

## **Resistance Management- a Key to the Transgenic Era**

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### **Resistance is a serious threat to transgenic cottons**

Australian cotton growers are very familiar with the devastation that the industry can suffer from insecticide resistance, as in the Ord in the 1970s. Similarly, cotton growers are also well aware of the importance of transgenic cotton for reducing pesticide use in the industry. Unfortunately, transgenic plants are no less subject to selection for resistance than classical insecticides.

A major pest of cabbage, the diamondback moth, has already evolved resistance to *Bacillus thuringiensis* (Bt) sprays in many cropping areas around the world. Bt-resistant diamondback moth larvae can be selected by and are completely resistant to transgenic plants that carry exactly the same Bt gene ("CryIA") as is used in transgenic (INGARD) cotton (Metz, Roush, Tang, Shelton, and Earle, 1995 Molecular Breeding 1: 309-317). There is every reason to suspect that a few cotton bollworms carry a resistance gene that is very similar to that in diamondback moth. Further, in contrast to insecticide sprays, the Bt toxin is continuously expressed in transgenic plants, which means that every insect feeding on them will be selected for resistance. This persistent exposure offers the potential of even stronger selection for resistance than would come from sprays. There is also evidence that genes for resistance to Bt may be more common than were genes for resistance to chemical insecticides, which could also give faster resistance. Thus, resistance management is at least as critical to transgenic crops as it has been for chemical insecticides.

### **Resistance Management Works!**

The resistance threat is serious, but we have learned important lessons from the past that, if heeded, can provide the key to a prosperous transgenic era. The resistance management strategy for synthetic pyrethroids and other pesticides adopted for Australian cotton in the early 1980's is truly a remarkable accomplishment. It almost certainly extended by 3-10 years the usefulness of pyrethroids to the industry, as is clear from comparisons to other countries and from computer simulation models. However, the same simulation models also suggest that the strategy was instituted too late to have provided maximum benefits.

If the strategy had been introduced at the first use of pyrethroids, their long-term effectiveness could have been much higher, especially throughout the last 10 years. After the initiation of the strategy, sorghum generated large numbers of pyrethroid-susceptible bollworm moths. That is, it served as a "refuge" and thereby played a major role in diluting resistance. However, prior to the strategy, applications of pyrethroids for sorghum midge helped to select for resistance in bollworms (Forrester, Cahill, Bird, and Layland, 1993, Bulletin of Entomological Research, Suppl. 1). Similarly, it is clear from the "spike" in resistance at the start of the 1986 growing season that the lack of stubble cultivation (with consequent mortality of resistant pupae) during the winter of 1986 (the year of record low cotton prices) also accelerated the decline of pyrethroids (Forrester, et al. 1993), probably by about 3 years.

The good news is that it is not too late to learn from history and apply these lessons to making transgenics work for much longer than pyrethroids. Three important features are: (1) refuges, ie., host plants without Bt toxins, (2) destruction of pupae under transgenic cotton, and (3) adopting and maintaining a resistance management program from the very first use of the transgenic crops.

### **Genetics of Resistance and Selection**

To better illustrate the importance of refuges, it is necessary to discuss a little genetics. The genetics of resistance to Bt toxins is somewhat like that for eye colour in humans. Each of us has two genes for eye colour; if the two genes are the same, we are homozygotes; if they are different, we are heterozygotes. My mother has blue eyes and since blue eyes is a recessive trait, she is a "blue-blue homozygote". My father and I have brown eyes, which means that I must be a "blue-brown" heterozygote. Further, my father's eyes are very brown, suggesting that he is a "brown-brown" homozygote. In the cases studied so far, resistance to Bt, like blue eyes, tends to be recessive. However, like the blue trying to show through my moderately brown eyes, Bt resistance ("R") is not completely recessive, and indeed some heterozygotes (RS) can survive concentrations of Bt toxin that will kill all susceptible (SS) homozygotes.

When the resistance gene is rare, resistant homozygotes (with two R genes) are much more rare (probably by thousands of times) than heterozygotes (only one R gene). Therefore, it is the RS heterozygotes that are most important to selection for resistance. If the concentration of Bt is high enough to kill all of them, resistance will be a long time coming, but if the concentration in transgenic plants kills less than 90% of the heterozygotes, resistance could appear in as few

as 10 generations of exposure (3-4 years) if the percentage of the population developing in the refuges is small (Figure 1). Refuges are essential to provide Bt-susceptible (SS) insects that can mate with and dilute resistance in bollworms developing on transgenic hosts. Refuges have already proven effective with Bt-resistant diamondback moths in glasshouse trials.

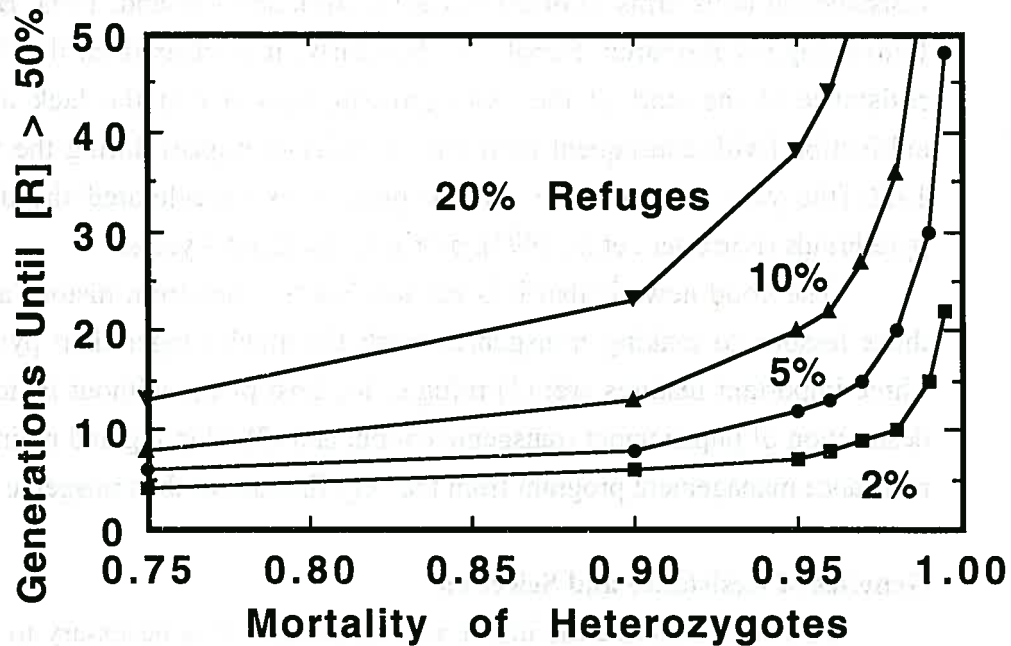


Figure 1. Effect of mortality of RS heterozygotes and percentage of the population escaping exposure ("refuges") on the evolution of resistance.

#### The need for more and larger refuge crops

As noted by Fitt (this proceedings), the current cotton varieties do not have a very high level of expression relative to Australian bollworm species; some bollworms even develop on the transgenic plants. *Helicoverpa armigera* is roughly 10-fold less sensitive to Bt than the principal American pest, *Heliothis virescens*, none of which have been observed to successfully develop on the current transgenic cottons. Thus, even if INGARD plants kill more than 95% of resistant heterozygotes of *Heliothis virescens* (and thereby justify a refuge of unsprayed cotton of as little as 4% in the USA), it is extremely unlikely that the transgenic plants are anywhere near as effective against *armigera*. On the basis of comparisons to the diamondback moth, it seems likely that mortality of *armigera* heterozygotes on the current cotton will be the range of 50-90%. Thus, refuges are even more critical in Australia than in America.

Further, the refuges of non-transgenic hosts already available in Australian cotton growing regions are not adequate. As illustrated with data collected by Gary Fitt and co-workers for the Namoi (Figure 2), alternate hosts for bollworms are currently especially thin in March, so this will be an important gap to fill in the refuge system. The destruction of pupae under transgenic crops will also enhance the effect of the March refuge. Fitt's experiments suggest that 75-90% mortality of pupae is feasible without significant damage to soil structure, which will have the effect of multiplying the late season refuge by 4-10 fold.

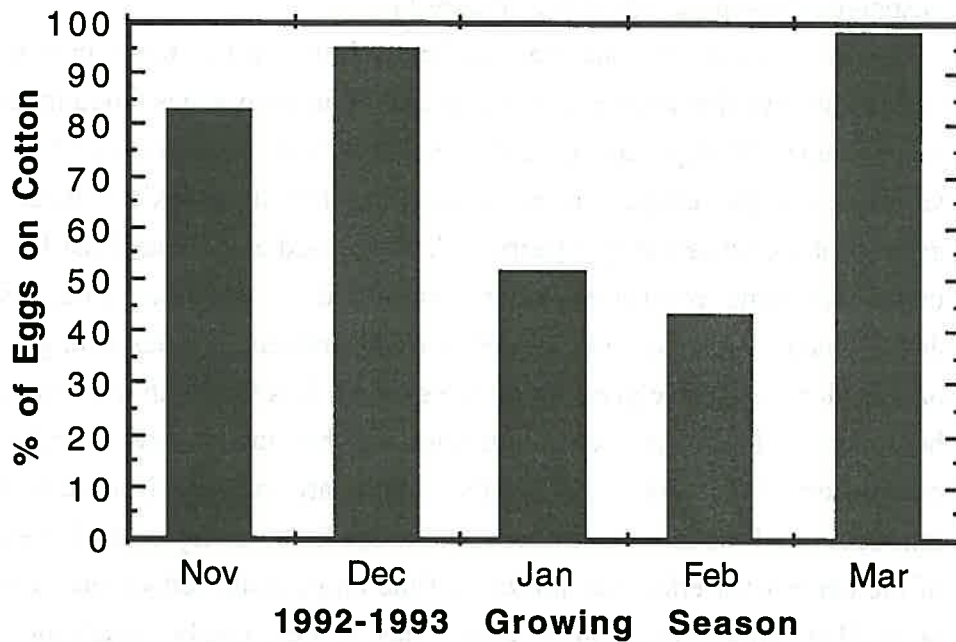


Figure 2. Percentage of *armigera* eggs on cotton compared to all crops. Data courtesy of Dr Gary Fitt.

As discussed by Fitt and Tann (these proceedings), cotton and especially pesticide-treated cotton are not the only choices for refuges, and are probably too costly to be the best choices. Pesticide-treated cotton is an especially poor choice because it will tend to use up both the pesticides and transgenics at the same time. The worst possible scenario for the use of multiple toxicants, such as transgenic plants and insecticides, is in such a mosaic pattern of one toxin or the other. So few insects are generated in either habitat that neither serves as a very effective refuge for the other. It is as if you split the population in half and selected for resistance to both toxicants at the same time. A far better approach is to use just one of the toxicants at a time, because you can always go back to the other if resistance evolves. For this reason alone, it is far too premature to shelve

resistance management strategies for chemical insecticides, and extremely important to develop alternatives to sprayed cotton as the refuge host.

### **Safeguarding the Future**

The current transgenic cottons do not produce enough Bt toxin to be conducive to resistance management. Under these circumstances, the only reliable way to manage resistance is with large refuges. At least 10% and preferably 20% of all bollworm eggs should be laid on hosts other than transgenic cotton and which are at least as favourable for bollworm development as unsprayed non-transgenic cotton (Figure 1).

In the long term, the most feasible solution to this problem is the use of cotton varieties that express more than one toxin (two genes "pyramided" in the same plant). Refuges are essential to dilute resistance even with pyramided varieties, but the refuges can be smaller than for single toxin cultivars. Toxin genes that would last only 5 years each when used alone could last for 31 years under the same conditions when pyramided. Pyramided lines are under development. However, the durability of pyramided varieties strongly depends on very low resistance gene frequencies (much less than 1 in a thousand) in the bollworms. The resistance frequencies for the current CryIA toxin will not remain low if the current INGARD cultivars are extensively used prior to the introduction of the more permanent pyramided lines. Every year of extensive use of the current varieties (say 80-90% of the crop) could reduce the durability of pyramided varieties by about 5 years. Thus, it is extremely critical for the future of the cotton industry that current single-toxin cultivars be used with restraint.

Fortunately, there are two new insecticide groups on the horizon, pyrroles and spinosads, that could be used to buy time until the pyramid breeding work is complete. In contrast to Bt toxins, these insecticides have no multiplier benefit. Like pyrethroids, they can be managed to last for some years, but clearly do not have the potential for a sustainable, environmentally-friendly industry. We might just as well use them now as later. The same cannot be said for the CryIA toxin, which would be best used later with a partner toxin in pyramided cultivars.

As was the case with pyrethroids, resistance usually appears rather suddenly, because selection can move quickly from undetectable frequencies (less than 1%) to control failures. Thus, it is essential this time around to adopt and stick to stringent resistance management plans prior to any hints of resistance. Resistance management truly will be the key to a long and profitable transgenic era.