

GENETIC MODELS TO ASSESS THE DEVELOPMENT OF COUNTER-RESISTANCE IN INSECT PESTS EXPOSED TO *Bt*-SUGARCANE

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Abstract

The use of transgenic sugarcane expressing insecticidal toxins is one potential method of reducing the damage caused by eldana in the South African sugar industry. This approach carries the risk that the pest may quickly develop resistance to the toxin, rendering transgenic sugarcane ineffective in controlling eldana. Insect resistance to the *Bacillus thuringiensis* (*Bt*) toxin is usually controlled using the so-called high-dose/refuge, or HDR strategy. In essence, this approach is based on the use of transgenic lines expressing high levels of the toxin, combined with planting refuges of non-transgenic plants to maintain a population of insects in which selection pressure for counter-resistance is absent. The speed at which insect populations develop resistance is dependent on factors such as the frequency of resistance genes in the initial population exposed to the toxin, the mode of gene action and the size of the non-transgenic refuges. A genetic model has been developed to examine the rate of development of resistance under different conditions. Results suggest that the current permit requirement for the planting of refuges may be inadequate, and that refuges comprising 50% of the planted area may be required to effectively delay the evolution of counter-resistance in insects exposed to *Bt*-crops.

Keywords: counter resistance, transgenic sugarcane, genetic model, *Bacillus thuringiensis*, refuge size

Introduction

The borer insect *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is the major pest of sugarcane in South Africa, but no published estimates of yield loss due to eldana are available. If it is assumed that 60% of the crop is affected by eldana causing a 5% loss of sucrose yield in affected cane, a direct loss in recoverable value to growers of R97.4 million would have occurred in the 2001/2002 milling season (industry sugar yield = 2.4 million tons, RV = R1352.14/ton (Anon, 2002)). On an industry basis of direct income totalling R5 billion (Anon, 2002), similar assumptions would reflect a loss of R150 million due to eldana.

Varietal resistance against the eldana borer is an effective way of minimising the damage caused by the insect, and breeding for resistance is one of the main goals of the SASEX plant breeding programme. Plant resistance to eldana is often associated with undesirable phenotypic traits - such as low sucrose yield or high fibre content - and the current selection of high yielding resistant varieties available to growers is limited. Developing transgenic sugarcane varieties expressing anti-insect toxins such as the *Bacillus thuringiensis* (*Bt*) toxin, is an alternative strategy for obtaining resistant varieties. This approach has been used successfully with crops such as maize, soybean and cotton, and in 2001 there were approximately 12 million ha of transgenic crops containing the *Bt* gene grown worldwide. (James, 2001).

A concern regarding the use of single gene resistance mechanisms, such as *Bt*, is that insect pests may rapidly develop counter-resistance to the transgene through natural selection. Various management strategies have been suggested to delay the evolution of counter-resistance by insects, and the current method of choice is the so-called *high-dose/refuge* (HDR) strategy. The HDR strategy is based on the assumptions that (a) resistance to the transgene is caused by recessive alleles carried within the insect population, (b) the dose of the transgenic toxin is high enough to kill the vast majority of insects heterozygous for the resistance allele, and (c), that planting a refuge of non-transgenic host plants will allow sufficient susceptible insects to survive and mate with resistant insects, so that the frequency of the resistance alleles does not increase in the population. Population genetic models using these assumptions have been developed to estimate the size of the refuge required to delay the evolution of counter-resistance (e.g. Arpaia *et al*, 1998, Rausher, 2001, Shelton *et al*, 2000), and results from these models have been used to make recommendations regarding the planting of transgenic crops and refuges. In South Africa, permits for commercial planting of *Bt* maize require that a 5% unsprayed refuge, or a 20% sprayed refuge, is established (M Vosges, personal communication¹). If transgenic *Bt* sugarcane is ever commercialised in South Africa, policy on refuge size and management of field plantings will need to be introduced, and genetic models of insect counter-resistance can be used to formulate the resistance management strategy.

Evidence from laboratory studies and field experiments suggest that some of the assumptions implicit in the current genetic models of counter-resistance may not apply in practice. Reports that fewer than 90% of cotton bollworm (*Helicoverpa zea*) and European corn borer (*Ostrinia nubilalis*) larvae are killed by commercial *Bt* crops (Gould *et al*, 1997), and that 3 to 5% of *Bt* maize stems in a trial in South Africa contained live diapause larvae of the African stalk borer, *Busseola fusca* (van Rensburg, 2001), suggest that significant numbers of heterozygous insects survive the toxin dosage present in these transgenic varieties. Gould *et al* (1992) suggest that at high toxin concentrations, resistance of tobacco budworm (*Heliothis virescens*) to *Bt* toxin is inherited as an additive trait, rather than a recessive one. Studies of Mediterranean corn borer (*Sesamia nonagrioides*) in Spain have shown considerable variation - up to 800% - in LC₉₀ levels (lethal concentration needed to kill 90% of individuals) between insect populations collected in different locations (González-Núñez *et al.*, 2000). These populations showed similar LC₅₀ levels, suggesting that the mode of resistance gene action may differ between populations. A similar discrepancy between LC₉₀ and LC₅₀ levels was also found between populations of corn earworm (*Helicoverpa zea*) in the USA by Siegfried *et al.*, 2000.

As model assumptions and input parameters will dictate the outcome of a simulation experiment, it is crucial that these are correctly specified. The aim of this study is to develop an improved genetic model of the evolution of counter-resistance to toxins in insect pests, taking various modes of resistance gene action into account. The model can then be used to develop refuge management strategies to prevent the evolution of counter-resistance to *Bt*-sugarcane in *Eldana saccharina*.

Materials and Methods

The equation for change in gene frequency described by Rausher (2001) was used as the basis of the model.

$$p_{x+1} = p_x(p_x W_{AA} + q_x W_{Aa}) / (p_x^2 W_{AA} + 2p_x q_x W_{Aa} + q_x^2 W_{aa}) \quad (1)$$

where p_x is the frequency of the wild-type allele *A* conferring susceptibility in generation x , $q_x (= 1 - p_x)$ is the frequency of the recessive allele *a* conferring resistance, and W_{ij} is the fitness of genotype ij .

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The fitness of each genotype is then derived (Appendix 1) as:

$$\begin{aligned} W_{AA} &= \beta; \\ W_{Aa} &= (1-\beta)(h-hc) + \beta(1-hc); \\ W_{aa} &= 1-c; \end{aligned}$$

where β = the proportion of land planted to non-transgenic refuge; h = level of dominance of the heterozygote (0 = completely recessive, 1 = dominant); and c = the fitness cost of counter-resistance. This differs from Rausher (2001), by the inclusion of a term in W_{Aa} to account for instances where the heterozygote is not completely susceptible (i.e. when $h > 0$).

The proportion of the population showing counter-resistance (CR) in any given generation is then:

$$CR_x = (1-p_x)^2 + 2p_x(1-p_x)h \quad (2)$$

A second modification to the model used by Rausher is to allow for a change in the fitness cost over time. If fitness costs are substantial, selection pressure will exist in favour of individuals with reduced cost, suggesting that c could change over time. In the present model, fitness cost is regarded as a quantitative trait, which responds to selection according to the equation $R = h^2S$ (Falconer, 1981), where R is the selection response, h^2 is the trait heritability, and S is the selection differential. The change in cost over time can then be given as:

$$c_{x+1} = c_x(1-R) \quad (3)$$

By appropriate substitution, the change in allele frequency of the gene causing counter-resistance can be calculated under conditions of varying initial gene frequency, mode of gene action, fitness cost and refuge size. A simulation programme was written in Microsoft-Excel[®], and the number of generations required until 50% of the insect population showed counter-resistance (G_{50}) was calculated for a range of scenarios. It was assumed that eldana has four generations per year (G Leslie, personal communication²), and the number of years until counter-resistance develops was calculated (Y_{50}). In all cases, it was assumed that the refuge was a separate planting of non-transgenic material as opposed to a mixed planting of transgenic and non-transgenic plants, and that the refuge was not sprayed with pesticide.

Results

Effect of refuge size - β

Initially, simulation to test the effect of refuge size, β , was done using the assumption that the resistance allele was completely recessive ($h=0$), that the initial gene frequency of the resistance allele was low ($q = 0.001$), and that the cost of counter resistance was significant ($c = 0.4$). The number of generations (G_{50}) and number of years (Y_{50}) for 50% of the insect population to develop resistance is shown in Table 1. It can be seen that when the resistance gene is completely recessive, even a small refuge can significantly delay the development of resistance in the population. For the case when there is no refuge, although the population will become resistant very quickly, it must be noted that the population size will be small. This means that there will be a lag phase before the population size increases sufficiently to cause noticeable damage to the crop. It would be possible to model the length of this lag period using parameters of insect biology and reproduction, but this will not be considered here.

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Table 1. The effect of refuge size, β , on the evolution of counter-resistance.
 G_{50} (Y_{50}) = number of generations (years) until 50% of the population is resistant.

β	G_{50}	Y_{50}
0	1	0.3
0.01	22	5.5
0.05	98	24.5
0.10	208	52.0

Dominance - $h = 0$;
 Allele frequency - $q = 0.001$;
 Fitness cost - $c = 0.4$.

Table 2. The interaction between level of dominance, h , and refuge size, β , on the evolution of counter-resistance.

h	β	G_{50}	Y_{50}
0	0.05	98	19.5
0.01	0.05	28	5.8
0.10	0.05	9	2.0
0.50	0.05	5	1.0
0.10	0.39	98	24.5
0.50	0.52	94	23.5

Allele frequency - $q = 0.001$;
 Fitness cost - $c = 0.4$.

Effect of dominance level - h

Simulations to test the effect of level of dominance were done using the assumption that a 5% refuge was planted (current requirement), that the initial gene frequency of the resistant allele was low ($q = 0.001$), and that the cost of counter-resistance was significant ($c = 0.4$). The number of generations and number of years for the development of counter resistance at different levels of dominance (h) is shown in Table 2. If only 10 percent of individuals heterozygous for the resistance allele survive ($h = 0.1$), the population will become resistant in nine generations. Thus small changes in the level of dominance result in large changes in the speed at which resistance can develop. If the resistance gene shows an additive mode of action ($h = 0.5$), the population will take only five generations to become resistant with a 5% refuge. This means that refuge sizes need to be substantially increased when the assumption of complete recessivity of the resistance allele is invalid. For a dominance level of $h = 0.10$, a refuge of 39% would be required to give a similar level of counter-resistance delay to that of a 5% refuge when the allele is completely recessive. When resistance shows additive gene action ($h = 0.5$), a 52% refuge would be required (assuming $q = 0.001$; $c = 0.4$).

Effect of initial gene frequency - q

The initial frequency of the resistance allele determines the proportion of the insect population that survives on the transgenic crop. The effect of initial gene frequency was simulated by assuming that the allele is not completely recessive ($h = 0.1$), and that the fitness cost of counter-resistance is significant ($c = 0.4$). The gene frequency used was $q = 0.001$ and $q = 0.01$. This corresponds to 2 out of 10 000 and 2 out of 1 000 individuals resistant to the transgenic crop respectively. Results in Table 3 show that by increasing the initial gene frequency tenfold reduces the period of population susceptibility by about half. Under these conditions, an increase in refuge size from 39% to 46% would double the 'lifespan' of the transgenic crop.

Table 3. The interaction between resistance allele frequency, q , and refuge size, β , on the evolution of counter-resistance.

q	β	G_{50}	Y_{50}
0.001	0.39	98	24.5
0.01	0.39	55	13.8
0.01	0.46	95	23.8

Dominance - $h = 0.1$;
 Fitness cost - $c = 0.4$.

Table 4. The interaction between refuge size, β , and fitness cost, c , on the evolution of counter-resistance.

β	c	G_{50}	Y_{50}
0.39	0.4	98	24.5
0.39	0.2	53	13.3
0.39	0.1	43	10.8
0.52	0.2	98	24.5
0.58	0.1	96	24.0

Dominance - $h = 0.1$;
 Allele frequency - $q = 0.001$

Effect of cost of counter-resistance - c

The cost of counter-resistance was simulated assuming an initial gene frequency of 0.001, a level of dominance of 0.1, and a refuge size of 39%. Results of decreasing the costs of counter-resistance are shown in Table 4. Reducing fitness costs by 50% or 75% respectively would require an increase in refuge size from 39% to 52% or 58% to give a similar level of counter-resistance delay.

It can be shown that if the cost of resistance is constant, then a refuge size of $1-c$ would prevent the development of counter-resistance, irrespective of the level of dominance (see Appendix 2). If fitness costs are high, refuges are then highly effective in delaying evolution of counter resistance. Thus for example, if the cost of resistance was 0.6, a refuge of 40% would completely prevent the development of counter-resistance. This, however, no longer holds if selection pressure results in a response to selection for fitness cost (see Materials and Methods). For a population with $q = 0.001$, $c = 0.6$, a refuge size of 40% and a 0.1% selection response per generation ($R = 0.001$), resistance would develop in 275 generations, or in 99 generations if the selection response was 1% per generation ($R = 0.01$. Data not shown).

Average- and high-risk scenarios

In the absence of data for the model parameters for eldana, estimates representing average-risk and high-risk scenarios were assumed from data published for other species (see Discussion below). Refuge size required to delay the development of counter-resistance for 25 years was calculated for each scenario (Table 5). For the high-risk case, 75% of the crop area would need to be planted to non-transgenic refuge, and 50% for the average-risk case. This is considerably more than the 5% required by legislation.

Table 5. Refuge size, β , required under average- and high- risk assumptions for the delay of counter-resistance evolution.

Scenario	q	h	c	β	G_{50}	Y_{50}
'average-risk'	0.002	0.12	0.30	0.50	100	25.0
'high-risk'	0.005	0.20	0.08	0.75	99	24.8

q = allele frequency;
 h = level of dominance;
 c = fitness cost.

Discussion

The above results show that the rate at which counter-resistance evolves within populations exposed to toxins is highly sensitive to the assumptions made about the genetic control of resistance. In particular a small departure in mode of gene action from complete recessivity results in a rapid build-up of resistance, unless refuge size is large. (Table 2). For the range of variables used, the effects of fitness costs have a larger impact on counter-resistance evolution than the effect of initial gene frequency (Tables 3 and 4), but this difference is relatively small. All three variables, *viz.* gene frequency, level of dominance and fitness cost are important in determining the size of the refuge needed to appreciably delay the development of resistance.

At present, no estimates for these parameters are available for eldana in response to the *Bt* toxin. Estimates for *Bt* resistance alleles frequency in other insects vary from 0.0015 in cotton bollworm (Gould *et al.*, 1997) to 0.12 in diamondback moth (Tabashnik *et al.*, 1997). The allele frequencies used in the above simulations (0.001 and 0.01), thus represent the lower end of the observed variation in other species. Similarly, estimates of the cost of counter-resistance vary from negligible (Tabashnik *et al.*, 1997), to 0.41 (Tabashnik *et al.*, 1994) in diamondback moth. No estimates for level of dominance have been published, but reports of field survival of insects exposed to

transgenic crops (Gould *et al*, 1997, van Rensburg, 2001) suggest that resistance is not completely recessive, and Gould *et al* (1992), concludes that resistance may be due to additive gene action ($h \approx 0.5$) at high toxin doses. Irrespective of the actual value for the level of dominance, it appears that the current legislation that requires a 5% unsprayed refuge may be inadequate to delay the build-up of counter-resistance appreciably.

Published estimates of parameters used in the model suggest that the assumptions used in the average-risk and high-risk scenarios (Table 5) are not unrealistic. Although a refuge of 50% non-transgenic crop may seem large, if one assumes that the insect population will be reduced by half, and that the remaining insects will be evenly distributed between the transgenic plantings and the refuge, the amount of damage will be reduced by 75% compared to a completely non-transgenic scenario. Thus planting large refuges gives most of the potential benefit that could be gained from the transgenic crop, and significantly reduces the risk of the insect pest developing counter-resistance. Even when refuge size is 75%, the level of damage in the crop as a whole will be reduced by nearly half while the insect population remains susceptible.

In order to make more precise recommendations on refuge size, it would be of benefit to have estimates of level of dominance, fitness costs of resistance and allele frequency of *Bt* resistance alleles in eldana. Methodologies to estimate these parameters have been developed (e.g. Andow and Alstad, 1998). Until these estimates are available, it should be assumed that a refuge size of 50% would give an adequate delay in counter-resistance evolution in eldana exposed to *Bt*-sugarcane. This is considerably higher than that required by the current permits, but would still lead to a significant reduction in the level of damage caused by eldana. It is in the interests of the South African sugar industry to be responsible users of biotechnology, and should *Bt*-sugarcane ever become commercialised in South Africa, SASA and SASEX should take an active role in promoting appropriate legislation regarding refuge size and management.

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APPENDIX 1 (modified from Rausher, 2001)

Let β be the proportion of land planted to the non-transgenic refuge, and $1-\beta$ be the proportion planted to the transgenic crop. Then the fitness of the three genotypes in refuge, transgenic and total area is:

Genotype	Transgenic	Refuge	Total (W_{ij})
AA (susceptible)	0	1	$(1-\beta)0 + (\beta)1 = \beta$
Aa (heterozygote)	$h-hc$	$1-hc$	$(1-\beta)(h-hc) + \beta(1-hc)$
aa (resistant)	$1-c$	$1-c$	$(1-\beta)(1-c) + \beta(1-c) = 1-c$

APPENDIX 2

If $\beta + c = 1$, then $c = 1 - \beta$,

$$\begin{aligned}
 \text{Substitute into } W_{Aa} &= (1-\beta)(h-hc) + \beta(1-hc) \\
 &= (1-\beta)(h-h(1-\beta)) + \beta(1-h(1-\beta)) \\
 &= h\beta - h\beta^2 + \beta - h\beta + h\beta^2 \\
 &= \beta
 \end{aligned}$$

$$\begin{aligned}
 \text{Also, } W_{aa} &= 1-c \\
 &= 1-(1-\beta) \\
 &= \beta
 \end{aligned}$$

Because $W_{AA} = \beta = W_{Aa} = W_{aa}$,

$$\begin{aligned}
 p_{x+1} &= p_x(p_x W_{AA} + q_x W_{Aa}) / (p_x^2 W_{AA} + 2p_x q_x W_{Aa} + q_x^2 W_{aa}) \\
 &= p_x(p_x + q_x) / (p_x^2 + 2p_x q_x + q_x^2) \\
 &= p_x / (p_x + q_x) \\
 &= p_x
 \end{aligned}$$