

## Host Plant Effects Associated with Cry1A Resistance in *Helicoverpa armigera*

Lisa Bird<sup>1,2</sup>, and Ray Akhurst<sup>1\*</sup>

<sup>1</sup>CSIRO Entomology, GPO Box 1700, Canberra ACT, Australia, 2601

<sup>2</sup>Department of Botany and Zoology, Australian National University, Canberra ACT, Australia, 2601

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Transgenic cotton, producing Cry1Ac at two stages with significantly different levels of expression, and sorghum and pigeon pea, alternatives to conventional cotton for the mandated refuges, were used in these experiments. Although fitness costs in a Cry1A-resistant strain of *H. armigera* were evident for each of the plant species tested, there was some variation in the characteristics of the fitness costs. The fitness cost was recessive in all but the young, high expression cotton. Resistance was more dominant in older cotton and was also incomplete. The dominance and incompleteness of Cry1A resistance in older cotton together with the recessive nature of fitness costs indicate that the risk of increasing the frequency of resistance alleles rises through the season.

### Introduction

The high dose/refuge strategy has been identified as the best option for resistance management in transgenic cotton (*Bt* cotton) expressing toxins from *Bacillus thuringiensis* (*Bt*) (2). The aim of this management approach is to kill all, or as many as possible, of the heterozygotes to minimize the probability of heterozygotes mating together to generate resistant homozygotes. However, the high dose component of the strategy is not applicable for *Helicoverpa armigera* (Lepidoptera: Noctuidae) on *Bt* cotton. *H. armigera* is much less susceptible to the Cry toxins than *Heliothis virescens* (Lepidoptera: Noctuidae) (3) and the seasonal decline in Cry1Ac expression in INGARD™, the Australian counterpart of BOLLGARD™, allows the survival of susceptible insects in the latter part of the growing season (4). If a proportion of the population bearing no resistance alleles is able to survive on *Bt* cotton, then larvae bearing one resistance allele will also survive on *Bt* cotton and selection for resistance would be enhanced through survival of heterozygotes.

Resistance management for *H. armigera* in *Bt* cotton in Australia is based on the refuge strategy. Refuge theory makes three major assumptions: that the initial frequency of resistance alleles is low; that sufficient mating occurs between adults emerging from the transgenic fields and the refuges, and that the toxin concentration is high enough during the period of plant development during which larvae are actively feeding to make resistance functionally recessive. However,

despite the apparent violations of these assumptions in some cases (e.g. pink bollworm in Arizona (5, 6)), an increase in the frequency of resistance to *Bt* toxins in field populations caused by exposure to commercially grown *Bt* crops has yet to be documented. There is some evidence that this may be due to variable fitness which can contribute to delaying or even reversing the evolution of resistance.

The aim of our study was to evaluate host plant effects associated with resistance to Cry1Ac and assess their impact on the expected benefits of the refuge strategy as it relates to *H. armigera*.

### Materials and methods

Although we used a laboratory selected strain in our glasshouse experiments, we have evidence that the allele conferring Cry1A resistance in this strain is also present in the field (Akhurst and Bird, unpublished data). We conducted a series of 4 backcrosses and re-selections to reduce the background variability between the susceptible and resistant strains (1). We assessed fitness on high and low Cry1Ac expressing cotton and on two alternative refuge species by placing a single first instar larva of *H. armigera* on each caged plant, allowing the larvae to feed on all plant tissues. The alternative refuge species, sorghum and pigeon pea, were tested at flowering, with flowering cotton as the control.

\* Corresponding author. Mailing address: CSIRO Entomology, GPO Box 1700, Canberra, ACT, Australia 2601. Phone: 61 2 62464123. Fax: 61 2 6246 4202. E-mail: ray.akhurst@csiro.au.

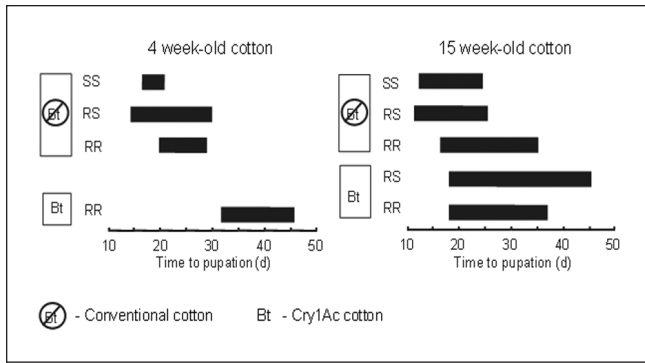


FIG. 1. Development times of Cry1A-resistant, -susceptible and heterozygous *H. armigera* feeding on conventional and Cry1Ac cotton.

## Results and discussion

There was no survival of susceptible (SS) or heterozygous (RS) *H. armigera* on Cry1Ac cotton plants 4 weeks after germination (range of Cry1Ac content = 3.2-4.1 ppm); approximately 30% of homozygous resistant (RR) insects survived to pupation. However, at 15 weeks (Cry1Ac content = 1.4 ppm) both homozygous resistant and heterozygous individuals survived to pupation. These data suggest that Cry1A resistance in *H. armigera*, although functionally recessive in younger plants, becomes partially dominant as the plants age.

Fitness costs were evident both in terms of survival on conventional plants (Table 1) and of development time (Fig. 1). The fitness cost as assessed by survival was partially dominant on young cotton, becoming increasingly recessive as cotton aged. It was appreciable on sorghum, but less evident on pigeon pea, and was recessive on both. Resistant *H. armigera* developed more slowly on young cotton, with 90% of the susceptible insects pupating before even 10% of the resistant insects pupated. However, there was a much greater overlap of development time at week 15 on conventional cotton. No statistically significant fitness cost, as shown by fertility, was evident among the insects feeding on conventional cotton.

TABLE 1. Survival of *H. armigera* from first instar to pupation on conventional plants.

Host plant	% survival to adult		
	Susceptible	Heterozygote	Resistant
4 week-old cotton	93 <sup>a</sup>	56 <sup>b</sup>	35 <sup>c</sup>
10 week-old cotton	88 <sup>d</sup>	78 <sup>d</sup>	57 <sup>e</sup>
15 week-old cotton	95 <sup>f</sup>	94 <sup>f</sup>	78 <sup>g</sup>
Sorghum	87 <sup>h</sup>	87 <sup>h</sup>	56 <sup>i</sup>
Pigeon pea	93 <sup>j</sup>	99 <sup>j</sup>	79 <sup>k</sup>

The superscript letters across the row indicate statistical significance ( $P < 0.05$ ) for insects on the same plant type.

Variable fitness costs in Cry1A-resistant *H. armigera* can impact on the refuge strategy. The fitness cost is recessive, or at best partially recessive, because the RS genotype is not at a significant disadvantage compared with the SS genotype in the absence of toxin, particularly on older conventional cotton (Table 1, Fig. 1). The lower fitness of the RR compared with the SS genotype would induce only a weak selection differential for decline in resistance in the refuge habitat.

There is some potential for early season temporal separation of RR genotypes because they develop much more slowly on early season Cry1Ac cotton (Fig. 1). However, the more critical issue is the synchrony between the RS and the SS in refuges, because the RR genotype is expected to be extremely rare, particularly when transgenic crops are first commercially released. Since the developmental fitness cost is recessive, temporal separation of the RS and SS genotypes is unlikely to contribute to increasing resistance frequency. Our data also indicate greater overlap of emergence of the pest from refuge and transgenic crops in the latter part of the season. This combined with merging field cohorts would create a continuum of emergence from the refuge that should ensure an abundance of susceptible genotypes at any given period when toxin expression was declining.

Resistance is deemed to be complete only if the treatment has no effect on the resistant insects (4). Incomplete resistance militates against the development of resistance. Our data showed that the contribution to resistance management conferred by the presence of incomplete resistance on young Cry1Ac cotton is removed as the level of toxin expression declines, because the RR genotype is as fit on mature *Bt* cotton as it is on conventional cotton (Table 2).

TABLE 2. Dominance status of Cry1A resistance in *H. armigera* on 4 and 15 week-old cotton.

Host plant	%survival to adult	
	4 week	15 week
Conventional cotton	35 <sup>a</sup>	72 <sup>c</sup>
INGARD cotton	15 <sup>b</sup>	62 <sup>c</sup>

The superscript letters in each column indicate statistical significance ( $P < 0.05$ ) for insects on plants of the same age.

## Conclusion

The factor that contributes most significantly to the evolution of resistance is an increase in the survival of heterozygotes relative to homozygous susceptible individuals. The observed increase in the level of dominance of resistance on crops with declining expression means an increase in the heritability of resistance because of the strong selection differential in favour of the RS genotype. This represents the greatest danger in terms of increasing Cry1A resistance allele frequency and highlights the need for large, well planned refuges.

With the introduction of two-gene cotton in the form of Bollgard II™ the efficacy of Cry1A should, in theory, be protected. However, the unexpectedly high frequency of a Cry2Ab resistance allele in Australian populations of *H. armigera* (R. Mahon, pers. comm.) indicates that efforts to contain resistance to *Bt* cotton in *H. armigera* must be maintained and this might not be an easy task.

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