

**ECOLOGY AND BIOLOGY OF COTTON
BOLLWORM IN REFERENCE TO MODELING
Bt RESISTANCE DEVELOPMENT
IN A Bt COTTON / Bt CORN SYSTEM**

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Abstract

Marketing of cotton and corn expressing Bt toxins in the Cotton Belt is a goal of seed companies. Cotton bollworm infests both crops during several generations and both crop species may express similar types of Bt toxins. Resistance evolution in bollworm to CryIAC toxin in cotton is expected to be affected by the culture of CryIAb corn, and visa versa. A wide area stochastic computer simulation model was developed to examine resistance evolution relationships in a cropping system containing both Bt cotton and Bt corn. This paper presents parameters selected for use in the model and discusses aspects of bollworm resistance evolution to Bt toxins.

Introduction

Transgenically altered cotton, expressing the CryIAC toxin derived from *Bacillus thuringiensis*, was planted on ca. 2.65 million acres in 1997 (Cotton Grower 1997). According to Demaske (1997) the adoption of Bollgard™ cotton significantly reduced synthetic insecticide use. Furthermore, Bt cotton has helped allay grower concerns over the inability to economically control caterpillar pests resistant to insecticides, particularly tobacco budworm, *Heliothis virescens* (F.). Growers have readily adopted Bt cotton and have been generally satisfied with the benefits. The current EPA approved label for Bt cotton requires a 4% unsprayed or 20% sprayed refuge to slow the evolution of resistance in pest insects to the Bt toxin.

Transgenically modified Bt corn has also been developed and was used on 14 million acres in 1998 (Farm Journal 1998). Bt corn is intended to reduce damage by European corn borer, *Ostrinia nubilalis*; southwest corn borer, *Diatrea grandiosella*; other *Diatrea* species; cotton bollworm (a.k.a. corn earworm), *Helicoverpa zea*; fall armyworm, *Spodoptera frugiperda*; and other insect pests. Transformation events used in corn express CryIAb, CryIAC, and Cry9c insecticidal proteins (Table 1). Currently the most commonly planted Bt corn hybrids,

employing Yieldgard™ gene events (Mon-810 and Bt-11 from Monsanto Company), are those expressing CryIAb throughout the entire plant, including the ear, for the entire season. In the southern U.S. these ear expressing genotypes are best suited for insect management programs (Table 2), however, selection of resistance in bollworm on these genotypes can take place. The other CryIAb gene event (E-176 from Novartis and Mycogen companies) express toxin only in the green tissues and the titre of toxin declines as plants reach the reproductive stage. These genotypes are not adequately efficacious against southern insect complexes and, since they express minimal toxin in the ear, place little selection pressure on bollworm (Table 2). The CryIAC event DBT-418 (Dekalb) will likely see limited commercial use due to the companies ownership change involving Monsanto. Corn hybrids expressing Cry9c toxin from event CBH-351(AgrEvo) have been recently introduced. This toxin appears to be efficacious against Pyralid stalk borers but has low toxicity to cotton bollworm and, therefore, should have little effect on the genetics of this insect (Table 2). Concern that the ear-expressing CryIAb corn events (Yieldgard) and CryIAC cotton will impose significant selection for resistance evolution in bollworm has led EPA to severely restrict the planting of corn cultivars with Yieldgard genes. Current efforts by Monsanto and others are encouraging EPA to allow corn hybrids containing MON-810 and Bt-11 gene events to be deployed in the Cotton Belt at much higher use levels (ILSI 1998).

Cotton bollworm is well adapted to the agroecosystems in which it occurs. It colonizes many cultivated and wild hosts and is an important pest of many vegetable, field, and ornamental crops. Across the southern U.S., bollworm is able to exploit, at least to some extent, most crop plants that flower or fruit when egg laying moths are present. The bollworm's physiology, reproductive biology, and behavior provides the plasticity necessary to thrive in a modern agricultural environment and, likewise, will provide populations with the ability to adapt to Bt crops, under favorable circumstances.

Resistance evolution is an ecological phenomenon and must be viewed in an ecological context. The evolution of resistance to plant-expressed Bt toxins is determined by many interacting factors. With cotton bollworm some factors are reasonably well quantified and others are poorly understood. Gould and Tabashnik (1998) lists the following ecological and genetic factors as critical in determining the rate of resistance evolution to Bt toxins:

- Number of generations per year exposed to Bt in transgenic crops or sprays,
- Percentage of the insect pest population exposed to Bt in transgenic crops or sprays in each generation,
- Toxin-induced mortality of individuals heterozygous for resistance alleles,

- Adult movement and mating patterns,
- Larval movement,
- Initial frequency of resistance alleles in the population, and
- fitness of individuals carrying resistance alleles in both the presence and absence of toxin.

The relative effect of these factors can be influenced by interactions among them, therefore, an understanding of a total effect must consider such interactions. Due to the complexity of these interactions computer simulation models must be employed to explore, through sensitivity analyses, the relative importance of each factor. Because many aspects of cotton bollworm genetics, ecology, and biology are not well understood, assumptions must be made when assigning values to important parameters in a model. Consequently, under these circumstances computer models should not be viewed as providing reliable predictions of the time frame in which resistance will develop. Rather, they should be viewed as providing insights into the relative importance of individual factors and of interactions among factors in determining the relative rate at which resistance evolves. Those examining results from computer simulation models should remain aware of this important distinction.

Recently, Dr. Mike Caprio at Mississippi State University and our team at North Carolina State University have independently used computer simulations to study resistance evolution to Bt toxins in cotton bollworm in an ecological setting containing both corn and cotton. Both efforts have employed region-wide stochastic models that incorporate bollworm traits and a diverse ecosystem that includes varying proportions of both Bt and non-Bt cotton and corn, as well as other host plants. At NCSU, we have also made a considerable effort to evaluate bollworm biology on non-Bt and ear-expressing Bt corn, in order to provide data for model parameters. This paper, and the companion paper by the same authors presented at this conference (immediately following this paper), report on portions of this work. The objective of this paper is to present important factors relevant to resistance evolution in cotton bollworm and to discuss the values assigned to them for our simulation model.

For convenience we have organized our discussion into four categories related to resistance evolution in cotton bollworm: 1) environmental aspects, 2) genetic factors, 3) aspects of bollworm biology, and 4) dose of toxin in the plants.

Discussion

Simulated Environment

Cotton bollworm primarily infests corn, cotton, and soybean throughout its southern range. Corn and cotton are universally recognized as primary hosts. Soybean appears to be a more favorable host in the coastal plain of the southeast and mid-Atlantic states than in the mid-south

(Stinner et al. 1980). Other hosts may be locally important hosts, such as peanut and grain sorghum. The model represents a region of 5760 acres partitioned into a matrix of 576 fields (24 X 24) of 10 acres each. The host crops are cotton and corn with, by default, 55% of the fields planted to corn and 45% to cotton. Weedy field margins provide additional host plants for the first generation. By default 10% of the population develops on weeds in the first generation. Each corn field was considered to have 220,000 ears available for bollworm infestation during the second generation. The default proportion of cotton fields that are Bt is 25% and 75% of corn fields are Bt. Non-Bt off-types within Bt fields were not considered although up to 2% off-types may be typical (Gould and Tabashnik 1998). Soybean and other cultivated and wild hosts are currently not included in the model but will be incorporated in future simulations. Proportions of crops and the Bt/non-Bt ratio of each can be controlled at any level when investigating these variables. The default spatial arrangement of crops within the regional matrix is random, and is re-randomized at the beginning of each season. When investigating the effects of the spatial arrangement, the field assignments can be controlled in any manner. A season lasts 123 days and accommodates 3 complete, and a partial 4th, bollworm generations. The crop mix and season length represented by the model parameters we evaluated, and therefore model output, most closely represents a mid-Atlantic coastal plain environment.

Hosts diversity has likely contributed significantly to maintaining insecticide susceptibility in the bollworm for long periods. Early generations of bollworm are rarely treated with insecticide in any crop, and corn and soybean receive relatively little insecticide throughout the season. Although later generations of bollworm in cotton are intensively controlled using insecticide, corn and soybean act as important refuges from selection for resistance and serve to preserve genes for susceptibility in the population. In a similar manner, host diversity can be expected to mitigate the evolution of resistance in bollworm to Bt toxins. However, the benefits of host diversity to resistance management for Bt-toxins may be reduced by the deployment of both Bt cotton and Bt corn that express the same Bt gene or similar genes that allow cross-resistance; even without cross resistance the use of Bt corn will reduce the refuge effect given by non-Bt corn. Further, the scope and intensity of selection for adaptation to plant expressed Bt toxins is likely to be greater than in the case of insecticides because both types of Bt corn and Bt cotton under consideration express the toxins throughout the season and in all plant tissues. As mentioned previously, the number of generations per year exposed to Bt-toxins in transgenic crops or sprays, and the proportion of each generation exposed, will influence resistance evolution. Because ear stage corn is a very suitable host for bollworm, when corn is available a disproportionate segment of second generation bollworm larvae develop in ear-stage corn,

allowing selection almost proportional to the percentages of Bt corn and non-Bt corn planted in a region.

For each bollworm generation, the non-Bt host plants contribute insects to a refuge population. For the refuge to be effective, moths produced from Bt crops must mate with moths produced from the refuge. The refuge benefits provided by host diversity depend on the number of moths produced from Bt and non-Bt hosts, and the proportion of the moths from the Bt sources that interbreed with moths from the non-Bt sources. The simple existence of non-Bt hosts in the agroecosystem does not guarantee that they will serve as a significant and functional refuge for susceptible genotypes that is of value for resistance management. Production of moths from non-Bt hosts must occur in close proximity to, and at the same time as, production from Bt hosts. High mobility in the target pest, such as with cotton bollworm, can be very influential in reducing the importance of closely placing the refuge to the Bt crop, as long as mating does not occur before movement out-of or into fields.

Genetic Factors

The rate of resistance evolution will be greatly influenced by: 1) the initial frequency of alleles conferring resistance; 2) the fitness of individuals carrying one or more R alleles, including functional dominance; and 3) number of resistance alleles. Prior to exposure of a population to Bt toxins, the rate of genetic mutation to resistance alleles, and the fitness cost of being resistant in the absence of selection, combine to determine the initial resistance allele frequency. In our model, we assume no prior exposure, a mutation rate of 5×10^{-6} , and a fitness cost of 5% per copy of the resistance allele. Thus, an initial R-allele frequency of 1×10^{-4} is produced. These values are assumptions since there are no bollworm data available. The assumptions are based on available data for other insect species, particularly Bt resistant tobacco budworm, *Heliothis virescens* (Gould et al. 1995, 1997). In the model, fitness is assumed to be functionally co-dominant, so that on Bt plants the fitness of heterozygous genotypes (RS) is set as intermediate between susceptible (SS) and homozygous resistant (RR). Since most historical instances of field resistance to insecticides have involved a single gene with two alleles, we also considered Bt resistance in bollworm to be controlled by one such gene. In addition, since CryIAb and CryIAc toxins are very similar, and their effects on bollworm are also similar, we assumed that resistance alleles confer complete cross resistance to Bt cotton and Bt corn.

The unknown status of these genetic values is an important concern to scientists and EPA. In the absence of recorded cases of resistance, a maximum value for initial gene frequency can be experimentally estimated, under different assumptions about dominance (Andow and Alstad 1998, EPA SAP IRM Subpanel, US EPA 1998). However, other aspects of resistance genetics requires study on resistant insects. Typically, this has been done using laboratory

colonies of resistant insects and this can be most helpful. However, laboratory selection for resistant bollworms could result in a population(s) that does not adequately represent the genetics and substructure components of resistant bollworms developing in the wild. The model gives consideration only to major genes while potential small contributions by minor genes is ignored. Minor genes could give an important survival benefit on Bt corn and Bt cotton, especially when the toxin dose is marginal, as is now the case. Total bollworm mortality observed on Bt plants may also include indirect effects of insect pathogens and other confounding factors associated with injury by the Bt toxin. Hypothetically, selection may promote genes that enhance survival (e.g. associated with the immune system) but are usually not viewed as “resistance genes” since they do not directly affect the activity of the toxin. Default genetic values selected for the computer simulation model are believed to be conservative but realistic, in light of current knowledge. Variations of these values were included in simulations and indicated the impact of departures from the default on model output. Model results from an array of genetic values give an estimate of the scope of potential error resulting in incorrect assumptions of genetic values.

Bollworm Biology

Resistance evolution may be influenced by many factors associated with bollworm biology, including: 1) movement, 2) differential development rates on Bt and non-Bt plants, 3) biotic and abiotic mortality factors (other than Bt intoxication), 4) number of yearly generations, 5) host range, and others. Some of these have been addressed earlier in the paper.

Bollworm movement in the model allows for high exchange of moths between crops in most generations, and especially between the 2nd and 3rd annual generations. This movement is largely affected by the insects ability to move and by changes in host phenology. First generation is assigned mainly to whorl stage corn with about 10% developing in weeds and cotton. Second generation mainly remains in, or moves to, corn with most larvae developing in ears but with a residual population remaining in cotton. The third and fourth generations mainly occupy cotton with a residual population remaining in corn. Most movement in the model is controlled by two forces. Firstly, the probability that a moth will move from its field is affected by the phenology of the crop in that field, with a minimum probability of 0.1 (default) if the field is at phenological peak. Secondly, the probability that a moth will move to a particular field is determined by the relative phenology of the crop in that field compared with crops in nearby fields, and the distance from the source field. The default maximum distance a moth can move in one night is 8.7 fields (ca. one mile).

During the 123-day season, the model encompasses three full insect generations and a partial fourth. The model accommodates slower development on Bt corn and Bt cotton for surviving susceptible larvae, so that larval

duration is extended by a default of 6 days. Starting on day 58 (approximately July 30th), the proportion of pupae entering diapause increases through the season, and half of the larvae that reach pupation on day 86 (August 27th) diapause. One consequence of delayed development is that the susceptible larvae surviving on Bt crops are more likely to enter diapause in the third generation, whereas larvae developing on non-Bt crops are more likely to attempt a fourth generation.

Default values for replacement rates are 1.5X for generation F₀ on whorl stage corn and weeds, 75X for generation F₁ in corn ears, and 10X for generation F₂ on cotton. These parameters were initially derived from literature sources and field study. However, replacement parameters were adjusted in the model so that output closely matched bollworm population dynamics in the mid-Atlantic coastal plain, as indicated by field data, historical light trap moth catches (Bacheler unpublished data), and a previously developed bollworm model (Stinner et al. 1977). These replacement rates incorporate the effects of background mortality rates (environmental resistance). Other biotic and abiotic mortality factors included in the model consist of cannibalism, intoxication from eating Bt plants, insecticide applied to cotton (when eggs or larvae exceed thresholds), and general overwintering mortality. Where these, and other, mortality factors disproportionately affect insects of different genotype (susceptible, heterozygote, or resistant) they affect the rate of resistance development. Cannibalism gives a good example of this relationship. Cannibalism by bollworm infesting corn ears is widely recognized and has been documented (Caron et al. 1978). Generally, in an encounter between two larvae the larger bollworm usually eats the smaller bollworm. In corn ears the first larva to get into an ear usually eats later arriving larvae. The fitness of heterozygous (RS) and resistant (RR) larvae assumed in the model allows for more rapid development in Bt corn ears (compared with SS). Therefore, in encounters of similar age larvae of different genotype, the more resistant larva usually cannibalizes the more susceptible larva, giving a positive survival differential to resistance genes. Cannibalistic encounters are more likely under higher populations. Thus, the model predicts the occurrence of density-dependent selection for resistance. The model only considers cannibalism in corn ears; however, cannibalism or other types of interspecific competition presumably occurs in bollworm in other settings, especially under high populations.

Overwintering mortality of diapaused pupae is assumed to be equal for all insect genotypes developing on all plant types. A default value of 96.6 % mortality was derived from data in Caron et al. (1978). Large fluctuations in this value among years and location are expected; variation in this value in simulations indicated the effect of the value on resistance evolution.

Toxin Dose

Current EPA-approved Bt cotton and Bt corn resistance management options are based upon the refuge-high dose strategy (EPA 1997). This approach requires that all susceptible genotypes (SS) and almost all heterozygous genotypes (RS) are killed on Bt plants and that random mating among populations minimizes matings between individuals carrying resistance alleles. (Georghiou and Taylor 1977, Tabashnik and Croft 1982). A non-Bt host, a refuge, supplies relatively large numbers of susceptible genotypes. Strictly, a high dose is one which kills more than 95% of heterozygotes and thus makes resistance alleles functionally recessive. In the absence of data on heterozygote fitness, a high dose has been defined as 25X the dose required to kill susceptible larvae (EPA SAP IRM Subpanel, US EPA 1998); this definition relies upon resistance not being generally dominant. Without a refuge to supply susceptible genotypes the rate of resistance evolution is proportional to the dose (Tabashnik and Croft 1982); the higher the dose the faster resistance develops. However, with adequate immigration of susceptible genotypes, a dose that kills most heterozygous (RS) genotypes may significantly reduce the rate of resistance evolution (Comins 1977, Tabashnik and Croft 1982). Refuges must supply at least 500 susceptible genotypes for each one resistance carrying genotype anticipated (EPA SAP IRM Subpanel, US EPA 1998) and these insects must be situated in time and space so as to assure their mating with those carrying one or more R genes. This has implications for the manner in which the refuge is cultured and where it is situated in respect to the Bt crop. At lower doses where greater numbers of heterozygous (RS) individuals survive, greater numbers of susceptible (SS) genotypes are needed to counterbalance the increased residual of R genes.

The toxin dose in Cry IAc Bt cotton is sufficiently high to qualify as high dose versus tobacco budworm (Gould et al. 1995). Likewise, CryIAb Bt corn qualifies as a high dose for European corn borer and other Pyralidae (ILSI 1998). However, bollworm survival on Bt cotton or Bt corn appears to range from ca. 75% to 95% and this does not qualify as a high dose (Mahaffey et al. 1995, Lambert et al. 1996, Horner U. MD, per. communication, Storer, unpublished data). This relatively high natural tolerance in bollworm has been documented to several Bt toxins (Stone and Sims 1993). Parameter values in our simulation model reflect this tolerance. The default relative survival of susceptible genotypes on both Bt cotton and Bt corn is set to 25%. Homozygous resistant genotypes (RR) survive at 100% and heterozygous (RS) survival is 62.5%, the mid-point between susceptible and resistant survival.

Model simulations were conducted to evaluate the sensitivity of model parameters and to investigate resistance evolution under differing proportions of Bt cotton and Bt corn development. The following paper by the same authors will report on the outcome of these investigations.

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Table 1. Gene events and toxin types currently used in Bt corn.

| Brand | Company* | Bt gene event | Toxin type | Promoter |
|-----------|------------|---------------|------------|----------|
| Naturgard | Mycogen | E-176 | CryIA(b) | PEP |
| Maximizer | Novartis | | | |
| Yieldgard | Monsanto | Mon-810 | CryIA(b) | e-35s |
| Yieldgard | Novartis** | Bt-11 | CryIA(b) | e-35s |
| BtExtra | Dekalb | DBT-418 | CryIA(c) | e-35s |
| Starlink | AgrEvo | CBH-351 | Cry9c | e-35s |

* Company commercializing gene with or without seed company partners, ** Monsanto gene licenced to Novartis.

Table 2. Generalized efficacy characterization of Bt corn gene events.

| Bt gene event | 1st gen. ECB * | 2nd gen. ECB * | 3rd gen. ECB* | 2nd gen. <i>Diatrea</i> ** | 2nd gen. <i>H. zea</i> *** |
|---------------|----------------|----------------|---------------|----------------------------|----------------------------|
| E-176 | High | Low-mod | Low | Low | Low |
| Mon-810 | High | High | High | High | Mod. |
| Bt-11 | High | High | High | High | Mod. |
| BtExtra | High | Low-mod. | Low | Low | ? |
| Starlink | High | High | High | High(?) | Low |

* European corn borer, *Ostrinia nubilalis*; ** southwestern corn borer, *D. grandiosella*; southern cornstalk borer, *D. crambidoides*; and/or sugarcane borer, *D. saccharalis*; *** cotton bollworm (corn earworm).