GENETICS OF BT RESISTANCE

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Overview

Resistance is an ongoing concern with the management of *Helicoverpa armigera* in the Australian cotton industry. In response, resistance management strategies (RMS) are in place to either prevent, or retard further development of, resistance to either chemical insecticides or to the Cry1Ac protein in transgenic plants. While these strategies have been successful at slowing down the rate at which resistance has developed to insecticides, they have neither prevented the ultimate spread of resistance to most field populations nor the evolution of new mechanisms of resistance that make resistance increasingly difficult to manage.

RMS are built on knowledge of the genetics of resistance and ecology of insects. Where knowledge is lacking we make assumptions about resistance based on general theories of how new genes evolve in populations. Our work with Bt resistance aims to test these assumptions so that we can refine or validate our knowledge and theories. In this way, we can devise strategies for the cotton industry that work, and do so at minimal cost to the grower.

Dr Ray Akhurst and coworkers (these proceedings) have selected a strain of *H. armigera* that is resistant to the Cry1Ac toxin. Larvae from this strain can feed and survive on Bt plants. We are testing the assumption that Bt resistance genes will be recessive. While our preliminary data suggest that the resistance gene is not recessive, we conclude that the current RMS for Bt cotton is resilient and the R gene should not be a major threat while the planted area remains restricted.

Genetics and Resistance

The RMS strategies for Bt cotton and insecticides have much in common. Both stress the need for 'pupae busting' and for alternative methods of pest control. In contrast, insecticide strategies limit exposure to the insecticide by alternating or rotating chemical groups so that each generation of *H. armigera* is exposed to a different toxin. This is not possible with Bt cotton where the toxin (Cry1Ac) is present all season long. So in this situation, selection pressure for resistance is reduced by allowing part of the population each generation to escape exposure to the toxin. This occurs in two ways: refuges of non-Bt cotton must be grown and also the total area of Bt cotton has been limited to below 30% of total hectares of cotton sown.

Two assumptions built into the Bt cotton refuge strategy are that (1) Bt resistance genes are rare in *H. armigera*, and (2) Bt-resistance genes [R] are recessive, i.e. individuals carrying only one copy of the gene (RS) would be reasonably susceptible to Cry1Ac, compared with fully resistant individuals (RR). Refuges of non-Bt cotton provide a source of susceptible individuals (SS) that dilute any resistant populations (this keeps the R genes rare) and the susceptible individuals (SS) mate with the resistant (RS) individuals (this keeps the RR individuals rare) (Roush et al., 1998).

Testing our Assumptions

Dr Akhurst's resistant strain was isolated from field populations. While it is not possible to determine accurately the frequency of the R gene in field populations of *H. armigera*, Akhurst and coworkers (these proceedings) estimate it to be present in approximately 1/1000 individuals. This is about three-fold more common than is normally assumed for resistance genes in field populations (1 in million or less). At such frequency (1/1000) it would not take long for resistance to evolve to detectable levels in field populations.

Whether resistance leads to problems with pest control under practical circumstances depends in part on the relative dominance of the R gene. When genes are at low frequency most individuals carrying the R gene will be heterozygous, RS. Thus, it is critical to know if, and when, RS individuals have greater survival on Bt cotton plants than SS individuals. This assumes that the resistance factor in the resistant strain is caused by changes to a single gene. While this may not be strictly true, genetics theory tells us that this is a reasonable assumption.

We crossed the resistant strain (assumed to be RR) with a laboratory susceptible strain (SS) to produce heterozygous offspring (RS). These three strains (RR, SS and RS) were bioassayed with Cry1Ac to determine their mortality at different concentrations of toxin. The ratio of the dose that kills 50% of individuals of the RR or RS strain compared with the SS strain is a convenient measure of the degree of resistance expressed by the strains. One complication is that the apparent toxicity of Cry1Ac (and indeed of insecticides) varies with the technique used in the bioassays. We used two methods and the resistance ratios were:

Method	Genotype	Resistance Factors
Diet incorporation	RS	10 to 17 fold
	RR	200 fold
Diet overlay	RS	10 to 30 fold
	RR	70 to 100 fold

Thus, the RS individuals are intermediate between the susceptible and fully resistant individuals, not recessive as had been assumed.

Survival of RS Individuals on Plants

Tests of dominance or recessiveness in laboratory studies can be misleading because the relative dominance of an R gene in the field can depend on the concentration of the toxin to which the insect is exposed. It can also change with the size of the larvae. We know that cotton plants have higher levels of Bt toxin before squaring. After this time, the toxin levels starts to decline so that by late season many susceptible larvae can also survive and grow on plants (Fitt et al. 1998). Larger larvae are more tolerant of Bt toxin than are smaller ones. Thus, we can predict that RS individuals may survive on plants at some stages of plant growth or at some larval instars although at present we cannot define these parameters in more detail under field conditions. Akhurst and coworkers (these proceedings) observed RR individuals could feed and grow on Bt resistant plants in the laboratory, although their growth was slower that SS individuals.

We have done some preliminary trials with neonate larvae of different genotypes and placed them on conventional and Bt cotton plants grown under controlled. The plants were pre-squaring. Two hundred individuals of a given genotype were placed on a group of 6 plants. Not all larvae could be recovered so the results are expressed, as a percentage of larvae surviving after six days compared with number of SS larvae placed on conventional plants. Two replicates were available for the RS individuals.

Genotype of larvae	**Plant Type**	**% Survived**	**Av wgt survivors (g) after 6 days**
SS	Conventional	100	2.1
	Bt	25	0.6
RS	Conventional	85,100	3.1, 3.4-
	Bt	61, 68	1.2, 1.1
RR	Conventional	78	1.6
	Bt	83	0.75

At the concentration present in the plants, the SS individuals had low survival and most individuals were small. Both RS and RR individuals had high survival and the RS larvae grew appreciably more than the RR individuals. These preliminary data suggest that at the critical time that plant expression starts to drop RS individuals have a fitness advantage over SS individuals. At this time the gene is functionally dominant in field populations.

Impact on Resistance Management for Bt Cotton

The combined results from Akhurst and coworkers and from our genetic studies indicate that two key assumptions underlying the RMS for Bt cotton do not hold. The R gene appears not be as rare as first thought, and secondly the R gene is not always recessive and probably is functionally dominant under field conditions at certain times.

While at first this may seem to be a threat to the RMS, the strategy is actually robust enough to 'manage' a dominant gene. Elements of the strategy, such as pupae busting, and the use of new chemical insecticide groups as part of pest management of heliothis, are both as effective against dominant as well as recessive Bt genes. In addition, the conservative approach to limiting the area sown to Bt cotton has also been valuable and needs to be maintained until 'two gene' Bt cottons are available. The remaining areas of conventional cotton, and other hosts of *Helicoverpa* outside those dictated by the refuge strategy, act as additional refuges of susceptible individuals. The cautious approach of the industry to the implementation of Bt cotton has bought the industry time.

References

Fitt, G. P., J. C. Daly, C. L. Mares. and K. Olsen. 1998. Changing efficacy of transgenic Bt Cotton – patterns and consequences, pp189-196, M. P. Zalucki, R. A. I. Drew and G. G. White [eds.], Pest Management – Future Consequences, Vol 1, University of Queensland Printery, Brisbane, Australia.

Holt, H. E. 1998. Season-long monitoring of transgenic cotton plants - development of an assay for the quantification of Bacillus thuringiensis insecticidal protein, pp. 331-335. In Proceedings, 9th Australian Cotton Conference, 12 - 14 August 1998, Broadbeach, Queensland. Australian Cotton Growers' Research Association, Wee Waa, Australia.

Roush, R.T., G.P. Fitt, N.W. Forrester and J.C. Daly. 1998. Resistance management for insecticidal transgenic crops: theory and practice, pp 247-257. In Pest Management – Future Challenges (Eds) M.L. Zalucki, R.A.I. Drew and G.G. White, University of Qld, Brisbane.