

**EVOLUTION OF REGION-WIDE RESISTANCE
IN COTTON BOLLWORM TO BT COTTON
AS INFLUENCED BY BT CORN:
IDENTIFICATION OF KEY FACTORS
THROUGH COMPUTER SIMULATION**

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Abstract

A stochastic, spatially-explicit computer model has been developed to simulate the evolution of resistance in cotton bollworm to Bt cotton in an agroecosystem that includes Bt and non-Bt corn. The model incorporates important aspects of insect biology and behavior, of agronomy and of agricultural operations. The model has been used to examine the effects of altering values for the parameters for which we have insufficient field data or which are inherently variable in nature.

Sensitivity analyses of the proportion of both corn and cotton fields that are planted to Bt, the initial frequency of resistance alleles, the functional dominance of resistance alleles, the fitness of susceptible larvae on Bt plants, the ratio of corn to cotton fields, and the spatial distribution of Bt crop deployment, among others, play a key role in determining the rate of evolution of resistance to Bt crops. Field determination of the mean values for these parameters, and the natural variation in these values, is crucial before predictions can be made of resistance evolution. In reducing risk, insect resistance management plans should pay heed to the consequences of uncertainty revealed by this model.

Introduction

The deployment of transgenic Bt cotton and Bt corn selects for resistance in populations of insects that feed on the crop and are affected by the toxin. The cotton bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) feeds both on cotton and on corn, often utilizing them sequentially. If Bt corn is deployed in the same region as Bt cotton, all 3 to 5 generations of bollworm can be subjected to selection for resistance to Bt. Furthermore, all evidence indicates that the dose of Bt experienced by cotton bollworm in ear-stage corn and in cotton does not meet the

requirements for a high-dose resistance management strategy to be effective for this insect. There is much natural variation among populations of bollworm in susceptibility to Bt (Stone and Sims, 1993), but in general Bt corn and Bt cotton kill around 75% - 95% of larvae, and delay the development of survivors by around 7 days (Mahaffey *et al.*, 1995, Lambert *et al.*, 1996, Horner, University of Maryland, personal communication, Storer, unpublished data). Concerns about resistance in bollworm have led the Environmental Protection Agency to severely limit the planting of Bt corn in cotton-growing counties. It is currently unclear how resistance to Bt transgenic crops may evolve in this insect in situations where the populations can be exposed to Bt corn and Bt cotton. Here we describe a spatially-explicit computer simulation model and use it to examine the role in Bt resistance evolution of important genetic, biological, agronomic and operational factors in a mixed corn/cotton agroecosystem.

Methods

A stochastic computer model has been developed that simulates the region-wide resistance genetics and population dynamics of cotton bollworm in a mixed cotton and corn agroecosystem. The model uses the structure developed by Peck *et al.* (in press) for simulating resistance evolution in the tobacco budworm (*Heliothis virescens*) to Bt cotton in the mid-South. The companion paper by the same authors presented at this conference (immediately preceding this paper) describes how the model simulates the seasonal life history of the insect as it applies to much of the south-eastern United States, and to eastern North Carolina in particular.

Fields are arranged in a grid, and each field is assigned one of four crop types (non-Bt cotton, Bt cotton, non-Bt corn, and Bt corn) each year. During the year, the phenology of each crop develops and their relative attractiveness to moths alters. The insect populations move between fields tracking host suitability. Resistance alleles are modeled as conferring complete resistance both to Bt cotton and to Bt corn (*i.e.* complete cross resistance). Effects of Bt crops on heterozygotes, and thus the functional dominance of the resistance allele, can be manipulated. Density-dependent selection occurs in Bt corn ears due to the combined action of larval cannibalism and delayed development (and thus greater probability of being cannibalized) of susceptible larvae feeding on Bt. Cotton fields, both Bt and non-Bt, are sprayed with a pyrethroid when larval thresholds are exceeded according to extension recommendations for North Carolina cotton. The model thus accounts for the aspects of the insects' life history, behavior and genetics, as well as crop effects and farm operations thought to be relevant in resistance management (Georghiou and Taylor, 1976; ILSI 1998).

The companion paper discusses the rationale behind the choice of default parameter values. The model's complexity

allows flexibility and experimentation to determine how resistance may evolve under different assumptions and different scenarios, by using non-default parameter values.

The model tracks the resistance allele frequency and insect population in each field, as well as the overall region-wide resistance allele frequency. A region is modeled as a 24x24 grid of 10-acre fields. The model is run for up to 15 simulation years to determine the time until the region-wide resistance allele frequency reaches 25%.

By altering parameter values for different model runs, it is possible to investigate what the effect of each parameter is on resistance development. As in a controlled experiment, all parameters are held constant while testing a range of values for the parameter of interest. A list of parameters tested, the default value for each, and the range of values tested is shown in Table 1.

Results and Discussion

Default Parameter Values

Figure 1 shows how resistance develops through time when the proportion of cotton fields that are Bt is 25%, and the proportion of corn fields that are Bt is increased from the 1% currently permitted up to 75%. Under the default assumptions the model suggests that deployment of 75% Bt corn in cotton growing regions would produce a region-wide resistance allele frequency of less than 5% in 10 years. However, the allele frequency rises rapidly at this point, and a region-wide allele frequency of 25% is reached less than two-years later. Figure 2 shows the spatial distribution of allele frequencies when the region-wide allele frequency has reached 25%. The allele frequency in each field is represented on a gray scale, with darker fields having a higher r-allele frequency. At the point in time shown, several fields have reached an allele frequency of 50%, which would be seen as a loss of control of this insect in Bt fields. Figure 2 shows considerable variation in allele frequency among fields, which indicates that monitoring of allele frequencies should be carried out on a fine spatial scale.

Initial Resistance Allele Frequency

The initial frequency of alleles conferring complete resistance to Bt in bollworm is unknown. Extensive monitoring of Bt crops throughout the insect's range has not produced any definitive cases of resistance, but past use of Bt sprays on crops fed upon by bollworm means that some selection is likely to have already taken place. Figure 3 shows the effect of a wide range of values for initial frequency on time to 25% region-wide resistance. The extreme sensitivity of the model to this parameter highlights the immediate need for field estimates of this value.

Functional Dominance

Again, since no resistant insects have been identified, it is not possible to characterize the functional dominance of any

resistance alleles. Our best indications of the likely range come from colonies of tobacco budworm (*Heliothis virescens*) that have been selected for Bt resistance at North Carolina State University (Gould *et al.*, 1995). These colonies show a functional dominance of between 0.35 and 0.65 at Bt concentrations that kill 75% of susceptible larvae. Model output shown in Figure 4 suggests that within this range of values, this parameter has a very important effect on resistance development: the time to 25% resistance is doubled if functional dominance is 0.35, or nearly halved if functional dominance is 0.65, compared with the default assumption of 0.5 (*i.e.* additivity). Different alleles for resistance have different values for functional dominance, and the value for any one allele may differ in different environments.

Bollworm Survival on Bt Cotton

Decreasing the survival of insects on Bt crops, compared with their survival on non-Bt crops, increases the selection pressure for resistance. If survival of susceptible bollworms in Bt cotton is altered from the default assumption of 25%, and assuming the functional dominance of resistance alleles remains at 0.5, then resistance evolution will be affected as indicated in Figure 5. If the survival of susceptibles on Bt, compared with their survival on non-Bt, drops much below 15% then the model suggests that resistance could evolve far more rapidly. However, if relative survival does drop this low, it is likely that most forms of resistance would become rather more recessive. Figure 4 indicates that such a shift in functional dominance should counteract the greater selection pressure. Resistance management must account for the variation in relative bollworm survival on Bt cotton that has been experienced throughout the cotton belt since the first deployment of Bt cotton, and the relationship between this parameter and resistance evolution rates.

Proportion of Region that is Corn

The relative amount of corn and cotton in a region is also critical in determining how resistance evolves. As the proportion of the total corn + cotton acreage that is corn decreases, the time until resistance criteria are reached also decreases (Figure 6). With the proportion of corn and cotton fields planted as Bt set at 75% and 25% respectively, a decrease in total corn means a decrease in total Bt. However, this does not result in smaller selection pressure for two reasons. Firstly non-Bt cotton is sprayed when larval populations reach threshold, whereas corn fields are never sprayed. This means that the refuge provided by non-Bt cotton is less effective than the refuge provided by non-Bt corn. Hence decreasing the total amount of corn relative to cotton also decreases the total refuge production. Secondly, as the amount of corn decreases and the amount of cotton increases, the density of larvae per acre of corn during the ear-feeding generation becomes greater. This causes the intensity of cannibalism and hence the selective advantage of being resistant, to rise. Generally, the ratio of corn:cotton is lower in more southerly states in the south-east, so the model suggests Bt resistance may develop faster

in Georgia than in Virginia. It must be noted though that relative crop phenologies, and hence crop usage patterns by bollworm populations, also change geographically, so caution must be taken in interpreting the output from this model for other regions.

Proportion of Farms Adopting Bt Technology

As with any agricultural innovation, not all farmers will adopt Bt transgenic crops. It has been proposed that non-adopting farmers may provide a portion of the refugia required to slow resistance development. Figure 7 shows the effect of incomplete adoption of Bt technology on the time to region-wide resistance allele frequency of 25%, when, region-wide, Bt corn is planted in 50% of corn fields and Bt cotton is planted in 50% of cotton fields. Defining a farm as a 5x5 block of fields, the chart shows the comparison between all farms adopting Bt technology (*i.e.* 100% adoption of 50% Bt) and two-thirds of farms adopting Bt technology (*i.e.* 67% adoption of 75% Bt). As deployment of Bt become more patchy, region-wide resistance develops slightly more rapidly. However, Figure 8, which shows the spatial distribution of allele frequencies at the time the region-wide allele frequency is 25%, demonstrates that farms that use Bt heavily are not protected from resistance development by farms that do not use Bt, but create local populations with very high levels of resistance (allele frequency = 0.7 in some fields). Even with an insect that is as mobile as bollworm adults are, populations from non-adopting farms do not interact sufficiently with populations from adopting farms to slow resistance. These data highlight the need to consider the spatial aspects of population genetics when exploring resistance management plans.

Conclusions

The proportion of both corn and cotton fields that are planted to Bt, the initial frequency of resistance alleles, the functional dominance of resistance alleles, the fitness of susceptible larvae on Bt plants, the ratio of corn to cotton fields, and the spatial distribution of Bt deployment, among others, play a key role in determining the rate of evolution of resistance to Bt in populations of the cotton bollworm in regions where both corn and cotton are grown. Field determination of mean values for these parameters, and natural variation in these values, is crucial before predictions can be made of resistance evolution. In reducing risk, insect resistance management plans should pay heed to the consequences of uncertainty revealed by this model.

The model has yet to be exhaustively analyzed. In particular, the interactions between parameters have not been investigated, so the effects of altering more than one parameter value at a time are unknown. Furthermore, the seasonal life history of the bollworm is modeled as it applies specifically to eastern North Carolina; the complexity of the model enables scenarios specific to

different geographic areas to be tested. Finally, with two-toxin transgenic crops in development, the model can be adapted to simulate resistance development to these new varieties in agroecosystems made more complex by their introduction.

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Table 1. Default values and range tested for several operation, genetic and biological parameters affecting the rate of evolution of resistance to Bt.

Parameter Description	Default Value	Range Tested
Proportion of corn fields as Bt	75%	1% - 75%
Proportion of cotton fields as Bt	25%	5% - 75%
Initial frequency of resistance alleles	10^{-4}	10^{-6} - 10^{-2}
Functional dominance of resistance alleles	0.5	0.1 - 0.8
Susceptible survival on Bt cotton	25%	5% - 35%
Proportion of region as corn	55%	35% - 65%
Percentage adoption of Bt technology	100%	67%, 100%

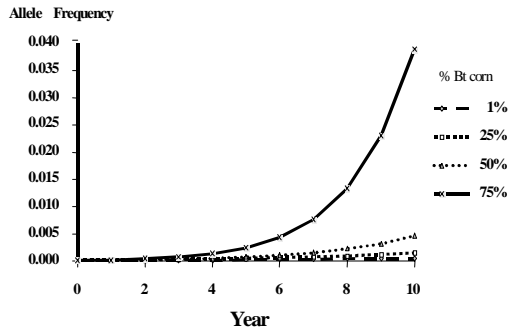


Figure 1. Evolution of resistance, as increasing resistance allele frequency through time, at 4 proportions of corn fields planted to Bt. Bt cotton represents 25% of cotton fields throughout. Default values from Table 1 apply to other parameters.

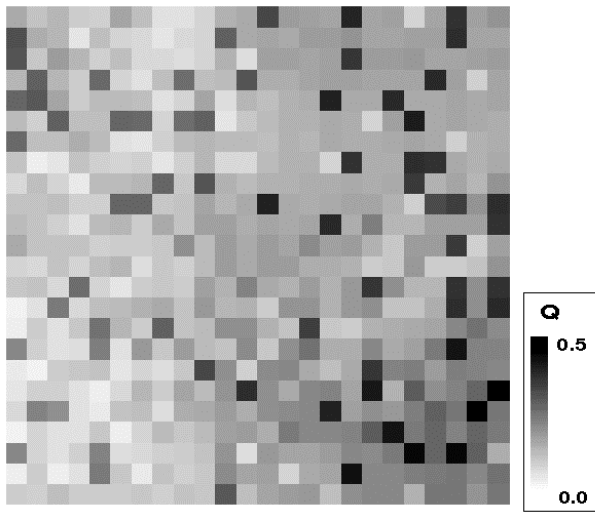


Figure 2. Spatial distribution of resistance allele frequencies for fields when region-wide frequency is 25%. Fields throughout the region are randomly assigned a crop each year. Bt cotton is planted in 25% of cotton fields; Bt corn is planted in 75% of cornfields. The gray scale indicates the allele frequency in each field (Q). Maximum frequency, represented by black, is 0.5; minimum (white) is 0.0. Default values from Table 1 apply to other parameters.

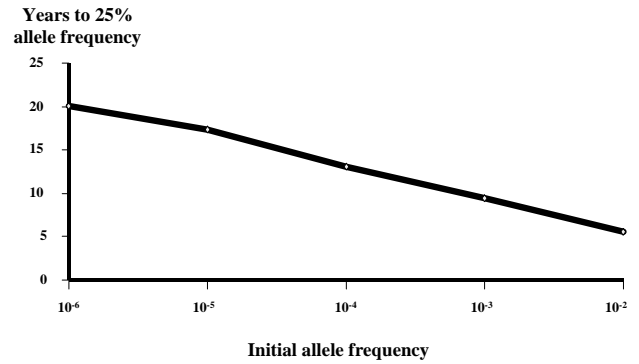


Figure 3. Years until region-wide resistance allele frequency reaches 25%, at different initial allele frequencies. Default values from Table 1 apply to other parameters.

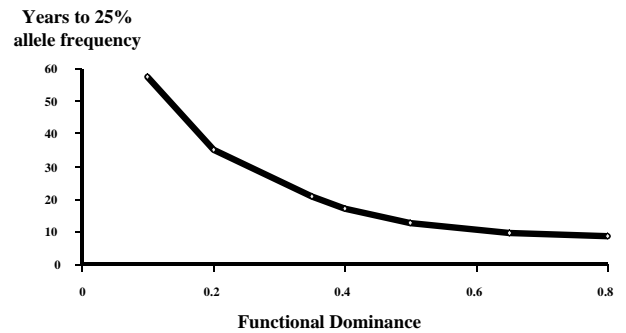


Figure 4. Years until region-wide resistance allele frequency reaches 25%, at different values for resistance allele functional dominance. Default values from Table 1 apply to other parameters.

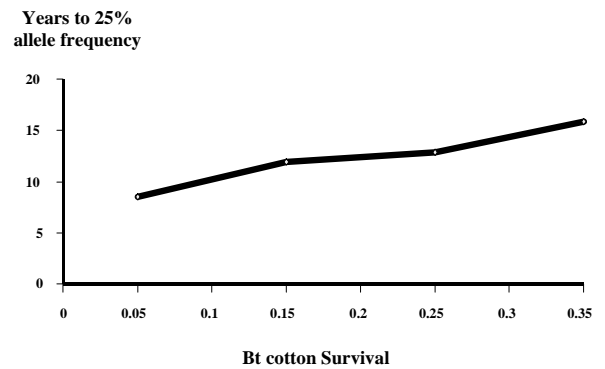


Figure 5. Years until region-wide resistance allele frequency reaches 25%, at different levels of survival of susceptible larvae on Bt cotton. Default values from Table 1 apply to other parameters.

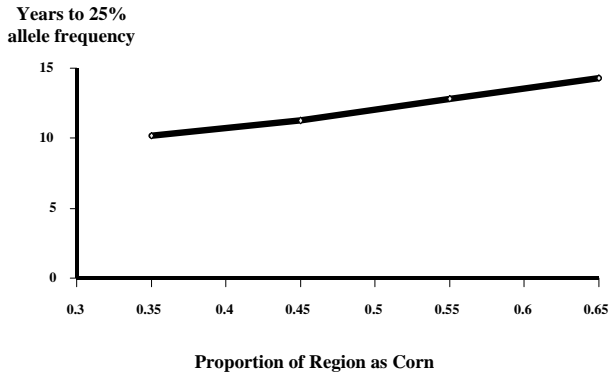


Figure 6. Years until region-wide resistance allele frequency reaches 25%, at different proportions of total corn + cotton acreage planted as corn. Default values from Table 1 apply to other parameters.

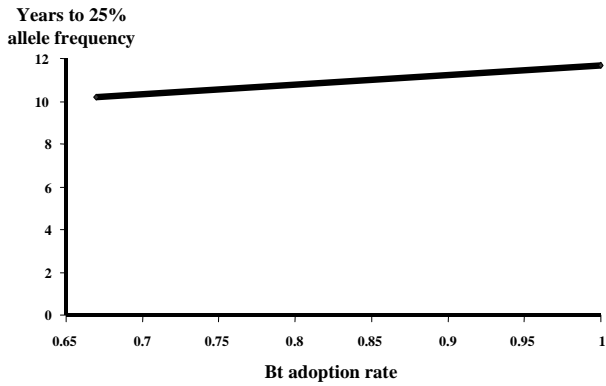


Figure 7. Years until region-wide resistance allele frequency reaches 25%, at two levels of Bt technology adoption. Refuge levels on farms adopting Bt are set so that in both cases the region-wide total amount of Bt corn is 50% of corn fields, and the region-wide total amount of Bt cotton is 50% of cotton fields. Default values from Table 1 apply to other parameters.

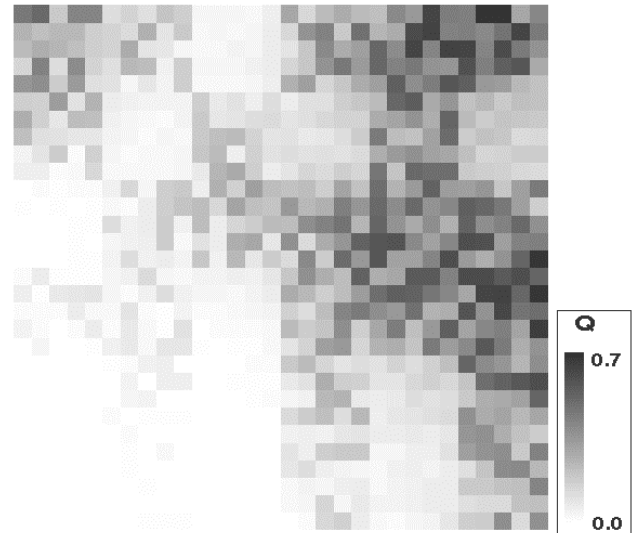


Figure 8. Spatial distribution of resistance allele frequencies for fields when region-wide frequency is 25% and only 67% of farms use Bt crops. Farms (5x5 field blocks) adopting Bt plant Bt cotton on 75% of cotton fields, and Bt corn on 75% of cornfields. Non-adopting farms plant only non-Bt corn and non-Bt cotton. The gray scale indicates the allele frequency in each field (Q). Maximum frequency, represented by black, is 0.7; minimum (white) is 0.0. Default values from Table 1 apply to other parameters.