

Refuges - a key element in transgenic cotton management

Gary P. Fitt

**CSIRO Division of Entomology and
CRC for Sustainable Cotton Production, Narrabri**

With the imminent release of cotton varieties expressing the INGARD gene to control *Helicoverpa* the time has come to consider in detail the elements of the proposed resistance management plan for transgenic cotton. The characteristics and value of cotton varieties with INGARD and the critical need for a pre-emptive resistance strategy has been discussed widely over the past few years. Through collaboration of numerous entomologists and industry the components of a strategy have been identified and researched and details of the resistance strategy for 1996-97 have been finalised by the TIMS (Transgenic and Insect Management Strategy) Committee with input from researchers, growers, consultants and industry partners. Details have been widely publicised (Fitt 1996, Forrester, this proceedings). The strategy focuses squarely on *Heliothis armigera* as the pest most likely to evolve resistance to INGARD varieties, just as it has to conventional chemicals. Of the four main components (planting window, refuges, overwinter cultivation to destroy pupae and late season *Helicoverpa* control), the concept of providing refuges to assist resistance management is perhaps the most novel aspect of the whole strategy, and the most critical component. It is also likely to be the most challenging aspect for growers and the most difficult to implement effectively. Here I will briefly explain the concept of a refuge, why we need them, precisely what they are, how they work and what they will mean for growers.

What is a refuge and why do we need them ?

Refuges have been an integral, though not always obvious, part of resistance management for many decades. Simulation models and experimental data have shown that the provision of refuges can dramatically delay the occurrence of resistance in certain circumstances by diluting the selection for resistance happening

in other parts of the pest's environment. Simply put, a refuge is an area where the pest can breed without being exposed to a pesticide and so without experiencing selection pressure which would lead to resistance.

We have used refuges historically in the pyrethroid resistance management strategy where in the early years at least we saw dilution of resistance each spring as some *H. armigera* migrated into cotton areas from other cropping regions - the refuges. Eventually those refuges were also contaminated by resistance genes through the movement of moths out of cotton areas as well. However, the best example of how a refuge can assist with resistance management is our second pest species, *Helicoverpa punctigera*. *H. punctigera* is regularly treated with pesticides in cotton and other crops and is physiologically capable of becoming resistant. However, this species has a huge range of host plants and widespread populations outside of cropping regions. These natural refuges generate a large proportion of the total *H. punctigera* population and routinely provide sufficient migrants to the cropping areas that any selection for resistance is rapidly diluted. Unfortunately with *H. armigera* we do not have such large and reliable natural refuges, we must provide our own if we wish to pre-empt the emergence of resistance. However, the prospect of cotton growers producing crops to ensure *H. armigera* survives probably seems anathema to most.

So why do we need refuges? We have to accept that Bt resistant *H. armigera* probably already exist, but at very low frequencies, so low that we can't detect them. We also have to accept that INGARD crops will impose intense selection pressure on each generation of *H. armigera* which feeds on it. In the early years of INGARD cotton very small numbers of Bt resistant *H. armigera* may survive, too few for us to notice. If these resistant survivors are allowed to mate with one another they could establish the nucleus of a resistant population. Simulation studies (by Rick Roush and others) and some experimental data have shown that without deliberately added **refuge populations**, Bt resistance could appear in only three to four years after the

first **widespread** use of INGARD cotton. With effective resistance management, including refuges, varieties with the INGARD gene could remain effective for decades. However, refuges **WILL NOT PREVENT** resistance occurring. By producing large numbers of unselected, susceptible moths which can mate with the few resistant ones, we can effectively counter the selection occurring in INGARD crops and dramatically **SLOW DOWN** the evolution of resistance, so that it does not become an issue for many years. Ensuring that two resistant survivors do not meet one another for mating is the primary aim. So in the case of transgenic cotton the refuge can be any crop or non-crop habitat where *H.armigera* can breed and survive without any exposure to the Bt proteins.

Characteristics of a refuge.

To be most effective a refuge must:

- 1) *produce adult H. armigera*. That is, larvae must feed and develop, pupate in the soil and successfully emerge to mate. So the refuge must be something that *H. armigera* will lay eggs on and which is suitable for larvae to develop. It also means the refuge must be managed so that it is attractive to moths and can support larvae: so it will need water and fertilizer. If the refuge is much less attractive than the INGARD crop itself then it will not be an effective refuge. A crop which does not produce moths IS NOT a refuge.
- 2) *have no use of Bt sprays*, since this would simply impose selection pressure in the refuge as well.
- 3) *produce moths during the period when Ingard crops are growing* - to maximise the chances for random mating and hence dilution to occur it is important that refuges are producing moths at least during the cotton growing period when INGARD cotton might also produce a few survivors. Since *H. armigera* is most common on cotton during the second half of the growing cycle this is the crucial time

to have productive refuges. Ideally the refuges will also produce pupae which overwinter and provide dilution in the following spring as well, but this is not yet a firm requirement of the strategy. We might well see such a requirement in the future.

4) *the refuge needs to be nearby the INGARD crop - at least on every farm where INGARD cotton is grown.* We know quite a lot about the movements of *Helicoverpa* adults, enough to know that they do move over substantial distances before and after mating (see paper by Gregg et al, this proceedings). Some moths will stay in INGARD crops to mate, others will move several kilometres away. So we do not assume that the refuge on each farm will produce moths to dilute resistance just on that farm. All the refuges spread around cotton areas will contribute to a pool of susceptible moths moving throughout the region. To some extent then the refuges operate collectively rather than individually. If you have a refuge, but your neighbour doesn't, then you are both likely to suffer in the long run.

Refuge options for 1996-97

The refuge options proposed for 1996/97 are as follows. For each 100 hectares of INGARD cotton planted, a grower is required to plant a minimum of one of the following:

- * An area of conventional irrigated cotton of **10 hectares**, which will be untreated for any reason with products that control *Helicoverpa*; or,
- * An area of conventional irrigated cotton of **50 hectares**, which can be conventionally managed for *Helicoverpa* and other pests; or,
- * An area of irrigated sorghum or corn of **20 hectares**, which will be untreated for any reason with products that control *Helicoverpa*, and managed to flower from January 15 to February 28.

These refuge options and their relative areas are derived from simulation models which indicate the proportion of the population which needs to be in a refuge (see

Roush, this proceedings); and experimental results which show the relative numbers of *H. armigera* pupae which may be produced under different crops (see Fitt and Tann, this proceedings).

Unsprayed, non-transgenic cotton (refuge option 1) is an excellent refuge in that it will produce numbers of moths over a long period, at least as long as the INGARD crop. Refuge option 2 - conventionally sprayed non-transgenic cotton - acknowledges that, with current pesticides and resistance levels, commercial cotton does produce *Helicoverpa*. But their numbers are much less than from an unsprayed crop and so the area needs to be relatively larger, five times larger in fact. While sprayed cotton refuges might appear to be the least costly option, we should nevertheless aim for unsprayed refuges for the future since these could require much smaller areas and maximise the potential to reduce pesticide use on most cotton produced.

Refuges of unsprayed irrigated sorghum and corn (refuge option 3) can also produce many moths but only over a period of two to three weeks. For that reason these refuges need to be managed to flower over a much longer period to be as effective as a cotton refuge. To achieve that will require multiple plantings of these crops.

Growers will be aware that the refuges proposed for Australia are different, and importantly larger, than those proposed for the USA where Bollgard cotton is now commercial. This reflects the simple fact that we have a much more difficult pest and resistance system to deal with. The main target for Bollgard cotton is *Heliothis virescens*, a species with a history of resistance development to pesticides, but one which is quite susceptible to Bt proteins. Both our species (*H. armigera* and *H. punctigera*) are 10 times harder to kill with Bt than *H. virescens*. This does not mean our species are already resistant, they simply interact differently with the Bt proteins and have a different level of natural tolerance. We already know from field trials that

while cotton with the INGARD gene kills *H. armigera* very well through most of the season, there is some survival of *H. armigera* late in the season; these are only small numbers and do little damage but they show that we do not have a very high level of expression of the Bt proteins in our varieties. Although I have not discussed this issue here, a high level of expression of the toxin is the other important component of the refuge/ high dose strategy (see Roush, this proceedings). Because our species are more difficult to kill, our refuges become even more critical as the major plank of the management strategy, hence they need to be larger and we must ensure they are planted and managed carefully.

Other Refuge Options.

The refuge options proposed for the first year may well change as we gather more information about other possible refuges and as the resistance management strategy evolves. Numerous other refuge possibilities are being researched since there are many crops which can produce *H. armigera*. Crops vary widely in their potential to generate moths. Our aim is thus to find the refuge which generates the highest numbers of moths over a long period of time from the smallest possible area and at the least cost to growers. Achieving all these things is not simple. In addition to the options proposed above, we are quantifying the refuge value of grain legume crops such as pigeon pea and adzuki bean, of lucerne and associated cotton managed with food sprays, and of seedmixtures involving the summer legume *Dolichos lablab*. The latter option seeks to use what is basically a green manure crop as a refuge by mixing with it a range of plants which are hosts for *Helicoverpa* (see Fitt and Tann, this proceedings). In each case the basic measure of value is simply the number of *H. armigera* pupae produced per unit area and over time compared to what is produced by unsprayed cotton at the same site. So unsprayed cotton is the standard for comparisons of these options. Those refuges which produce more pupae/ ha are more valuable for resistance management.

The Dolichos option described briefly above shows some promise, but may generate unacceptable agronomic problems with weed management and has been labelled the “jungle” option by some researchers. However, the point is that there are many ways to implement effective refuges; sprayed cotton is just one and from the point of view of achieving sustainable systems for the future we should be looking more to the use of Envirofeast managed cotton or unsprayed areas of other crops as refuges for resistance management of INGARD crops.

Why must all growers have a refuge?

Whilst we know that many *H. armigera* are produced on crops other than cotton in cropping systems of northern NSW and southern Qld, it would be unwise for the cotton industry to rely on these other crops (sorghum, corn, sunflowers, legumes etc) as our refuges. They are often, or usually, grown by non-cotton growers and so the areas may vary widely from year to year in response to market changes and climate and would not be related to the areas of INGARD cotton being grown. It may also be that in future these other crop hosts could be sprayed with Bt sprays or perhaps themselves be engineered to produce Bt. They would then not be refuges at all. By ensuring that every cotton grower using INGARD is required to provide a refuge the responsibility for resistance management will be effectively shared among those who gain the benefit from transgenic pest management and the long-term sustainability that reduced pesticide use and environmental pressures should bring.

All the other crops hosts of *H. armigera* where Bt sprays are not used, will act as refuges and so contribute further stability to the resistance strategy employed within the cotton industry, but we cannot rely on those other crops to always be there.

What will refuges mean for growers?

Whatever type of refuge is used it will inevitably impose a cost on growers. This might be a cost of lost yield on unsprayed cotton or other crops through allowing *H. armigera* to feed on them, or the lost opportunity to grow additional hectares of INGARD cotton through using the much larger sprayed cotton refuge option. Whatever the cost, it should be viewed as part of the cost structure of growing the INGARD crop, and as long-term insurance for the technology, rather than being viewed as an add-on cost in the short term. Hopefully the full costs of providing refuges to ensure the long-term viability of INGARD will be reflected in the cost of the technology to growers. At the time of writing (June 1996) the cost of INGARD protection has not been announced, though of course speculation is rife!

Conclusions.

From the point of view of pest management I believe the best perspective on transgenic cotton is to see it as a new platform on which to build better IPM systems, to utilise other technologies which were not compatible with the use of hard chemicals, and to fully develop a more environmentally friendly face for cotton production. This emerging era of a cotton industry in which pest management is based on transgenic Bt/ Envirofeast/ beneficials/ soft chemicals offers strong possibilities for a sustainable future, provided Bt resistance does not quickly muddy the waters. Refuges are a critical component of the pre-emptive resistance management strategy which builds on the fact that we have no Bt resistance now. It is only one component however; the other components are also important. Mandatory cultivation of crop residues to ensure no survival of pupae under INGARD crops is also critical and will multiply the value of the refuges. We are still learning how best to use refuges and growers and consultants can look forward to some interesting and challenging times ahead during the early years of varieties with INGARD as the management strategy evolves.