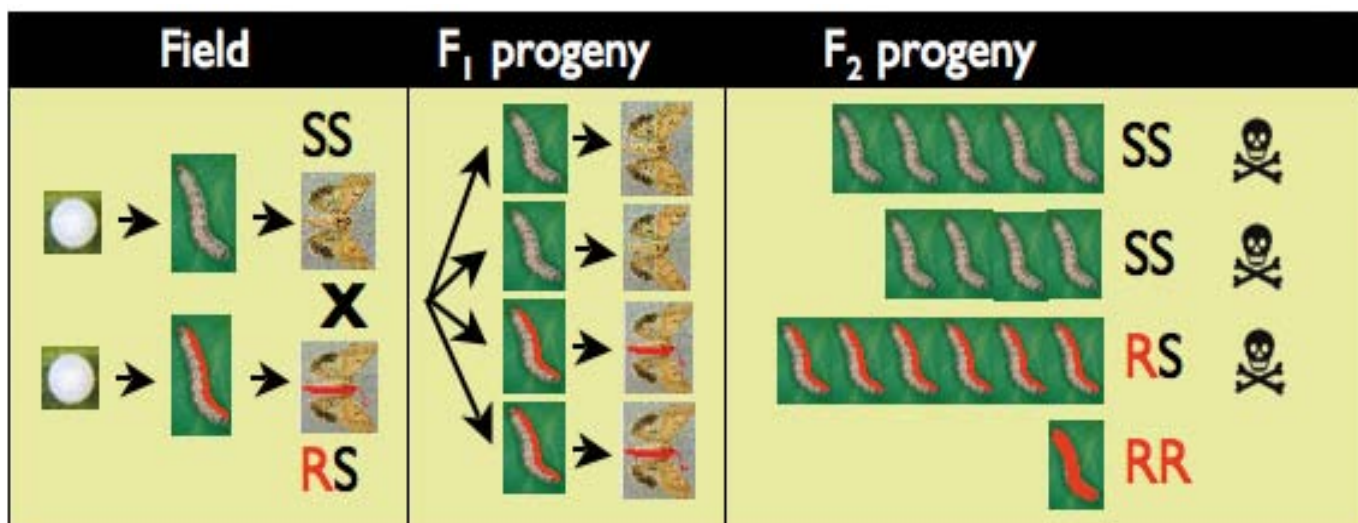
We have tested five of the 12 *H. armigera* isolates that are resistant to Cry2Ab and found that resistance was due to alleles at the same locus. Although we have not tested the remaining isolates, the development and survival of these lines in the initial F2 screen is similar to that of the five allelic strains.

While extrapolating a little, it is reasonable to assume that all instances of resistance so far detected are due to alleles at that locus. Importantly, the first isolates were detected prior to the widespread use of Bollgard II, so we believe that the resistance was present at a detectable frequency prior to the introduction of Bollgard II.

The frequency of Cry2Ab resistance alleles does not appear to be increasing

A critical question is whether the frequency of Cry2Ab resistance alleles in natural populations of *H. armigera* is in-

FIGURE 1: F2 screen for rare resistance alleles in *Helicoverpa armigera*



Parents are collected in the field, usually as eggs or larvae, and here one of them is indicated with one copy of the resistance allele. Their F₁ progeny are sib-mated to produce the F₂ generation. If resistance is completely recessive, in the F₂ generation only 1/16 of the larvae are expected to be homozygous for the rare resistance allele (RR), and the remaining homozygous susceptible (SS) and heterozygote progeny will be killed by the discriminating dose of toxin.

creasing now that we are growing Bollgard II. A regression analysis on our data demonstrates that there is no significant trend in frequencies over years. The difference between data collected before and since Bollgard II became widely adopted is also not statistically significant (the frequency of resistant alleles in 2002–04 was 0.0035 versus 0.0047 in 2005–07).

Data collected by Monsanto over several years using an alternative version of the F2 screen known as an F1 test (crossing field

individuals with individuals from a colony resistant to Cry2Ab — the SP15 colony) suggests that the frequency of alleles in natural populations may be two or three times higher than that found using F2 screens. The frequency of alleles detected using these F1 screens also has not increased over time. This data is currently being analysed by Monsanto in preparation for publication. In the coming season CSIRO will also investigate the frequency of SP15-like Cry2Ab resistance alleles using F1 crosses.

What are the characteristics of the Cry2Ab resistance?

This resistance to Cry2Ab in *H. armigera* is potent. Resistant insects are able to survive a dose that is much greater than that found in transgenic plants. In limited trials, the Cry2Ab-resistant insects also thrived on laboratory-grown experimental cotton that expressed only Cry2Ab. Fortunately, all of the Cry2Ab resistant insects are susceptible to Cry1Ac. In the labora-

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tory, Cry2Ab resistance is recessive, which is favourable.

But based on the frequency of surviving (and functionally resistant) insects in F2 tests, some dominance may be present. We are currently investigating this. If dominance is indeed present, then the threat to Bollgard II changes.

Many forms of resistance render the carrier less fit in environments that do not expose it to the insecticide/toxin. This 'fitness cost' can lead to a decline in the frequency of the resistance. We have compared the performance of resistant and susceptible insects in a range of environments that we considered would expose any 'weakness' of the resistant strain.

Despite extensive analyses we failed to detect an effect of Cry2Ab resistance in *H. armigera* on larval growth, survival, pupae size, moth emergence, time to pupation, time to emergence, or fertility. Therefore whenever Cry2Ab resistant insects are favoured, the frequency of resistance alleles may increase, and in the absence of fitness costs, remain at that frequency until the next set of conditions arise to favour another cohort of resistant individuals.

How might a high baseline frequency affect resistance development?

If the resistance detected for *H. armigera* proves to be recessive, as shown for five isolates in the laboratory (but note our concern about dominance in field insects), only homozygous resistant insects will be able to tolerate Cry2Ab toxin. With a resistance frequency of 0.005, homozygous individuals that are capable of surviving on cotton expressing Cry2Ab toxin will occur at the square of that frequency ($0.005^2 = 0.000025$) and therefore be extremely rare.

The frequency of resistance to Cry1Ac is also rare and Cry2Ab resistant insects are susceptible to Cry1Ac. So the frequency of insects resistant to both toxins would be exceedingly small. But the concentration of both toxins in Bollgard II varies markedly which may provide opportunities for insects that are resistant to only one toxin.

In particular, homozygous Cry2Ab resistant insects may be favoured on Bollgard II whenever the Cry1Ac concentration declines to levels that Cry1Ac-susceptible insects can tolerate. Such events are more likely to occur late in the season on mature Bollgard II cotton. We have observed lim-

ited survival of Cry2Ab resistant insects on early squaring field-grown Bollgard II in the laboratory, but numbers increased on late season cotton.

What can the industry do to help preserve Bt-cotton?

It is critical that the industry maintains the rigour of the Resistance Management Plan for Bollgard II, including improving the efficacy of refuges. We are continuing to develop and implement more sensitive tests to detect changes in the frequency of resistance alleles. In the short-term this may involve an abridged version of the F2 tests that will enable an increase in the number of alleles scored.

Longer term, the likely answer will be fundamental research to isolate the gene conferring resistance which should allow the development of DNA-based techniques to score resistant alleles. Such techniques will enable vastly increased numbers of insects to be tested for resistance and therefore enable the detection of subtle changes. Importantly, this approach may enable an increase in frequency to be detected earlier, and so provide the industry with more time and opportunities to undertake remedial action to slow the rate of evolution of resistance.

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