Physiologically based demographics of Bt cotton–pest interactions
II. Temporal refuges, natural enemy interactions

Andrew Paul Gutierrez a,∗, John J. Adamczyk Jr. b, Sergine Ponsard a, c, C.K. Ellis a

a Division of Ecosystem Sciences, 151 Hillgard Hall, University of California, Berkeley, CA 94720, USA
b Southern Insect Management Research Unit, USDA-ARS-MSA, P.O. Box 346, Stoneville, MS 38866, USA
c Laboratoire Dynamique de la Biodiversité, University P. Sabatier, Toulouse, France

Received 4 March 2004; received in revised form 26 May 2005; accepted 17 June 2005
Available online 19 September 2005

Abstract

An holistic demographic physiologically based age–mass structured model of transgenic Bt cotton expressing one or two toxin of the bacterium Bacillus thuringiensis Berliner (Bt) and 10 of its pests as affected by generalist natural enemies is developed. The goal of the model is to assess factors favoring the development of resistance in pest species. Among the pest included in the model are the pink bollworm (a primary pest of cotton), and several secondary pests of the family Noctuidae (tobacco budworm, bollworm, fall armyworm, beet armyworm, cabbage looper, soybean looper) as well as a plant bug, boll weevil and whitefly. The pests have widely varying tolerance to the toxin.

Resistance to the Bt toxin is assumed recessive, autosomal and controlled by a single diallelic gene. Spatial refuges of non-Bt cotton have been mandated to increase heterozygosity in Bt cotton, but spatial refuges may exist for many pest species in non-Bt and/or sylvan hosts. In addition, temporal refuges may arise within Bt cotton due to innate pest tolerance to the toxin that increases with larval age, and variable toxin concentrations in the plant over time and with plant subunit age. Mortality rates and non-lethal effects that increase larval developmental time and decrease adult fecundity vary with Bt toxin concentration. All genotypes are affected but resistant genotypes are assumed less affected than susceptible ones. The effects of spatial and temporal refuges on the development of resistance and pest densities in one and two toxin cottons are examined over several consecutive seasons.

Resistance to Bt toxin is more likely to develop in stenophagous highly susceptible pests such as pink bollworm and budworm than in polyphagous highly tolerant pests such as fall armyworm, beet armyworm and soybean looper that have large temporal refuges that slow and possibly reverse resistance development.

Predator longevity feeding on Bt intoxicated prey is decreased ca. 28% [Ponsard, S., Gutierrez, A.P., Mills, N.J., 2002. Effects of Bt-toxin in transgenic cotton on the adult longevity of four heteropteran predators. Environ. Entomol. 31, 1197–1205.] creating tradeoffs between the mortality caused by the toxin and that due to reduced predation. Despite reduced natural enemy efficacy,
levels of susceptible pests (budworm) are reduced, while levels of Bt tolerant and immune pests (e.g. fall armyworm, Lygus) increase. The use of pesticide for supplementary control of tolerant pests in Bt cotton may further disrupts natural enemies and increase pest levels.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Model, Bt cotton, Pests, Natural enemies, Resistance, Refuges, Risk

1. Introduction

Several insect species are serious pests of cotton worldwide, and transgenic cotton expressing toxins of the bacterium *Bacillus thuringiensis* Berliner (i.e. Bt) are increasingly being used to control some of them. Development of resistance to Bt toxins in pests is viewed as a potential problem (see also Gould et al., 1992, 1997; Tabashnik et al., 2000) stimulating the development of resistance management programs based on spatial refuges of non-Bt cotton to preserve susceptibility (see Carrère et al., 2001a). In the USA, planting of spatial refuges of non-Bt cotton has been mandated (EPA, 2000).

Cottons expressing the Cry1Ac δ-endotoxin (i.e. Bt toxin) have given good control of tobacco budworm (*Heliothis virescens* (F.), TBW) and pink bollworm (*Pectinophora gossypiella* Saunders) (Williams, 2000), but variable control of bollworms, beet armyworm (*Spodoptera exigua* (Hubner)) and other noctuid pests is occurring (Luttrell et al., 1999) requiring insecticides use for their control (Mahaffey et al., 1995; Lambert et al., 1996; Turnipseed and Sullivan, 1999; USDA, 2000; EPA, 2000; Gianessi and Carpenter, 1999; Benbrook, 2000; Ra et al., 2002). Why this is occurring is unresolved. Other potential pests are in the family Noctuidae (bollworm (BW, *Helicoverpa zea* (Boddie), fall armyworm (FAW, *Spodoptera frugiperda* (J.E. Smith)), cabbage looper (CL, *Trichoplusia ni* (Hubner), soybean looper (SBL, *Pseudoplusia includens* (Walker))) and include plant bugs (e.g. *Lygus hesperus* Knight, Heteroptera) and whitefly (WF, Homoptera) (see Table 1). Most of these species are secondary pests in cotton con-

<table>
<thead>
<tr>
<th>Table 1 Summary of cotton pest characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>Pest status (host type)</td>
</tr>
<tr>
<td>Host specificity</td>
</tr>
<tr>
<td>Tolerance to Bt</td>
</tr>
<tr>
<td>Natural refuge</td>
</tr>
<tr>
<td>Fecundity</td>
</tr>
<tr>
<td>Regulation by natural enemies</td>
</tr>
<tr>
<td>Fruit feeders</td>
</tr>
<tr>
<td>Pink bollworm (PBW)</td>
</tr>
<tr>
<td>1 (F) (F)</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Very low</td>
</tr>
<tr>
<td>Small</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Bollworm (BW)</td>
</tr>
<tr>
<td>2 (F)</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Bollworm budworm (TBW)</td>
</tr>
<tr>
<td>2 (F)</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Deltolators</td>
</tr>
<tr>
<td>Beet armyworm (BaAW)</td>
</tr>
<tr>
<td>2 (L)</td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Large</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Soybean looper (SBL)</td>
</tr>
<tr>
<td>1 (L)</td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Large</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>Lygus bug (Lx)</td>
</tr>
<tr>
<td>1 (F)</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Very high</td>
</tr>
<tr>
<td>N/A</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Very low</td>
</tr>
<tr>
<td>Boll weevil (Lx)</td>
</tr>
<tr>
<td>2 (S)</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Large</td>
</tr>
<tr>
<td>Low-moderate</td>
</tr>
<tr>
<td>Whitemy (WF)</td>
</tr>
<tr>
<td>–</td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Low-moderate</td>
</tr>
<tr>
<td>Natural enemies</td>
</tr>
<tr>
<td>Generalist predators</td>
</tr>
<tr>
<td>–</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Whitemy parasites</td>
</tr>
<tr>
<td>–</td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
</tbody>
</table>

---

Notes:

a. Abbreviation of the common name.

b. 1, primary pest; 2, secondary pest.

c. Plant subunit attacked: F, fruit; L, leaves; S, vascular system.

d. Not included in the analysis.
trolled by the action of natural enemies, but insecticide uses can disrupt this and lead to outbreaks and to insecticide resistance (Eveleens et al., 1973; Elderd et al., 1973; Gutierrez et al., 1975; van den Bosch, 1978; Nussly and Sterling, 1994; Sansone and Smith, 2001; Naranjo, 2001). Secondary pests such as bollworm and Lygus may be considered primary pests in areas where cropping patterns, alternative crops and insecticide use increase their abundance (e.g. the south-eastern USA). To examine how the Bt cotton technology affects the dynamics and the development of resistance in all pest species simultaneously requires a holistic model of cotton and its pests that captures the biological subtleties of their interaction.

1.1. Modeling Bt cotton–pests—natural enemy interactions

A review of the literature and the mathematical structure of the basic model were presented for pink bollworm in Gutierrez and Ponsard (2005), and here only minimal additional details are given for the other pests.

The demographic models of Bt cotton and its pests have the following properties: they are physiologically based, age (and mass) structured, and the underpinning dynamics model is characterized as a distributed maturation time model (see Vansickle, 1977). A time invariant form of the model is used for cotton and Bt immune pests (Lygus and whitefly and its parasitoids), while a time varying distributed maturation time model is used for all noctuid species to accommodate the time varying Bt concentrations in their food that affect developmental times, fecundity and survival. The similarity of the resource acquisition and allocation biology across plant and animal species allows a generic model structure to be used for analogous processes (Gutierrez and Baumgartner, 1984). For the noctuid pests, only parameter changes and modifications in linkages to cotton subunits are required. Two dynamics models are required to simulate the number and mass dynamics of each pest genotype in single toxin Bt cotton for a total of six per noctuid species (and 18 in a two toxin system). The models for Lygus and whitefly are reviewed in Gutierrez et al. (1977b) and Mills and Gutierrez (1996), respectively, and are not discussed further here.

Model dynamics are driven by weather and edaphic factors and realistically capture the bottom-up effects on crop growth and pest dynamics as well as the top-down effects of natural enemies. Time and age in the models are in degree-day units (dd) above 12◦C for cotton and 12.2◦C for noctuid pests (e.g. Table 2 for length of developmental stages). Pest species may be included in any combination in the simulation runs using Boolean variables in a setup file. Similarly, the number of Bt toxins in the run may be determined by an integer variable (i.e. 0–2). The model may simulate several consecutive years with the linkage between years being the surviving winter diapause pest populations.

1.2. Noctuid pest biology on Bt cotton

1.2.1. Over-winter survival, migration and fecundity

In the Central Valley of California, the development of noctuid larvae and pupae (e.g. beet armyworm) is arrested by low fall temperature with adult emergence occurring in spring when temperatures warm (Hogg and Gutierrez, 1980). Approximately, 0.5% of overwintering larvae are assumed to survive the winter (e.g. for BW, see Roach and Adkisson, 1971; for BAW, see Hogg and Gutierrez, 1980). The emergence period is roughly equal the larval developmental period, and the pattern is sigmoid and predicted using an invariant distributed maturation time model (Vansickle, 1977). In a Bt cotton monoculture, the frequency of the resistance allele in spring adults is assumed that of the overwintering population.

The number of migrant noctuid adults caught in light traps in California grows exponentially from early spring until late summer (Hogg and Gutierrez, 1980) providing an increasing source of immigrants to cotton. In a heterogeneous environment, susceptible adults

<table>
<thead>
<tr>
<th>Stage</th>
<th>Beet armyworm</th>
<th>Bollworm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>0–52.2</td>
<td>0–52.2</td>
</tr>
<tr>
<td>I</td>
<td>117.4</td>
<td>148.2</td>
</tr>
<tr>
<td>II</td>
<td>192.6</td>
<td>223</td>
</tr>
<tr>
<td>III</td>
<td>247.8</td>
<td>296</td>
</tr>
<tr>
<td>IV</td>
<td>313.0</td>
<td>371</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>445</td>
</tr>
<tr>
<td>Pupa</td>
<td>490.0</td>
<td>650</td>
</tr>
<tr>
<td>Pupa</td>
<td>550.0</td>
<td>696</td>
</tr>
<tr>
<td>Adult</td>
<td>980.0</td>
<td>1040</td>
</tr>
</tbody>
</table>
may come from spatial refuges of cotton and other non-Bt crops as well as wild hosts. These adults dilute the frequency of resistance in cotton populations. In other areas, migration from distant locations may occur (Gould et al., 2002). The model allows light trap data to be used as a basis for computing daily immigration rates.

The number of eggs deposited by all females of a population is summed across all genotypes (n) and ages (a). If \( N_n(a, t) \) is the number of adults of genotype \( n \) of age \( a \) at time \( t \), then the total eggs (\( E(t) \)) deposited by all genotypes may be computed as follows:

\[
E(t) = 0.5 \sum_n h(a) N_n(a, t)
\]  

(1)

The constant 0.5 corrects for sex ratio, \( 0 < \phi_s \leq 1 \) is the Bt tolerance scalar of the \( n^{th} \) genotype compared to conventional cotton, \( h(a) = ca/da^s \) predicts the maximum per capita fecundity rate per unit time with species-specific constants \( c \) and \( d \) (Bieri et al., 1983).

Assuming panmixia, the number of the new eggs allocated to each genotype population is computed from all age larvae, we first compute their feeding rate and mass available (\( \alpha_F \times k_{larv} \times k_{trav} \) preference matrix, cf. Wilson and Gutierrez, 1980). The demand-driven Gutierrez–Baumgärtner functional response model (Eq. (2) ) is used to estimate the total fruit attacked (\( S(t) \)) per larva at a specific age \( i \) and time \( t \) at temperature \( T \) (Gutierrez and Ponsard, 2005).

\[
S(t) = D(t) f(u) = D(t)(T) \left(1 - \exp\left(-\frac{\alpha F^*(i)}{D(t)(T)}\right)\right).
\]  

(2)

The components of (2) are defined below. To simplify the notation, we ignore \( t \) and \( T \) except in definitions.

The available fruit (\( F^*(i) \)) across all ages (\( i = 1, \ldots, k_i \)) corrected for species preferences (\( \theta(i,j) \)) across all age larvae (\( j = 1, \ldots, k_{larv} \)) is computed as:

\[
F^* = \sum_{j=1}^{k_{larv}} \sum_{i=1}^{k_i} \theta(i,j) F(i,j).
\]  

(3)

Previously attacked fruit are assumed to have a low preference.

The total demand \( D = D(t) \) for fruit (\( F \) = number, \( M \) = mass) by larval population \( N_j(t) \) of genotype \( n \) with per capita demand \( D_n(j,t) \) at age \( j \) is computed as:

\[
D = \sum_n \sum_j D_n(j)n_n(j).
\]  

(4)

Substituting \( F^* \) and \( D \) in Eq. (2), we can compute the attack rate by all larvae \( N_n \) at time \( t \).

To compute the number of fruit attacked by \( j \)th age larvae of the \( n \)th genotype in the face of competition from all age larvae, we first compute their feeding rate (\( S_n(j,t) \)) in the absence of competition from other age classes (Eqs. (2) and (5)).

\[
S_n(j) = f(a) D_n(j)N_n(j)
\]  

(5)

\[
S(j) = \sum_j S_n(j)D_n(j)N_n(j).
\]  

(6)

The supply/demand ratio (\( \phi_n(j) \)) for the \( j \)th larval age class equals:

\[
0 \leq \phi_n(j) = \frac{S_n(j)}{D_n(j)N_n(j)} < 1.
\]  

(6i)

The mortality rate of fruit of age \( i \) (\( F_{atkd}(i,j) \)) is computed using the total fruit attacked by all larvae \( S(i,t) \), the total weighted fruit available for attack \( F^*(i) \) (see Eq. (2)), and the weighted number of \( i \)th age fruit \( F^*(i,j) \).

\[
F_{atkd}(i,j) = \frac{S(i,j)}{F^*}.
\]  

(7)

The mass of fruit of age \( i \) attacked (\( M_{atkd}(i,j) \)) is simply the mass available (\( M(i,j) \)) multiplied by the fraction...
of fruits (numbers) attacked in the age-class.

\[ M_{\text{matkd}}(i) = M_i \left( \frac{F_{\text{matkd}}(i)}{P_{\text{matkd}}(i)} \right) \] (7ii)

Buds, flowers and small bolls attacked by noctuid larvae abscise, but larger bolls remain and are available for multiple attacks that may occur at high pest densities. The same models (2–7) can be extended for multiple species attacking fruit or other subunits.

Food acquisition shortfalls as measured by \( 0 \leq 1 - \phi_n(j, t) < 1 \) cause mortality in larval stages and emigration of adults at rates proportional to the short-fall. The concentration of toxin in the species diet changes with the ages of fruit attacked and this has time varying effects on the species vital rates. Modeling the feeding biology of noctuid defoliators incorporating preferences can be approached in the same manner (i.e. modifying Eqs. (2)–(7)). For example, Bt tolerant beet armyworm (BAW) prefers older leaves low in the canopy that have low toxin levels (Hogg and Gutierrez, 1980), while the moderately tolerant cabbage looper (CL) prefer young to middle aged leaves with higher toxin concentrations (see Gutierrez et al., 1975; Wilson et al., 1981). Species may feed on non-preferred leaves when preferred ages are in short supply. As an aside, mortality of older leaves has less impact on plant growth and yield than does mortality of young leaves.

1.2.3. Bt toxin effects on noctuid larvae

The susceptibility of noctuid species to Bt toxin varies widely, it decreases with larval age (Stewart et al., 2001) and with toxin concentration that decline with plant and plant subunit ages and other factors. These factors allow more susceptible genotype individuals to survive creating temporal refuges for susceptibility. Survivors of all genotypes experience sublethal effects, the degree varying with their level of susceptibility. Among the effects are longer developmental times and decreased fecundity (Ashfaq et al., 2000; Liu et al., 1999, 2001; Abel and Adamczyk, 2004). Pests such as plant bugs (Lygus sp.) and whitefly are refractory to the toxin. The order of tolerance to Bt toxin of fruit pests is plant bugs \( \gg \) fall armyworm \( \gg \) bollworm \( \gg \) budworm \( \gg \) PBW, and the order of foliage pests is whitefly \( \gg \) soybean looper \( \gg \) beet armyworm \( \gg \) cabbage looper. Fig. 1 shows the survivorship of larval stages on Bt cotton plotted on normalized developmental time. Also shown is the proportional change in developmental time of survivors on Bt cotton and maximum species fecundity on conventional cotton.

The data summarized in Fig. 1 is a synthesis of data from many sources. Ali and Young (1996) found that the LC50 for bollworm (BW) larvae increases with age with late 4–5th instars being largely immune (see Adamczyk et al., 2000, 2001b). The LC50 in BW may vary 13–16-fold geographically (Stone and Sims, 1993). Stewart et al. (2001) found the 4-day survival of susceptible first instar BW larvae fed a diet containing extracts of non-Bt and Bt cotton leaves were 0.92 and 0.34, respectively, while survival rates using extracts of one-day old bolls were 0.83 and 0.1. Survival rates of 2nd instar BW larvae to pupation were 0.65 and 0.07 on non-Bt and Bt cotton leaves, respectively, and delays to pupation of 2nd instar larvae were 8.6 days when reared on artificial diet containing Bt cotton extracts. Some noctuid species are largely immune to Cry1Ac toxin (Moar et al., 1990; Nyouki et al., 1996; Luttrell et al., 1999). For example, BAW survival rates of 0.88.
and 0.93 on Bt and non-Bt leaves, and delays in 2nd instar to pupation of 0.46 days have been observed on Bt cotton. Survival rates of the highly tolerant \( S. frugiperda \) were 0.75 and 0.90 on Bt and non-Bt leaves and no developmental delays were found. Developmental times of susceptible BW and BAW larvae were 43 and 25% longer, respectively, on Bt cotton than on non-Bt cotton, and size at pupation was similarly reduced (cf. Ashfaq et al., 2000). Hogg and Gutierrez (1980) found a linear size-fecundity relationship in BAW, and this is used to scale Bt effects on fecundity in all noctuid species. Food age preferences (Wilson and Gutierrez, 1980; Wilson et al., 1980, 1981; Hogg and Gutierrez, 1980) also play a role in exposure to the toxin.

Eq. (8i) was fit to survivorship data on days feeding \( \theta_{feeding} \) on Bt cotton with species \( s \)-specific parameter \( v_{i,s} \) (Ashfaq et al., 2000). For example, in bollworm mortality in 1st instar larvae is roughly 10% on the first day of feeding and declines rapidly to 1% per day for large larvae on day 24. The mortality rate for BAW is 3.4% the first day of feeding and declines to 0.2% day\(^{-1}\) by day 13. Similarly, computations can be outlined for other noctuid species.

Toxin levels in the food consumed by larvae change daily and greatly influence pest vital rates. These time-varying concentration have important consequences for the control of each pests and for the development of resistance in them (i.e. time varying temporal refuges). In the model, plant subunit age and pest larval age are divided into seven and five age categories, respectively and used to compute larvae age-specific mortality rate \( \mu_{B,t} \). For species and genotype \( N_w \), we assume \( \mu_{B,t} \) (see Fig. 1) is the maximum mortality rate for larval cohort \( j \) (\( j = 1, \ldots, 5 \)) feeding on \( i \)th age fruit \( (i = 1, \ldots, 7) \) at time \( t \), while the realized mortality rate \( \mu_{B,t}^{*} \) \( (i, j, t) \) due to toxin in the diet is computed using (8ii):

\[
\mu_{B,t}^{*}(i, j, t) = (1 - 0.15) \mu_{B,t}(i, j, t) \times \frac{\left( \sum_{a} \mu_{B,t}^{N_w}(i, j, t)f_{M_{a,t}}(i, t) \right)}{\sum_{a} f_{M_{a,t}}(i, t)} .
\]  

(8ii)

The term \( (1 - 0.15) \) corrects for increasing tolerance of larvae to Bt with age \( j \), and the plant supply–demand ratio \( \phi_{l}(t) \) corrects toxin level for current plant growth rate. Larval age-specific survivorship is:

\[
\mu_{B,t}^{*}(i, j, t) = 1 - \mu_{B,t}(i, j, t) .
\]  

(8iii)

In a two Bt-toxin system, say Cry1Ac and Cry2Ab (e.g. Gore et al., 2003), computations for the second toxin follow the same pattern and the combined survivorship is:

\[
\mu_{B,t}^{*}(i, j, t) = \mu_{B,t}^{*}(i, j, t) \times \exp(\mu_{B,t}^{*}(i, j, t)) .
\]  

(8iv)

The sublethal effects of Bt toxin on fecundity and developmental time of survivors are species and genotype specific and are assumed to vary linearly with Bt toxin concentrations in the diet. For example, if the maximum developmental time of a susceptible genotype is 1.4\( \times \) that on conventional cotton, the developmental time falls to 1.2\( \times \) when the toxin concentration falls to half. Similar linear scaling is applied for larval growth rates and fecundity.

1.2.4. Effects of Bt toxin on generalist predators

In conventional cotton, Neesdy and Sterling (1994) found a total predation rate of 81% for bollworm eggs due to the combined action of Orius insidiosus, Orius tristicolor, Geocoris puntipes, Pseudomoscelis striatus and the ant Solenopsis invicta. Using partial ecological life tables, Sansone and Smith (2001) found that \( O. insidiosus \) was the most important predator of BW in conventional cotton, and fitting a curve to their data yields a decreasing egg-larval survivorship function \( \exp(\mu_{pred}(a)) \) on bollworm age \( a \) in \( dd \).

\[
0 \leq \exp(\mu_{pred}(a)) = e^{-0.0185 a} \leq 1 .
\]  

(9)

The daily age-specific mortality rate at time \( t \) varies with temperature \( T(t) \) (i.e. \( \Delta t(n(T)) \) = degree-days day\(^{-1}\) in unsprayed conventional cotton; hence, taking the derivative of (9) and correcting for \( \Delta t(T) \), the rate per day is:

\[
\mu_{pred}(t, a) = 0.0185 e^{-0.0185 a} \Delta t(T) .
\]  

(9ii)

or approximately 0.3 day\(^{-1}\) at 28.4 °C. For example, if BAW’s egg-larval period is 313 \( dd \), then total survival over the period is \( e^{-0.0185 \times 313} = 0.003 \). Similar computations can be made for other noocuid species. Most of the mortality occurs in the younger stages (i.e.
81.6% in the egg-first instar with a range of 70–94%) with 98.3–99.7% mortality occurring before pupation. This estimate concurs with the 25–30% day$^{-1}$ rate estimated in California cotton (Jones and Gutierrez, unpublished) where the same generalist predators are active (see Byerly et al., 1978).

Ponsard et al. (2002) showed that survivorship of O. tristicolor and Geocoris sp. adults collected in non-Bt cotton was reduced 27–28% when fed Bt intoxicated noctuid larvae. Similarly, Hilbeck et al. (1998) found that lacewing Chrysoperla carnea, an important predator in cotton, is also negatively affected when fed Bt toxin, but recent studies suggest the effect may be prey mediated (Romeis et al., 2004). These three predators cause most of the early stage pest mortality (Nuessly and Sterling, 1994). Ponsard et al. (2002) also tested larger adult hemipteran predators, Nabis sp., but no sublethal effects were found. The toxicity of Bt is pest size-dependent (e.g. Ali and Young, 1996), and hence there is likely a similar size relationship in predators.

In the model, daily predation rates on noctuid eggs and 1st–3rd instar larvae are assumed reduced 28% in Bt cotton (i.e. $\lambda = 0.72$, Eq. (9iii)) increasing pest egg-larval survivorship per day.

$$0 \leq 1 - \lambda \mu_{\text{pred}}(t, a) < 1, \text{ for } a < a_{\text{1st instar}} \quad \text{and} \quad (9\text{iii})$$

$$0 \leq 1 - \mu_{\text{pred}}(t, a) < 1, \text{ for } a \geq a_{\text{1st instar}}$$

In a two toxin system, $\lambda$ for each toxin is assumed the same but independent; hence, the combined effect ($\lambda_{1,2} = 0.72^2 = 0.52$) further decreases predation rates.

1.2.5. Insecticide mortality

When included, insecticides are applied biweekly, and are assumed to kill most pest eggs, larvae and adults, but not the pupae in the soil. PBW larvae burrow into fruit and are largely immune. If applied at time $t = t_{\text{spray}}$, insecticide toxicity decays from 0.95 to 0 in say 7 days ($t_{\text{decay}}$) at a rate characterized by the parameter $u$. Other decay rates can be used to reflect different products. The kill rate per day ($\mu_{\text{spray}}(t)$) at time $0 < t - t_{\text{spray}} \leq t_{\text{decay}}$ equals:

$$\mu_{\text{spray}}(t) = 0.95e^{-u(t-t_{\text{spray}})}.$$  \hspace{1cm} (10i)

The survivorship function for sprays is (Eq. (10ii))

$$\gamma(t) = \begin{cases} 0 < (t - t_{\text{spray}})/t_{\text{decay}} \leq 1 & \mu_{\text{spray}}(t) \text{ decay equals:} \\
0, & \text{if } t - t_{\text{spray}} \geq t_{\text{decay}} \\
\end{cases}.$$  \hspace{1cm} (11i)

The effect of spraying and Bt intoxication are incorporated in (9ii) yielding (11i).

$$\mu^{*}_{\text{pred}}(t, a) = \gamma(t)\lambda \mu_{\text{pred}}(t)$$  \hspace{1cm} (11ii)

The action of pesticides and Bt toxin are complementary as both kill pests, but both also adversely affect natural enemy efficacy (Eq. (11ii)) resulting in pest resurgence under certain conditions.

1.2.6. Total age-specific survivorship

The survival rate (i.e. $\eta_{\text{total}}$) of a cohort of pests (number and mass) at time $t$ of age $j$ from all factors is computed as follows.

$$\eta_{\text{total}}(t, j) = \eta_{\text{Bt}}(t, j) \eta_{\text{pesticides}}(t, j) \eta_{\text{natural enemies}}(t, j) \gamma(t)$$  \hspace{1cm} (12)

2. Simulation results

All simulations were run using meteorological data for Brawley, Imperial County, CA between 1 January 1983 and 31 December 2000 (UC/IPM, 2001). Weather data from other locations have also been used (e.g. Stoneville, MS) without changing the general predictions of the model. The dynamics of bollworm and beet armyworm are examined in detail, and the results used as a basis for comparison to the other species using a 2.5% refuge. High initial levels of resistance are
assumed simply to make the direction of changes in resistance obvious.

2.1. Bollworm and beet armyworm

2.1.1. Bollworm in conventional cotton

Populations of highly damaging large BW larvae (instars 3+) are usually low in unsprayed cotton in California because of effective natural enemies. This is predicted in our 17-year simulation (Fig. 2a). However, biweekly applications of insecticides kill not only BW, but also disrupt natural enemies resulting in a two- to three-fold increase in the BW densities (i.e. secondary pest outbreaks, Fig. 2b). Because we assumed no selection pressure and no cost of resistance in conventional cotton, the frequency of the allele for resistance to Bt remained unchanged over the 17 seasons (Fig. 2a and b).

2.1.2. Bollworm in Bt cotton—no refuge

In the absence of a spatial refuge for susceptibility, the number of large bollworm larvae decreases in Bt cotton even though the frequency of the resistance allele triples from the arbitrary high initial value of 0.15 to nearly 0.45 over the 17-years (Fig. 2a versus c). Resistance in BW is slowed by susceptible individuals surviving in the temporal refuge created by BW tolerance to Bt toxin. Within a season, resistance builds early when larvae must feed on young fruit with high toxin concentrations, but declines later as toxin concentrations fall. In the model, developmental time of resistant individual individuals increases 1.2-fold and fecundity decreases 20% (see Halcomb et al., 1996; Ashfaq et al., 2000), and surviving susceptible individuals have a 1.4× developmental time and a 40% decrease in fecundity. Despite the predicted buildup of resistance, BW populations fail to explode because the time varying sublethal effects on BW vital rates are augmented by natural enemy mortality, albeit impaired ca. 30%.

Applying insecticides biweekly in Bt cotton does not increase larval numbers (Fig. 2c versus d) compared to sprayed conventional cotton (Fig. 2b). The lack of increase is due to the combined effects of the pesticide on all exposed stages, the lethal and sublethal effects of the Bt toxin, and the residual predation on egg-larvae stages. The small difference between larval numbers in sprayed Bt cotton (Fig. 2d) and the higher numbers in sprayed non-Bt cotton (Fig. 2b) is a measure of the added effect of the Bt toxin. The frequency of resistance in BW in sprayed Bt cotton declines over time from the arbitrary initial value of 0.15 to roughly 0.07–0.08 is due to the interactions of longer developmental time, and hence greater exposure time to sprays, reduced fecundity, and the diluting effects on resistance of the temporal refuge. Note that the frequency of the allele for resistance in sprayed Bt cotton (Fig. 2d) increases early each season but then declines as more susceptible individuals survive due...
to late season declines in toxin levels. This pattern of within season resistance is also seen in Fig. 2c but is less obvious due to the scale used.

2.1.3. Beet armyworm in conventional cotton

BAW field populations in unsprayed California cotton are usually higher than bollworm populations, and this is seen in our simulation (i.e. 10–12×, Fig. 2a versus Fig. 3a). Assuming the same predation rate, BAW’s faster development, greater availability of food, lower toxin concentrations and higher fecundity easily explains this. Densities of BAW are usually not high enough to cause economic damage and they feed on older leaves. At peak densities, the simulated numbers of large BAW larvae range between 0.05 and 0.12 per plant across all years with the number surviving to pupation being 0.01–0.05 per plant (not shown). The frequency of the resistance remains unchanged in conventional cotton over the 17 seasons because no costs of resistance are assumed. As with bollworm, the biweekly applications of insecticide induce BAW outbreaks roughly 8–10× higher than in unsprayed cotton (Fig. 3a versus b).

2.1.4. Beet armyworm in Bt cotton—no refuge

The dynamics of BAW in Bt cotton and the frequency of the resistance are shown in Fig. 3c. As with bollworm, resistance in BAW builds but the rate is twice as fast (Fig. 2c versus Fig. 3c) reaching unity in 16 years. This is surprising as the temporal refuge for BAW is larger than in HW, at least until one factors in BAW’s higher vital rates. However, despite the development of resistance, BAW populations do not explode due to the tradeoffs between reduced predation and increased developmental times, decreased fecundity (1.1× in resistant and 1.2× in susceptible individuals) and lower direct mortality caused by the Bt toxin. These tradeoffs keep BAW populations lower than in unsprayed conventional cotton during the early years of the run, but noticeable population increases occur as resistance and fitness climb during later years (Fig. 3 (a versus c)).

In sharp contrast, biweekly applications of insecticide in Bt cotton result in rapid selection for resistance and a 8–10× increase in BAW populations (Fig. 3c versus d). These populations are five to seven times higher than in unsprayed conventional cotton (Fig. 3a) because of natural enemy disruption by Bt toxin and pesticide and increases in vital rates of Bt resistant individuals. Factors aiding the rapid selection for resistance are decreased fitness and longer developmental times of susceptible individuals that exposes them longer to all mortality factors, especially insecticide.

Fig. 3. Simulations of populations of large beet armyworm (BAW) larvae per plant with natural control over 17 seasons: (a) conventional cotton and no sprays, (b) conventional cotton with biweekly sprays, (c) Bt cotton, no refuge and no sprays and (d) Bt cotton, no refuge and biweekly sprays. The heavy line in (c and d) is the frequency of the allele for resistance to the Bt toxin. Weather data from Brawley, CA were used to run the model. Dashed horizontal lines are reference lines across treatments.
2.2. The effects of spatial refuges on the development of resistance

The effects of immigration into Bt cotton from various spatial refuges were incorporated as a daily reduction in resistance in BW and BAW adults. If we assume a 5% dilution rate per day, the frequency of the allele for resistance falls to near zero in 7–8 years in both pests (Fig. 4a and c), while at a 15% dilution rate only 3 years are required (Fig. 4b and d). In both species, simulated densities are very low due to the combined action of Bt mortality, the non-lethal effects on an increasingly susceptible population as well as residual predation. Population levels of both pests are half that in unsprayed conventional cotton (Figs. 2a and 3a versus Fig. 4a and c) suggesting the effects of Bt is greater than its reduction of predation rates. In either case, both populations are below damaging levels.

Adding insecticides to the scenario in Fig. 4c causes three- to four-fold increases in BAW populations and increases the rate of decline in resistance (Fig. 4c versus e). This suggests that the disruptive effects of the sprays on natural enemies are greater than the compensatory effect of the Bt toxin. Similar results accrue for BW but the results are not presented.

2.3. Two Bt toxin cotton—BAW

The effect of pyramiding two Bt toxins on BAW population size and the development of resistance is examined by systematically removing factors (e.g. predation, refuges and insecticide). Incorporating genes to express two toxins has been proposed to overcome potential failures of the one gene technology (Tabashnik, 1994a,b; Sachs et al., 1996; Gould, 1998). The increased lethality of two toxins has been demonstrated by Stewart et al. (2001) who found 0.93 and 0.39 survival of 2nd instar BAW larvae on one and two toxin cottons, respectively, with survival in bollworm being considerably lower. If resistance to each toxin (say, RR for Cry1Ac and SS for Cry2Ab) is selected independently, the likelihood of selecting resistant individuals is thought to decrease.

Fig. 4. Simulations of populations of large bollworm (BW, a and b) and beet armyworm (BAW, c–e) larva per plant with natural control over 17 seasons with different levels of refuges: (a) BW, 5% refuge, (b) BW, 15% refuge, (c) BAW, 5% refuge, (d) BAW, 15% refuge and (e) BAW, 5% refuge + biweekly sprays. The heavy lines in the figures are the frequency of the allele for resistance to the Bt toxin. Weather data from Brawley, CA were used to run the model. Dashed horizontal lines are reference lines across treatments.
In our analysis, we assume arbitrarily high resistance gene frequencies of 0.15 for Cry1Ac and 0.1 for Cry2Ab, and the sublethal effect on increase developmental time and decreases in fecundity of surviving susceptible BAW genotypes increases from $1.2\times$ with one toxin to $1.4\times$ with two toxins while the effects on resistant individuals are $1.1$ and $1.2\times$, respectively. We assume that survivorship from predation in a two toxin system is reduced from 0.72 in a one toxin system to 0.52 in a two toxin system (see Eq. (9i)).

### 2.3.1. No refuge

In the absence of natural enemies and spraying, the frequency of the more common resistance allele ($R$ for Cry1Ac) increases from 0.15 to 1 over 4 years while the second allele ($S = 0.1$ for Cry2Ab) requires 6 years. BAW populations explode to 30–40 large larvae per plant (Fig. 5a) as resistance, and hence average fitness increase in the absence of natural enemies. If we include natural enemies but not sprays, the frequency of $R$ increases from 0.15 to 1 and $S$ from 0.1 to 0.38 over the 17 years period with larval populations increasing with resistance (results not shown) but lower compared to Fig. 5a. The important result is that predation slows the development of resistance.

If we add biweekly spraying to the system with natural enemies, population numbers decline to 0.4–0.8 per plant (Fig. 5b) but resistance develops faster (5 and 7 years for $R$ and $S$, respectively).

### 2.3.2. Adding a refuge

Adding a 5% refuge to the system in Fig. 5b causes the frequency of resistance of both alleles to fall quickly to zero in 3 years (Fig. 5c), and BAW populations fall to less than 0.05 per plant due to the increased levels of susceptible genotypes, the direct effect of the sprays, and the residual action of predation. The action of these factors is increased by the longer developmental times of susceptible individuals that increase exposure to mortality factors and reduce fecundity. Increasing the refuge effect, say to 15%, drives the frequency of the resistance genes down faster and reduces populations further (not shown).

---

### Fig. 5. Simulation of populations of large beet armyworm (BAW) larvae per plant over 17 seasons in a two Bt-toxin system: (a) no refuge, no sprays, and no predation, (b) no refuge, with sprays and predation, (c) 5% refuge, sprays and predation, (d) 5% refuge, no sprays but with predation, (e) 5% refuge, sprays but no predation and (f) 5% refuge, no sprays and no predation. The heavy lines in (c and d) are the frequency of the allele for resistance to the Bt toxin. Weather data from Brawley, CA were used to run the model. Dashed horizontal lines are reference lines across treatments.
Removing the sprays but retaining natural enemies increases the time for resistance to fall to near zero in 7–8 years and BAW densities double (Fig. 5d). Comparing Fig. 5d with the same but single toxin scenario in Fig. 4c shows that little benefit accrues from pyramiding the two toxins, as the second toxin merely replaces the mortality that would have been caused by natural enemies.

However, adding spraying but removing natural enemies also gives the same levels of BAW (Fig. 5e versus d) suggesting a tradeoff between mortality caused by spraying and natural enemies. The rate of decline in resistance is greater with sprays.

Removing sprays and natural enemies results in BAW outbreaks (20–25 larvae per plant) with the frequency of allele R oscillating roughly between 0.6 and 0.95 while that of S declines to near zero due to the initial unequal level of resistance to the toxins (Fig. 5f). The populations in Fig. 5f are roughly 50% lower than those in Fig. 5a estimating the beneficial effects of the 5% refuge.

2.3.3. Analysis of the BAW simulation data

While graphical results are instructive, we need to examine the large trends in the data. Year-end values for 12 variables (x1–x12, Table 4) from the 17-year scenarios listed in Table 3 were written to a file, and the data analyzed using multivariate regression. The use of statistical analyses can be questioned for various reasons, but for heuristic purposes it provides a convenient way to summarize the effects of different factors on yield (bale = 480 lbs seed cotton) and season long pest density (log10 cumulative large BAW larvae per season, i.e. log10 larval days). Only variables or combinations of variables having significant slopes (p < 0.05) were retained in the model along with variables that were combined with significant variables (Table 4).

Yield, bales, x7 was significantly correlated only with the length of year in physiological time units (x2) (e.g. water and nitrogen were not limiting) and BAW rarely causes economic damage.

\[
bales = 6.132 - 0.0011 x_7 \\
r = 0.282, F = 21.89, \text{d.f.} = 288 \quad (13i)
\]

The slope for physiological time was negative suggesting yields decreased with season length, likely due to pest buildup in longer seasons. No other variables had a significant effect including the presence-absence of the Bt technology or of sprays. Average yield across all treatments was 2.60 ± 0.40 bales.

The regression model for log10 cumulative large BAW larvae is given in Eq. (13ii).

\[
\log_{10}\text{BAW} = 8.56 - 4.54 x_1 - 5.87 x_4 - 11.66 x_3 x_4 + 2.52 x_3 - 0.98 x_4 - 0.23 x_3 x_4 + 9.61 x_{12} \\
R = 0.68, F = 34.73, \text{d.f.} = 281 \quad (13ii)
\]

Table 3

<table>
<thead>
<tr>
<th>Run</th>
<th>Bt</th>
<th>BAW</th>
<th>Sprays</th>
<th>Dilution rate</th>
<th># of toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>0.05</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.05</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>0.10</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.10</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>0.15</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.15</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>0.10</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.10</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>0.15</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.15</td>
<td>2</td>
</tr>
</tbody>
</table>

* Dilution rate per day of the frequency of the allele for resistance.

Table 4

<table>
<thead>
<tr>
<th>Variables</th>
<th>Definition</th>
<th>Range of values</th>
</tr>
</thead>
<tbody>
<tr>
<td>x1</td>
<td>Year</td>
<td>1–17</td>
</tr>
<tr>
<td>x2</td>
<td>Total degree-days&gt;12°C</td>
<td>&gt;2500</td>
</tr>
<tr>
<td>x3</td>
<td>Bt presence-absence</td>
<td>0 or 1</td>
</tr>
<tr>
<td>x4</td>
<td>Biweekly spraying</td>
<td>0 or 1</td>
</tr>
<tr>
<td>x5</td>
<td>Sprays during season</td>
<td>6–8</td>
</tr>
<tr>
<td>x6</td>
<td>Bt toxins in plant</td>
<td>0–2</td>
</tr>
<tr>
<td>x7</td>
<td>Yield in bales</td>
<td>0.5–3.6</td>
</tr>
<tr>
<td>x8</td>
<td>Log10 large BAW days</td>
<td>–</td>
</tr>
<tr>
<td>x9</td>
<td>BAW presence-absence</td>
<td>0 or 1</td>
</tr>
<tr>
<td>x10</td>
<td>Allele dilution rate</td>
<td>0–1</td>
</tr>
<tr>
<td>x11</td>
<td>Frequency of allele R</td>
<td>0–1</td>
</tr>
<tr>
<td>x12</td>
<td>Frequency of allele S</td>
<td>0–1</td>
</tr>
</tbody>
</table>

* Scalar as proportion not diluted.

a Frequency at the end of the season.
Factors decreasing BAW larval numbers were the presence–absence (i.e. 0, 1) of the Bt technology ($x_1$, [0, 1]), spraying ($x_4$, [0, 1]), the number of Bt toxins ($x_6$, 0–2) and the interactions Bt x spraying ($x_3x_4$) and the number of sprays x number of toxins ($x_4x_6$). In contrast, level of resistance ($x_{11}$) and the number of sprays ($x_4$) increased BAW density. The refuge effect ($x_{10}$) failed to be significant either singly or in combination with other terms. Of the terms retained in the model, only spray ($x_4$) and the number of Bt toxins ($x_6$) were not significant in their own right. The beneficial effect of the spatial refuge ($x_{10}$) was non-significant likely due to the larger temporal refuge created by BAW tolerance to Bt.

2.4. Comparison of Bt effects across pest species

2.4.1. Pests of cotton fruit

Pink bollworm has a narrow host range, while fall armyworm > bollworm > budworm are polyphagous and feed on non-Bt crops and wild hosts. In addition, these noctuid pests are highly migratory; hence, the spatial refuge is likely much greater than the 2.5% assumed here for comparative purposes. The results are presented as total larvae $m^{-2}$ (i.e. per 6.6 plants).

In unsprayed California cotton, most noctuid pests, but not pink bollworm (Fig. 6a), are kept below economic levels by the action of natural enemies (e.g. Fig. 6c and e). In the Bt cotton simulations, resistance in budworm > bollworm increases despite the 2.5% spatial refuge (Fig. 6d and f) and their small modest temporal refuges. As resistance increases, there is a three- and four-fold increase in budworm and bollworm populations compared to unsprayed conventional cotton (Fig. 6c and e) versus (d and f). This occurs because the lethal and sublethal effects of the Bt toxin are less than the 28% reduction in predator efficacy. In contrast, there is a 10–15× decrease in pink bollworm despite resistance buildup (Fig. 6b) due to the large non-lethal effects on PBW vital rates. Compared to conventional unsprayed cotton, only pink had lower populations in Bt cotton after resistance developed.

Increasing the size of the spatial refuge increases the diluting effect on resistance in bollworm and budworm and if large enough would reverse resistance. Unfortunately, this would not decrease their population densities much as there is a tradeoff between their tolerance to the toxin and reduced predator efficacy.

Fig. 6. Pest dynamics $m^{-2}$ (6.6 plants $m^{-2}$) in conventional and Bt cotton with a small 2.5% refuge: pink bollworm (PBW, a and b), budworm (TBW, c and d) and bollworm (BW, e and f). The stepped lines in (b, d and f) indicate the level of resistance (right legend). Weather data from Brawley, CA for the period 1982–2003 (UC/IPM) were used to run the model. Dashed horizontal lines are reference lines across treatments.
Resistance in the highly tolerant fall armyworm decreases because of its large temporal refuge, but its populations more than double in Bt cotton due to reduced natural enemy efficacy resulting in Bt induced secondary pest increases (Fig. 7(a versus b)).

In general, the development of resistance in Bt crop with 2.5% refuges would occur at rates proportional to their susceptibility (pink bollworm = budworm > bollworm) and population increases in the noctuids would be related to their tolerance to Bt toxin (fall armyworm > bollworm = budworm).

2.4.2. Defoliating pests (Noctuidae)

As with fruit feeding noctuids, defoliator populations are largely kept in check by the action of natural enemies in conventional unsprayed California cotton (Fig. 7c and e). The order of tolerance to Bt toxin is soybean looper > beet armyworm > cabbage looper. Simulation results for soybean looper are similar to those for fall armyworm and are not shown.

In Bt cotton, the combination of the temporal refuge created by tolerance to the toxin and the small 2.5% spatial refuge assure that resistance declines in beet armyworm and cabbage looper (e.g. Fig. 7d and f).

Populations of beet armyworm and cabbage looper decline 12 and 2×, respectively, compared to conventional cotton, while those of highly tolerant soybean looper increased 2–3× (not shown, see fall armyworm, Fig. 7b). Declines in beet armyworm and cabbage looper populations are due to the combined non-lethal effects of the toxin on pest vital rates and the action of natural enemies, albeit impaired. Soybean looper populations increase because the non-lethal effects of the toxin on its vital rates are less than the reduction in the action of natural enemies.

2.4.3. Plant bugs and whitefly

Lygus bug levels in conventional cotton in California are normally low and not economic because of plant compensation and the action of natural enemies (Falcon et al., 1968; Gutierrez et al., 1975) (Fig. 8a). Increases in plant bug populations have been reported in Bt cotton in the USA (Hardee and Bryan, 1997; Naranjo, personal communication; Willers, personal communication), but the cause is unknown. The same generalist predators attacking lepidopterous pests also attack Lygus, and sprays are known to increase Lygus numbers. Lygus is assumed immune to the Bt toxin, and in our simulations Lygus nymph densities nearly
double and adult populations increase about 30% compared to conventional cotton due solely to decreased predator efficacy (Fig. 8b).

Whitefly densities are commonly low in unsprayed conventional cotton where they are attacked by generalist predators and stenophagous parasitoids (von Arx et al., 1984; Mills and Gutierrez, 1999; Naranjo, 2001). Data on whitefly increases in Bt cotton are anecdotal and may be associated with the use of insecticides to control other pests (e.g. von Arx et al., 1984). Non-target effects of Bt on parasitoids have been documented only in maize stemborer (Bernal et al., 2001), but unlike many lepidoptera, whitefly does not appear to transform the Bt protoxin (Fig. 8c); hence, any increases in whitefly densities would arise from the impairment of generalist predators in Bt cotton or as a byproduct of the use of insecticides against pests not controlled by Bt.

3. Discussion

There is increasing adoption of transgenic cotton expressing a Bt protoxin worldwide and in the USA, it has been fueled in part by the continued good control of tobacco budworm (TBW) and pink bollworm (PBW) (Williams, 2000). Highly toxic insecticides may dissipate quickly while Bt toxins are weak insecticides that are ever-present in the plant, albeit in time varying concentrations. Of major concern is the specter of resistance to Bt toxins, and to combat this possibility, spatial refuges of conventional cotton have been mandated to slow or prevent its development (Gould, 1986; EPA, 2000). The mandated refuge strategy was developed and implemented despite the fact that little is known about adult pest movement and migration among fields by any of the pest species (Vacher et al., 2003; Linacre and Thompson, 2004; Gutierrez and Ponsard, 2005) making the size of proposed refuges a rough estimate at best (Carriere et al., 2001). Furthermore, in China, Xu et al. (2002) found that the lack of a consistent high dose of the toxin in Bt cotton and the complexity of managing individual fields in the same region, made the use of the refuge strategy difficult to implement.

3.1. Stenophagous versus polyphagous pests

It is interesting that insecticide resistance did not develop in the highly stenophagous PBW despite the massive quantities of insecticides applied over several years, but resistance did develop in bollworm and budworm (van den Bosch, 1978). This occurred in part because PBW burrowed into the fruit where it created a temporal refuge from insecticides, while bollworm and budworm fed externally and received the full selective impact. However, in Bt cotton the predictions concerning resistance buildup are reversed due largely to PBW’s high susceptibility that virtually eliminates its temporal refuge and its lack of significant natural spatial refuges. This greatly increases the selection pressure for Bt resistance and would appear to make PBW a good candidate for the “high dose-refuge” strategy for resistance management (see Gould, 1986, 1998;
In practice, the very low level or even failure of Bt resistance to develop in Arizona is more likely due to the very large spatial refuge created by the 44–49% non-adoption of the Bt technology (Gutierrez and Ponsard, 2005).

In contrast, control of polyphagous noctuid and other pests in Bt cotton has not been fully dependable (Burris et al., 1994; Mahaffey et al., 1995; Lambert et al., 1996; Mascarenhas and Luttrell, 1997; Adamczyk et al., 1998; Turnipseed and Sullivan, 1999; Luttrell et al., 1999; Stewart et al., 2000; Ru et al., 2002 in China) often requiring supplemental insecticide treatments for their control. Spatial refuges for susceptibility in polyphagous pests exist in other crops and wild hosts, and temporal refuges for susceptibility exist within the crop due to differing levels of tolerance among pest species that increases with age and the time varying concentration of toxin in the plant (e.g. Ali and Young, 1996; Ashfaq et al., 2000; Olsen and Daly, 2000; Pitt, 1998; Greenplate, 1999; Adamczyk et al., 2001a). Pests such as plant bugs (Lygus) and whitefly are thought to be immune to the toxin. The order of tolerance to Bt toxin in fruit pests is: Lygus (Ly) > fall armyworm > bollworm > budworm > pink bollworm. The order of tolerance in foliage pests is whitefly (WF) > soybean looper > beet armyworm > cabbage looper. Surviving individuals have reduced vital rates (increased developmental time and reduced fecundity) that vary with species and genotype tolerance. Complicating the problem are adverse effects on natural enemies feeding on intoxicated prey (Hilbeck et al., 1998; Ponsard et al., 2002).

So how big should the refuges be? This question appears to remain in the realm of the imponderable. Vacher et al. (2003) posited that refuges for tobacco budworm (Heliothis virescens) should be ca. 25%, much higher than the mandated 5% refuge (EPA, 2000). However, many noctuid pests are highly migratory and have large refuges in alternative non-Bt hosts. In the extreme, many late season budworm moths captured in Louisiana and Texas were migrants whose larvae developed on corn (possibly Bt corn) in more northern climes, and the majority of over-wintering forms may be migrants (Gould et al., 2002). Our model suggests that the mandatory spatial refuge strategy may not be appropriate for polyphagous noctuid pests as their sizeable spatial and temporal refuges may be sufficient to stop the development of resistance, but there are other important considerations.

3.2. Factors affecting resistance development

The model predicts that without spatial refuges, pest resistance in Bt cotton could develop more rapidly in moderately tolerant pests such as beet armyworm than in moderately susceptible ones (e.g. bollworm) because resistant BAW genotypes have short development times and higher fecundity than more susceptible pests (e.g. BW, Fig. 2c versus Fig. 3c). In both cases, the temporal refuge slows the development of resistance but does not stop it. However, if the influx of susceptible individuals from spatial refuges is large (>5% day⁻¹), this in concert with temporal refuges causes resistance in BW and BAW to decline (Fig. 4a and c). This may explain the current lack of resistance in many noctuid field populations.

Insecticide use in conventional cotton is known to disrupt natural enemies and causing outbreaks of secondary pests (van den Bosch, 1978), but spraying in Bt cotton has additional complications. Bt tolerant pests may increase in Bt cotton because of moderate to high tolerance to BT and compromised natural enemy efficacy, and supplemental insecticide used to control them may exacerbate pest problems generally (e.g. plant bugs, fall armyworm, bollworm). This possibly explains the results of Mascarenhas et al. (1999) and Parker et al. (2000) and others in the USA, Qaim and Zilberman (2003) in India and Ru et al. (2002) in China.

Insecticide use may differentially influence the development of resistance in pests. For example, in Bt cotton without refuges, spraying may decrease the rate of resistance development in BW, but increases it in BAW (Fig. 2d versus Fig. 3d). This occurs because the BAW resistant genotype has a shorter development time and higher fecundity relative to susceptible BAW genotypes and to BW generally. This lessens exposure time to insecticides in resistant BAW genotypes that also have higher fecundity. However, if both a 5% spatial refuge and spraying are used in Bt cotton (Fig. 4c), resistance in BAW declines rapidly, but larval populations increase 2× compared to unsprayed Bt cotton (Fig. 4c). This increase in BAW is a measure of the added disruptive effects of sprays on natural enemy efficacy in Bt cot-
ton. Similar results accrue for BW and other noctuid pests.

3.2.1. Pyramiding technologies
Pyramiding genes for additional protoxins in cotton’s genome has been suggested for control of highly tolerant or refractory pests (Tabashnik, 1994a,b; Sachs et al., 1996; Gould, 1998), and there has been some implementation (Greenplate et al., 2000; Stewart et al., 2000, 2001). Pyramiding could produce a cocktail of toxins similar to the failed use of insecticide mixtures that historically led to multiple and cross-resistance to insecticides and to increased levels of secondary pests (van den Bosch, 1978). In our simulation study on BAW, pyramiding two Bt toxins yielded the same levels of pests as in the one toxin scenario (Fig. 4c versus Fig. 5d), because the second toxin merely compensated for the added decline in natural enemy efficacy. In other cases, pyramiding could increase selection pressure for resistance in highly susceptible species (e.g. PBW, budworm), increase the potential for the development of cross resistance to Bt toxins, decreased natural enemy efficacy (see above) and an increased risk of the development of cross resistance to Bt toxin among pest species resulting in different rate of resistance development and increased levels of some pests due to the reductions in natural enemy efficacy, increases that may require insecticide use against highly tolerant species (e.g. boilworm, fall armyworm, soybean looper, Lygus bugs). Furthermore, the use of insecticides to control Bt tolerant pests may increase pest numbers and in some cases accelerate the development of resistance in some species because resistant individuals have shorter developmental times that lower their exposure to insecticides and natural enemies, and in addition they have higher fecundity than surviving susceptible genotypes (Gould et al., 1991). In some noctuid pests, there is a tradeoff between their suppression by the Bt toxin and the loss of natural enemy efficacy—a tradeoff that can be viewed as a placebo effect as some pest populations would be about the same with and without the Bt technology (e.g. in California’s Great Central Valley). What is clear is that pest resurgence and secondary pest outbreaks would increase with widespread planting of Bt cotton, especially as larger monocultures develop (cf. Gould, 1998; Hilder and Boulter, 1999), and all of this would be greatly exacerbated if pest fitness increases with resistance.

Hence, it is increasingly relevant to asked whether farmers are being involved in a grand experiment in which very little a priori research had been devoted to examining potential consequences of extant (Carrière et al., 2001) and developing biotechnologies in pest control. To date, the negative impacts of Bt crops are largely unexplored or dismissed as speculations, while positive results, often using industry data, are broadly advertised (e.g. economists Qaim and Zilberman, 2003). While we caution that the predictions of models such as ours include assumptions that need to be fine-tuned, the assumptions are not irrational fears of biotechnology luddites, rather they constitute
a more rational and plausible scenario than economic models based on a blind faith in the wisdom of the invisible hand of the market (cf. Zadoks and Waibel, 2000) or generalities (Ortman et al., 2001) or analyses that ignore trophic and other complexities (Quim and Zilberman, 2003) that may lead to unexpected consequences (Obrycki et al., 2001, Quist and Chapela, 2001, see http://www.biotech-info.net/gene_flow.html).

Our model was designed as a mechanism for incorporating new findings, and for examining on an ongoing basis the Bt and other biotechnologies and agronomic practices as they are implemented (e.g. pyramiding technologies, cultural practices, insecticides) (see Gutierrez and Ponsard, 2005). Our multi-species analysis of the Bt cotton technology is a step toward resolving some of the large issues associated with biotechnology innovation in pest control.

Acknowledgements

We thank Professor Bruce Tabashnik for suggestions, and Dr. Eric Natwick for valuable discussions on the history of pink bollworm in Imperial County, California. We also wish to thank Professor Gordon Rauser, former Dean of the College of Natural Resources at Berkeley for stimulating our interest in transgenic crop protection technologies. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the University of California or the US Department of Agriculture.

References

Abel, C.A., Adamczyk Jr., J.J., 2004. The effect of different photoperiodic stages on expression of Cry1A in maize leaves and cotton bolls, and corresponding development of fall armyworm (Lepidoptera: Noctuidae) and southwestern corn borer (Lepidoptera: Noctuidae) development on maize whorl leaf profiles. J. Econ. Entomol. 97, 1727–1744.


Adamczyk Jr., J.J., Holloway, J.W., Church, C.E., Leonard, B.R., Graves, J.B., 1998. Larval survival and development of the fall armyworm (Lepidoptera: Noctuidae) on normal and transgenic cotton expressing the Bacillus thuringiensis Cry1A(c) δ-endotoxin. J. Econ. Entomol. 91, 539–545.


cost associated with resistance to transgenic cotton in the pink
bollworm (Leptolopha gelechialis). J. Econ. Entomol. 94 (4),
935-941.

Chiu, C.C., Henneberry, T.J., Weddle, R.C., Natwick, E.T., Car-
son, J.R., Valenzuela, C., Bixas, S.L., Stanan, R.T., 1996. Reduc-
tion of pink bollworm (Leptolopha gelechialis) popu-
lations in Imperial Valley, California, following mandatory
short-season management systems. J. Econ. Entomol. 89, 175–
182.

Ehler, L.E., Eveleens, K.G., van den Bosch, R., 1973. An evalua-
tion of some natural enemies of the cabbage looper in cotton

EPA, 2000. Bt plant pesticides biopesticides registration action
cotton_final.pdf.

Eveleens, K.G., van den Bosch, R., Ehler, L.E., 1973. Sec-
ondary pest outbreak induction by experimental insecticide
applications in cotton in California. Environ. Entomol. 2, 497–
505.

Falcon, L.A., van den Bosch, R., Ferris, C.A., Strombhrt, L.K.,
season-long cotton pest-control programs in California. J. Econ.
Entomol. 61, 56-66.

Fitt, G.P, 1998. Efficacy of INGARD cotton-patterns and its conse-
quences. In: Proceedings of ninth Australian Cotton Conference,
The Cotton Research and Development Corporation, Broad-
brush, Australia, pp. 233-245.

Gianessi, L.P., Carpenter, J.E., 1999. Agricultural Biotechnol-
y: Insect Control Benefits, National Center for Food and
Agricultural Policy, USA. http://www.ncap.org/pub/biotech/
insectcontrolsbenefits.pdf.

tolopha Notistant) survival on Bollgard and Bollgard II
cotton buds and flower components. J. Econ. Entomol. 94,
1445-1451.

Gould, F., 1986. Simulation models for predicting durability of
insect-resistant germplasm: a deterministic diploid, two-locus
model. Environ. Entomol. 15, 1-10.

Gould, F., 1998. Sustainability of transgenic insecticidal cultivars:
modeling pest genetics and ecology. Ann. Rev. Entomol. 43,
765–776.

Gould, F., Anderson, A., Jones, A., Stumerford, B.R., Heckel, D.G.,
frequency of alleles for resistance to Bacillus thuringiensis toxins
in field populations of Heliothres zeaefollicus. Proc. Natl. Acad.

Gould, F., Blant, N., Redd, M., Reutin, T., Lopez, J., Micinski, S.,
2002. Bacillus thuringiensis-toxin resistance management:
stable isotope assessment of alternate host use by Helicoverpa

Gould, F., Kennedy, G.G., Johnson, M.T., 1995. Effects of nat-
ural enemies on the rate of herbicide adaptation to resistant host

Gould, F., Martinez-Ramirez, A., Anderson, A., Ferre, J., Silva,

Greenleaf, J.T., 1999. Quantification of Bacillus thuringiensis insect
control protein Cry1Ac over time in bollgard cotton fruits and
terminals. J. Econ. Entomol. 92, 1379–1383.

Greenleaf, J.T., Penn, B.R., Sharphey, Z., Oosthuizen, M., Mann,
J., Reisch, B., Osborn, J., 2000. Bollgard II efficacy—quantifica-
tion of total lepidopteran activity in a gene product. In: Dugger,
P., Richter, D. (Eds.), Proceedings of Belweide Cotton Confer-
941–963.

models of predator-prey energetics: I. Age specific ener-
tics models—pupa aphid Acyrthosiphon pisum (Harris) (Homoptera:
Aphilidae) as an example. Can. Entomol. 116, 923–
932.

Gutierrez, A.P., Falcon, L.A., Leres, W.B., Lepizg, P., van den Bosch,
for Acala cotton and the efficiency of defoliators on its yields.
Environ. Entomol. 4, 125–136.

Gutierrez, A.P., Ghezelbash, R., Ellis, C.K. Analysis of the climatic
limits of pink bollworm in Arizona and California. Acta Oecol.,
in press.

Gutierrez, A.P., Pennard, S., 2005. Physiologically based demo-
graphics of Bt cotton-pest interactions: I. Pink bollworm resis-

of cotton production in California: Lygus hesperus injury—an eval-

Halcromb, J.L., Benedict, J.H., Cook, B., Ring, D.R., 1996. Sur-
vival and growth of bollworm and tobacco budworm on non-
transgenic and transgenic cotton expressing a Cry1Aa insecticidal
protein (Leptolopha Notianta). Environ. Entomol. 25, 250–
255.

Hander, D.D., Bryan, W.W., 1997. Influence of Bacillus thuringiensis-transgenic and nectarless cotton on insect popu-
lations with emphasis on the tarnished plant bug (Helicopera:
Miridae). J. Econ. Entomol. 90, 663-668.

Hilbeck, A., Baumgattert, M., Fried, P.M, Bigler, F., 1999. Effects of
transgenic Bacillus thuringiensis corn-fed prey on mortality and
development time of immature Chrysoperla carnea (Neuroptera:


Hogg, D.B., Gutierrez, A.P., 1980. A model of the flight phenol-
ogy of the bunt armyworm (Leptolopha Notianta) in Central
California. Hilgarkia 48, 1–36.

ural enemy conservation and planting date on the susceptibility
of Bt cotton to Helicoverpa zea in North Carolina. In: Dugger,
P., Richter, D. (Eds.), Proceedings of Belweide Cotton Confer-
931–942.


Liu, Y.B., Tabashnik, B.E., Meyers, K.S., Carriere, Y., Bartlett,
A.C., 2001. Genetics of pink bollworm resistance to Bacil-
lus thuringiensis toxin Cry1Ac. J. Econ. Entomol. 94, 248–
252.


