

Spatial Processes in the Evolution of Resistance in *Helicoverpa zea* (Lepidoptera: Noctuidae) to Bt Transgenic Corn and Cotton in a Mixed Agroecosystem: a Biology-rich Stochastic Simulation Model

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ABSTRACT A simulation model is developed to examine the role of spatial processes in the evolution of resistance in *Helicoverpa zea* populations to Bt corn and Bt cotton. The model is developed from the stochastic spatially explicit *Heliothis virescens* model described by Peck et al. (1999), to accommodate a spatial mix of two host crops (corn and cotton), and to reflect the agronomic practices, as well as the spatial and temporal population dynamics of *H. zea*, in eastern North Carolina. The model suggests that selection for resistance is more intense in Bt cotton fields than in Bt corn fields. It further suggests that local gene frequencies are highly dependent on local deployment levels of Bt crops despite the high mobility of the adult insects. Region-wide average gene frequencies depend on the region-wide level of Bt deployment, so incomplete technology adoption slows the rate of resistance evolution. However, on a local scale, *H. zea* populations in clusters of fields in which Bt use is high undergo far more rapid evolution than populations in neighboring clusters of fields in which Bt use is low. The model suggests that farm-level refuge requirements are important for managing the risk of resistance. The model can be used as an aid in designing plans for monitoring for resistance by suggesting the appropriate distribution of monitoring locations, which should focus on areas of highest Bt crop deployment. The findings need to be placed in the context of the input parameters, many of which are uncertain or highly variable in nature, and therefore, a thorough sensitivity analysis is warranted.

KEY WORDS *Bacillus thuringiensis*, transgenic crops, computer simulation, resistance management

IN RECENT YEARS, VARIETIES of corn and cotton expressing δ -endotoxin crystal proteins (Cry) from *Bacillus thuringiensis* (Berliner) (Bt cotton and Bt corn) have been widely deployed. These are effective at protecting the crops from attack by a variety of lepidopteran pests (Armstrong et al. 1995, Benedict et al. 1996). Bt crops can be valuable pest management tools, improving the efficiency and safety of agricultural production. They can also reduce the chemical inputs needed and thus may improve agricultural sustainability. Preserving their efficacy has become a high priority of entomologists, the biotechnology industry, and regulatory authorities (Environmental Protection Agency 1998).

Whenever pests are exposed to pesticides, selection occurs for alleles that confer resistance to those pesticides. In recent years, field and laboratory populations of insects resistant to Cry1 Bt toxins have been

reported (see Tabashnik 1994, Chaufaux et al. 1997, Perez and Shelton 1997, Luttrell et al. 1999, Huang et al. 1999, Burd et al. 2000, Tabashnik et al. 2000). With constitutive expression of the toxins throughout the plant and for the entire growing season, Bt crops have the potential to place the highest selection pressure for such resistance of any insecticide deployed to date (Wearing and Hokkanen 1995).

These concerns have led to much effort to develop practical resistance management plans that can delay resistance evolution for 10-15 yr while permitting significant use of the new technology (Environmental Protection Agency 1997). As we learn more about interactions among the biological, genetic, environmental, agronomic, and operational factors that influence the rate of resistance evolution (Georghiou and Taylor 1977a, b), we are learning that generalizations are rarely valid. This in turn has led to the development of sophisticated computer models and a demand for system-specific modeling tools. Integration of spatial population dynamics and population genetics enables the examination of how new insecticidal tech-

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nologies affect the area-wide pest populations, and how the pest populations can respond.

The management of insect resistance to insecticides has for many years stimulated the development of theoretical models to explore the forces that determine the rate at which such resistance evolves (e.g., Georgioui and Taylor 1977a, b, Taylor and Georgioui 1979, Tabashnik 1986, Roush 1989). The advent of transgenic insecticidal crops has further boosted this work (e.g., Gould 1994, Roush 1994, Alstad and Andow 1995, Onstad and Gould 1998). The role played by spatial processes has been explicitly addressed in several recent papers (e.g., Mallet and Porter 1992, Caprio and Tabashnik 1992, Peck et al. 1999)

Helicoverpa zea (Boddie) is a lepidopteran pest of several crops in the USA. Two of its major host crops are corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.). Bt varieties of these crops provide moderate control of *H. zea* (Mahaffey et al. 1995, Lambert et al. 1996, Benedict et al. 1996, Halcomb et al. 1996, Sims et al. 1996, Pilcher et al. 1997, Lynch et al. 1999, Storer et al. 2001). The toxins expressed in currently available Bt varieties of corn (Cry1Ab) and cotton (Cry1Ac) are very similar in structure and mode of action. Cross-resistance to these toxins has been reported in populations of the lepidopteran *Plutella xylostella* (Tabashnik et al. 1994) and *Heliothis virescens* (Gould et al. 1992). It is therefore a reasonable assumption that alleles that confer cross-resistance to both toxins exist at low frequencies in populations of *H. zea*. To address concerns about such resistance evolving in *H. zea*, a sound understanding of how resistance may evolve in this species is required to guide a practical resistance management strategy.

This paper describes a spatially-explicit computer model that simulates the population dynamics and population genetics of *H. zea* in a mixed cropping system. Resistance alleles, as genetic mutations of wild-type alleles, are likely to be rare. Therefore, random events, such as genetic drift, local extinction, and deviations from Hardy-Weinberg genetics, are likely to play significant roles in the onset of resistance (Caprio and Tabashnik 1992). To simulate these random events, a stochastic model of *H. zea* was developed, where the number of insects of each genotype in each field are integers, and several events are probabilistic rather than deterministic. The model integrates agronomy, the biology of *H. zea*, insect resistance genetics, and the effects of currently available Bt corn and cotton varieties on the insect. It simulates resistance evolution over a wide area of mixed crops and weed hosts. The model has its roots in both population genetics and population dynamics theory.

The population dynamics of *H. zea* in eastern North Carolina were first modeled by Stinner et al. (1974) (HEL SIM), and improved by Stinner et al. (1977b) (HEL SIM II). Their model incorporated several host crops and important life history processes to predict the size, location, and timing of damaging populations. The density-dependent effects of cannibalism on the size and timing of the adult population from ear-stage cornfields was modeled by Stinner et al. (1977a). The

principles and processes in these models were adapted for use in the present model.

The population genetics in the model were adapted from the model of Peck et al. (1999), a stochastic spatially-explicit model of resistance evolution to Bt cotton in *H. virescens* populations. In the current study, Peck's model is modified to accommodate a spatial mix of multiple hosts of the polyphagous pest, *H. zea*, with similar Bt toxins expressed in cotton and corn. The model incorporates the spatial and temporal population dynamics of *H. zea* as described for eastern North Carolina. Additionally, the effects of Bt crops on the insect population were altered to reflect the moderate-dose imposed on *H. zea* by the Bt crops (causing <100% mortality of susceptible insects) as opposed to the high dose (\approx 100% mortality of susceptible insects) imposed on *H. virescens*. This model represents the first biology-rich, stochastic, spatially-explicit model simulating the evolution of resistance to Bt toxins in multiple host crops. The model is parameterized using historical field data on *H. zea* biology in eastern North Carolina, and field data on the effects of Bt corn on *H. zea* that are presented in Storer et al. (2001). This paper describes the model and uses it to investigate the effect of deployment patterns of Bt crops on the spatial and temporal patterns of resistance evolution. A companion paper (Storer et al. 2003) describes a sensitivity analysis to look at the effect of altering the value of many of the model parameters on the model output.

Materials and Methods

The single-crop, high Bt dose, spatially explicit model presented by Peck et al. (1999) was modified as follows to simulate the more complex biology of *H. zea* and more complex agronomy in a mixed cropping system.

Agricultural Environment. A geographical area, representing a section of eastern North Carolina, is divided into a spatial field grid of 24 or 30 rows and 24 or 30 columns. Each field is 10 acres, for a total of 5260 or 9000 acres modeled. Preliminary model runs showed that region sizes above 12×12 had little effect on the model output, and that field sizes of 10 acres or larger performed similarly. Ten acres is an average field size for eastern North Carolina (J.W.V.D., unpublished data). The simulated region is planted to four host crops: Bt cotton, non-Bt cotton, Bt corn, and non-Bt corn.

Each year starts in mid-May with insects emerging as adults from diapaused pupae (Rabb et al. 1975). As the year progresses, the phenology of the crops and weeds advances. Early in the year, cornfields are in the whorl stage, cotton fields are prebloom, and weed hosts are available in and around each field. The weeds are used by a proportion, up , of insects. At the beginning of July, weeds become less suitable and available as hosts, and corn enters the reproductive stage, producing silks and ears. During 11 d in the middle of July, cotton starts to bloom and corn silks start to dry. During August, cotton reaches peak bloom and boll

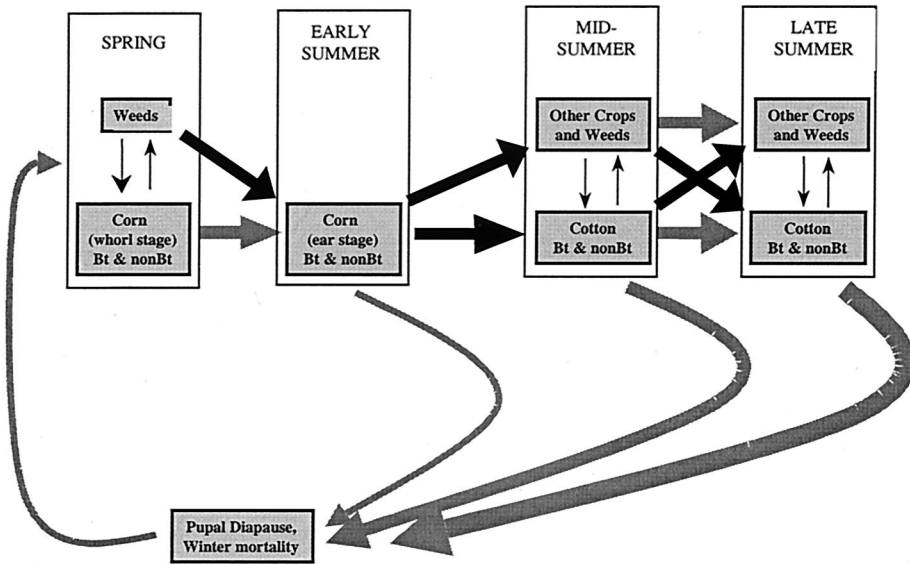


Fig. 1. Sequential host use by *H. zea* in eastern North Carolina through four generations. Dark arrows indicate where the insects switch hosts as the crop phenology changes through the year. As the season progresses an increasing proportion of insects enter diapause as pupae, as indicated by the increasing width of the gray arrows from later generations.

productions, while corn plants are unattractive and unsuitable as hosts. During the second half of August, through September, cotton fields mature. The season ends as the fields are harvested in October. This phenology follows that typical for eastern North Carolina (Fig. 1) (J.W.V.D, unpublished data), and here is followed deterministically for all fields.

Agronomic Operations. A proportion, P_{corn} , of the fields in the region is planted to corn. The remaining proportion of the fields ($1 - P_{corn}$) is planted to cotton. Bt_{CR} is the proportion of cornfields that are planted to Bt hybrids. Bt_{CT} is the proportion of cotton fields that are planted to Bt varieties. The spatial mix of crops can be fixed or randomized, and can be changed each year to simulate crop rotation. With random crop assignment, as used for the runs presented here, some fields can remain in the same crop for more than 1 yr, representing fields that are not rotated. Figure 2 shows an example of a random field layout with 55% of fields as corn (P_{corn}), 75% of which are Bt hybrids (Bt_{CR}), with the remaining 45% of fields as cotton with 75% as Bt hybrids (Bt_{CT}).

Insecticidal spray thresholds in Bt and non-Bt cotton are adjusted to correspond with the North Carolina Agricultural Extension Service recommendations for treatment of *H. zea* (Bacheler 1996a). In addition to the larval threshold in the *H. virescens* model, an egg threshold can also be employed. The number of eggs in a field is derived in the *H. zea* model from the number of neonate and 1-d-old larvae by assuming 90% natural mortality of eggs and 50% natural mortality of neonates in the first day after hatching (the number of eggs is thus assessed as 10 times the number of newly produced larvae plus twice the number of 1-d-old larvae). Using this modeling convenience, a

2-d delay between field scouting and field treatment is built in. These numbers reflect the number of eggs laid before egg mortality factors, such as parasitism and predation, take effect. Because in the field, only eggs on terminals are the primary target for scouting, the recommended egg threshold of 10 eggs per 100 terminals (Bacheler 1996b) is modified in the model to account for the distribution of eggs on cotton plants according to Farrar and Bradley (1985). The egg threshold for Bt fields is 10 times higher than for non-Bt fields, as recommended for North Carolina Bt cotton to allow for high larval mortality. The sprays



Fig. 2. Random crop layout: 55% of fields are corn; 45% are cotton. 75% of fields in each crop are planted to Bt cultivars. The region size is 30 fields \times 30 fields.

have a residual activity that declines linearly to zero over a period of 7 d, during which time the field is not sprayed, as recommended by Bachelier (1996b). In the model, spray efficacy is not affected by Bt ingestion (though see Brickle et al. 2001), and there is no cross-resistance between Bt and the insecticidal spray.

Insect Genetics. The genetics of resistance in the model (mutation rate, fitness costs, random-mating within fields) remain as described by Peck et al. (1999). A single diallelic locus controlled susceptibility (S-allele) or resistance (R-allele) in the insects. S-alleles mutated to R-alleles at a fixed rate. The mutation rate was applied probabilistically to each potential gamete at mating, whereby the number of S-alleles that mutated to R-alleles in each field each day was a random deviate drawn from a binomial distribution with population size as the number of S-alleles and probability 5×10^{-6} . This mutation rate is in keeping with that often reported for insects (Dobzhansky et al. 1977). As the dose of Bt in these crops is not high against *H. zea*, the assumption in Peck et al. (1999) of a recessive resistance allele is less robust. A default assumption of co-dominance between the susceptible and resistant alleles ($h = 0.5$) was made by extrapolating from the functional dominance of Bt resistance in the YHD2 strain of the closely related noctuid moth, *H. virescens* (Gould et al. 1995). At Bt concentrations that kill 75% of the susceptible strain (as Bt corn does with *H. zea*), two experiments with the YHD2 strain gave 25% and 50% mortality of heterozygous larvae. These mortality figures translate to functional dominance of 0.67 and 0.33, respectively. The average of these figures, 0.5, was used here. In this paper, Bt-susceptible homozygotes are designated SS, heterozygotes are designated RS, and homozygous resistant are designated RR.

Insect Biology and Effects of Transgenic Crops. The insect life cycle is divided into two stages: the egg, larval, and pupal stages of development are all modeled as one stage; the adult stage is modeled separately.

Egg/Larval/Pupal Mortality. Juvenile mortality is applied deterministically to the neonates, with survival calculated as the product of the survival rate to each of several mortality factors. The offspring surviving to adult produced per parent adult varies among generations. Overwintering adults produce fewer surviving offspring than do in-season adults. Fecundity may be lower, and environmental resistance for the first generation larvae is high because of less suitable food sources (whorl-stage corn and weeds) (Gross and Young 1977), greater vulnerability to inimical agents, and less benign weather conditions (Gross et al. 1976). Hence, in the model the replacement rate (R_1) for this generation is low. By contrast, the second generation is highly fecund, and ear-stage corn is an excellent host, so the replacement rate in the model is high (R_2). During subsequent generations, host suitability is lower, and environmental resistance is higher, so the replacement rates are reduced (R_3). In the absence of direct measurements of these replacement rates, the values used are set so that the seasonal

population dynamics in the absence of Bt crops mimic those typical for eastern North Carolina (Neunzig 1969; Stinner et al. 1974; Stinner et al. 1977b; J. S. Bachelier, personal communication). These replacement rates are used to calculate the expected number of neonates in each field each day. The actual number of neonates of each genotype is a random deviate from a binomial distribution with population size as the expected number of neonates and probability given by the expected genotypic frequency based on Hardy-Weinberg proportions (see Peck et al. 1999).

Larval survival on Bt relative to survival on non-Bt depends on the insect genotype, the crop type, and the crop phenology (see Table 1). Data in Storer et al. (2001) and Pilcher et al. (1997) suggest that the toxicity of Bt in whorl stage corn is much higher than toxicity in ear-stage corn. In whorl-stage Bt corn, the proportion of SS larvae that survive the Bt is given by S_{cu} and the proportion of RS larvae that survive the Bt is $2S_{cu}$ (the high toxicity makes the R-allele functionally recessive here). In ear-stage Bt corn, the proportion of SS larvae that survive the Bt is given by S_{cr} . In Bt cotton, the proportion of SS larvae that survive the Bt is given by S_{cr} . In ear-stage Bt corn and in Bt cotton, RS insects survive according to their relative fitness:

$$W_{RS} = h(W_{RR} - W_{SS}) + W_{SS} \quad [1]$$

where W_{RS} = fitness of genotype RS; W_{RR} = fitness of genotype RR; W_{SS} = fitness of genotype SS; and h = functional dominance of R-allele.

The relative fitness of genotype RR on all crops, W_{RR} , is equal to one – the fitness cost of resistance. For runs presented here, the fitness cost was set to balance the mutation rate so that the equilibrium R-allele frequency is the initial value, q_0 , as described by Peck et al. (1999). For the default parameter settings, this was calculated as 0.1.

On ear-stage corn, additional larval mortality occurs as a result of cannibalism (Stinner et al. 1977a). Cannibalism is generally of such intensity in this insect that usually only one larva will survive in each ear of corn. On non-Bt corn, that survivor is most likely to be the first one to enter the ear because it will be the largest larva during cannibalistic encounters. On Bt corn, resistant insects grow faster and reach a larger final size than susceptible insects. Resistant larvae are therefore likely to have a size advantage in most cannibalistic encounters even if they enter the ear later than susceptible larvae, and are thus more likely to win. Cannibalism therefore acts to increase the fitness differential between resistant and susceptible phenotypes on Bt corn. The role played by cannibalism in resistance evolution is both density-dependent and R-allele frequency-dependent. [See Storer (1999) for a full discussion of the role played by cannibalism in resistance evolution.] A brief description of the cannibalism model, and the equations used in this model for cannibalism mortality of each insect genotype on ear stage corn, are given in Appendix A.

Toward the end of summer, as cotton matures and the environmental resistance to *H. zea* rises, the suitability of cotton as a host declines. In the model,

Table 1. Parameters in the model, their default values, and the range, tested

Parameter name	Description	Default Value	Justification
T	Number of days per pest season	123 d	n/a
WS	Survival of overwintering diapaused pupae	3.4%	Caron et al. (1978)
μ_{DP}	Mean date of diapause induction	Day 86 (\approx August 27)	Stinner et al. (1977b)
P_{corn}	Proportion of fields in region planted to corn	55%	North Carolina Department of Agriculture and Consumer Services (2001)
K_{egg}	Mean egg threshold in one acre of non-Bt cotton (per acre)	60,000	Bachelier (1996a), Farrar and Bradley (1985)
K_{lar}	Mean larval threshold in one acre of cotton (per acre)	6,500	Bachelier (1996a)
wp	Percentage of insect population using weed hosts in spring	10%	Neunzig (1963)
S_{cw}	Percentage of SS larvae surviving the Bt in whorl-stage Bt corn	0%	J.W.V.D. (unpublished data), Storer (1999)
S_{cr}	Percentage of SS larvae surviving the Bt in ear-stage Bt corn	25%	Storer et al. (2001), Pilcher et al. (1997)
S_{ct}	Percentage of SS larvae surviving the Bt in Bt cotton	25%	Lambert et al. (1996), Mahaffey et al. (1995)
R_1	Replacement rate for first generation	1.5	Storer (1999), J. S. Bachelier (personal communication)
R_2	Replacement rate for second generation	75	Storer (1999), J. S. Bachelier (personal communication)
R_3	Replacement rate for third and subsequent generations	10	J. S. Bachelier (personal communication)
EM	Probability of emigrating from a suitable field	0.1	No data
ϕ^2_d	Variance for flight distance kernel	4.3 fields	No data
q_0	Initial region-wide frequency of resistance alleles	10^{-4}	Burd et al. (2001)
h	functional dominance of resistance allele	0.5	Gould et al. (1995), Burd et al. (2000)

neonate mortality rises linearly with time from mid August (model day 79) until the end of the season, on both Bt and non-Bt cotton.

Larval Development Rate. The development of susceptible insects on transgenic crops is delayed by 6–8 d (Pilcher et al. 1997, Storer et al. 2001). In the model, larval development follows a cohort-based distributed delay, each cohort consisting of the eggs laid in a single day. The delay is distributed normally about a mean dependent on the crop type and insect genotype. On non-Bt crops, the mean development duration for all larvae is 28 d. On Bt crops, the mean development duration for RR larvae is 28 d, and for SS larvae is 34 d. The standard deviation, also varies with insect genotype and plant type. On non-Bt crops, the standard deviation is 1.5 d. On Bt crops, the standard deviation for RR larvae is 1.5 d, and for SS larvae it is 1.8 d. The mean and standard deviation of development duration for RS larvae on Bt are intermediate between those for RR and SS on Bt according to their relative fitness (equation 1 above).

On ear-stage corn, the timing of adult recruitment is altered by the intensity of cannibalism (Stinner et al. 1977a). The cannibalism model by Storer (1999) showed that as cannibalism becomes more intense, insects entering ears later have a higher probability of being eaten than do those entering earlier. Consequently, the mean time to adult eclosion is reduced slightly at higher cannibalism rates.

Pupal Diapause. In Peck et al. (1999), insects that are in the pupal stage at the end of the season enter

diapause. This creates a high sensitivity to season-length when coupled with a developmental delay on Bt crops because it creates differential survival through the winter. In the present model, the proportion of insects that become diapaused pupae instead of becoming adults increases as the year progresses, starting at the end of July (day 58) and reaching the maximum probability at the end of September. As demonstrated by Rabb et al. (1975), this proportion follows an approximately cumulative normal curve through time. The mean diapause date is μ_{DP} , and the standard deviation is calculated as one third of the difference between the first diapause date and μ_{DP} . As diapause is primarily a response to photoperiod (Rabb et al. 1975), adjusting μ_{DP} effectively adjusts the time of the start of the season.

Diapaused pupae suffer winter mortality related to environmental and biotic factors. The level of survival, WS , is applied stochastically to the diapaused population, whereby the number of pupae of each genotype surviving winter in each field is a random deviate from a binomial distribution with population size as the number of diapaused pupae of each genotype going into winter, and probability WS .

Adult Movement. The adult movement distribution kernel of Peck et al. (1999) is modified by the relative field attractiveness, which changes with time. Field attractiveness is dependent on relative crop phenology: cornfields in the whorl stage are 10 times more attractive than prebloom cotton fields (all fields also have suitable weed hosts available at this stage); corn-

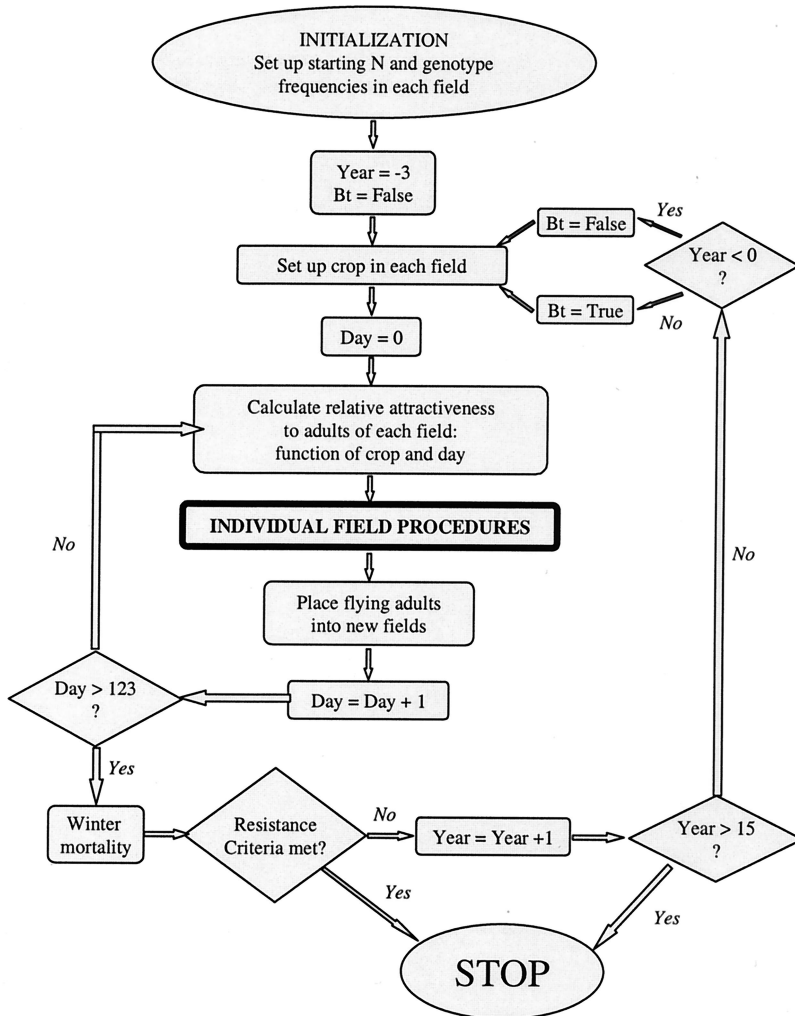


Fig. 3. Model flow over an entire run. The individual field procedures are shown in Fig. 4.

fields in the ear stage are 100 times more attractive than prebloom cotton fields; and cotton fields in bloom and fruiting stages are 75 times more attractive than postsilking cornfields. The relative attractiveness of the different hosts and host phenology for oviposition is based on the data in Johnson et al. (1975). After calculations of the distance and attractiveness of each field, the proportions of dispersing adults flying to each field in the distance kernel are modified so that they sum to 1.0.

Sources of Stochasticity. Stochasticity occurs within seasons, as within each field the number of neonates assigned to each genotype is probabilistic, and the number of genetic mutations from susceptible to resistant alleles is probabilistic. Additional stochasticity occurs between years as the number of diapaused pupae surviving winter is also probabilistic. Finally, the spatial matrix of crops is randomly determined at the start of each model year, based on the proportions of the four crop types.

The Subroutines and Model Flow. Figure 3 outlines the model flow over an entire run. The model starts by assigning crops to fields in the required proportions of Bt corn, non-Bt corn, Bt cotton, and non-Bt cotton. A random number (drawn from a normal distribution) of adults is then assigned to each field in the region, in the genotypic proportions determined by the required initial R-allele frequency. The model is run for 3 yr without Bt in the system so that the R-alleles become distributed through the region according to the biology of the insect. Because there are no detailed field data on population substructure in *H. zea*, running the model without Bt creates a simulated population substructure based on insect movement and genetic drift (Peck et al. 1999). A period of 3 yr was chosen for this null run because the average and standard deviation for both population sizes and R-allele frequencies do not change systematically within a run without Bt after this time. The values for these measures at 3 yr are representative of the values after 3 yr.

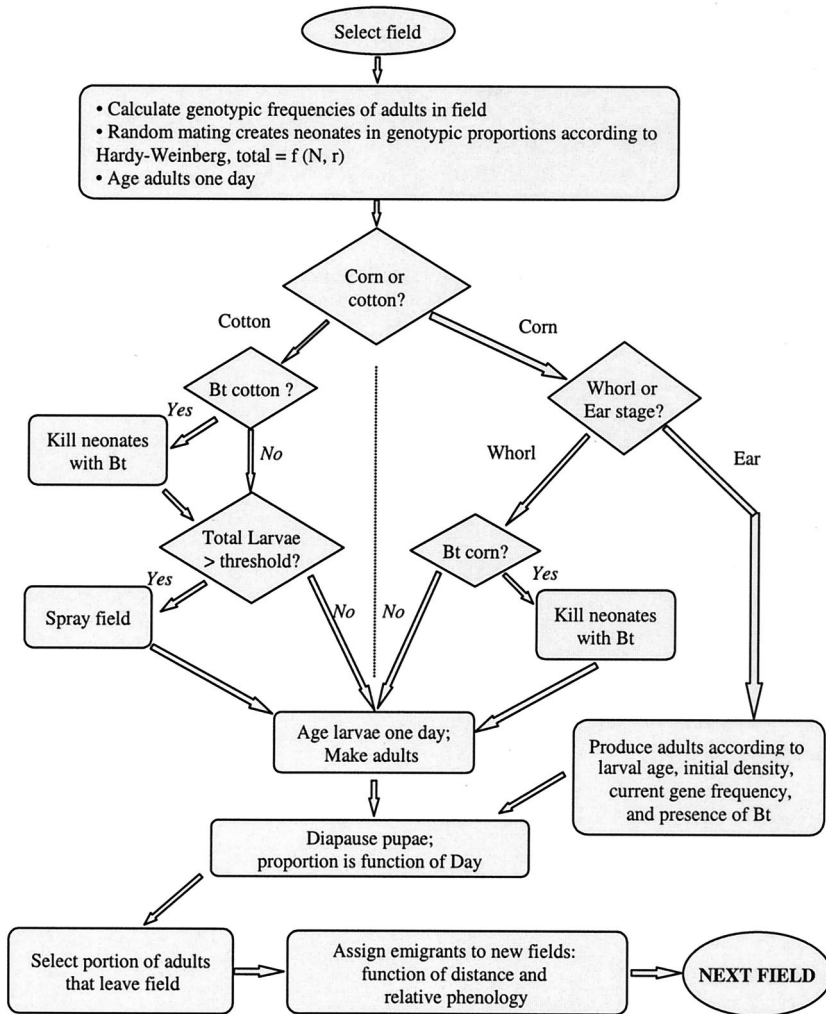


Fig. 4. Model flow in each field each day.

For each time-step, the program flow follows that outlined in Fig. 4. Each of the processes described above can occur in each field at each time step.

Model Outputs. Yearly output gives the total number of diapaused pupae in the region and the average R-allele frequency in this pupal population. Ancillary output includes the average number of times non-Bt cotton fields were sprayed during the year. Optional daily output consists of the average number of adults and the average R-allele frequency, both across all fields and across the fields planted to each of the four crops. In addition, the model gives the variance among fields for each of these variables.

A matrix giving the crop, the diapaused pupal population, and the R-allele frequency in each field is produced at the end of the year when the average R-allele frequency first exceeds 0.25, or at any other time of interest during a model run. Using these data, a measure of the variation in R-allele frequency among fields, the adjusted coefficient of variation in R-allele frequency, is calculated as:

$$cv^* = \frac{s_q^2}{\bar{q}(1 - \bar{q})} \quad [2]$$

where cv^* = adjusted coefficient of variation, \bar{q} = region-wide R-allele frequency, and s_q^2 = among-field variance in R-allele frequency. This statistic thus gives a measure of population subdivision (Wright 1978).

The program is written in C++, running in a UNIX environment. The runs presented here were made on a SUN SPARC Workstation.

Model Parameterization. The model is highly flexible with many agronomic, genetic, and biological parameters whose effects can be investigated. The choice of default parameters is made to best represent what is known about the life system of *H. zea* in eastern North Carolina. This information is drawn from historical information and from published literature. Table 1 gives the default value for each of the parameters. The effects of varying these parameters on the model

output are discussed in a companion paper (Storer et al. 2003).

Model Validation. To validate the insect population dynamics of the model, the output was qualitatively compared with field data. The temporal pattern of adult numbers during the final year before Bt deployment was qualitatively compared with actual light-trap data of *H. zea* in the region (J. S. Bacheler, personal communication). Larval infestation data presented by Neunzig (1969) and Storer et al. (2001) was used to validate the population dynamics in corn. The number of times non-Bt cotton fields are normally sprayed with insecticides for *H. zea* in North Carolina was used to validate population dynamics in cotton. The genetics of the model cannot be validated in the absence of documented resistance evolution in the field.

Model Runs. The model was used to investigate the effects on the population dynamics and genetics of increasing deployment of Bt corn and Bt cotton from a minimum of 1% of corn fields and 5% of cotton fields, up to 75% of each. For these runs, crops in the selected proportions were assigned to fields at random at the start of each model year. With 1% of cornfields as Bt, the model simulated the strict limitations imposed by the U.S. Environmental Protection Agency on planting Bt corn in cotton-growing regions up to the 1997 season (Environmental Protection Agency 1997). With 75% of both crops planted to Bt varieties, the model simulated the highest deployment of Bt crops envisaged for eastern North Carolina, accounting for required refugia and nonadoption of the technology.

A 4×4 factorial design was used to investigate the effects of the acreage of both Bt corn and Bt cotton. The model output from these runs was the region-wide R-allele frequency each year for 15 yr, or until an R-allele frequency of 50% is reached. Each set-up was run three times. Analysis of variance (ANOVA) for the log-transformed region-wide R-allele frequency after 8 yr of Bt crop deployment (q_8) was conducted as a completely randomized design using PROC GLM in SAS (SAS Institute 1990), with the probability level for rejecting the null hypothesis set at 0.10.

Spatial Pattern of Crops. For the default runs above, crops were assigned to fields at random each year. This created a distribution of fields that can by chance contain areas of locally high Bt deployment, and areas of locally low Bt deployment. Areas of high Bt deployment can spur the evolution of resistance because of the unselected population being small. However, current resistance management plans require each farm to have its own refuge, and include recommendations for the maximum distance between Bt crops and the nearest refuge. This forces the distribution of refuge and Bt fields to be more uniform. By controlling the spatial layout of fields, this model was used to assess the role of the refuge structure in resistance evolution in the highly mobile pest, *H. zea*.

Farm-Level Refuge. The model was used to simulate a farm-level refuge requirement by dividing a 30×30 -field region into 36 farms, each occupying a square of 5×5 fields. Each farm contained the same pro-

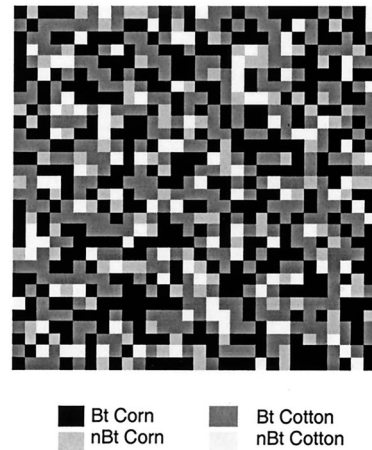


Fig. 5. Crop layout with farm-level refuge. Region is divided into farms of 25 fields. Within each farm, 55% of fields are corn 45% are cotton. Within each farm, 75% of fields in each crop are planted to Bt cultivars. Crop positions are randomized within farms. The region size is 30 fields \times 30 fields.

portion of each of the four crops, assigned to fields randomly. This ensured that Bt fields always have non-Bt fields in close proximity. An example of such a crop layout is shown in Fig. 5. Three model runs were made with these assumptions at three levels of Bt corn deployment ($BtCR = 25, 50,$ and 75%) across three levels of Bt cotton deployment ($BtCT = 25, 50,$ and 75%) in a 3 by 3 factorial design. The 8-yr region-wide R-allele frequencies were compared with those from similar runs using the default spatial assumptions by ANOVA.

Incomplete Adoption of Bt Crops. As with any agricultural innovation, not all farmers will adopt Bt transgenic crops. It seems reasonable to expect that farms that do not adopt Bt technology could provide some of the refuge for those that do, and that consequently the on-farm refuge requirements could be reduced to account for the nonadopting farms. To simulate this, the 30×30 field region was again divided into $36 \times 5 \times 5$ farms. For these runs, a number of farms did not adopt Bt crops. An example of such a crop layout is shown in Fig. 6. The farms that adopted Bt were the same each year, but positions of crops within farms were randomized each year. Analyses of variance were conducted to compare the region-wide 8-yr R-allele frequency at different levels of Bt adoption when the proportion of Bt crops used on Bt-adopting farms is fixed (so the regional Bt proportion depends on adoption rate). Additional analyses compared the region-wide 8-yr R-allele frequency at different levels of Bt adoption when the proportion of Bt crops used region-wide is fixed (i.e., on-farm Bt proportion depends on adoption rate). The contribution of non-adopting farms to resistance management in adopting farms was assessed by examining the spatial distribution of R-alleles in the different Bt-adoption scenarios.

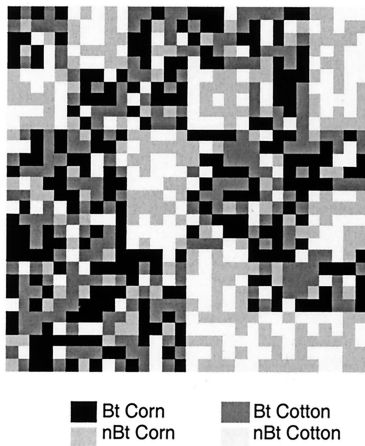


Fig. 6. Crop layout with incomplete adoption of Bt crops. Region is divided into farms of 25 fields. Within each farm, 55% of fields are corn and 45% are cotton. 67% of farms adopt Bt technology; within each adopting farm, 75% of fields in each crop are planted to Bt cultivars (so 50% of fields in each crop are Bt across the region). Crop positions are randomized within farms. Region size is 30 fields \times 30 fields.

Results and Discussion

The relative abundance of moths over a season in the model (Fig. 7) is qualitatively comparable with actual light-trap data of *H. zea* in the region (J. S. Bachelier, personal communication), with larval infestation data presented by Neunzig (1969). Light trap and larval infestation in eastern North Carolina show high variability in timing and size from year to year and location to location. However, the model output is in agreement with the general patterns in these data, such as 3–4 population peaks per year, with the largest being in early- to mid-August, and continual adult presence over the entire summer. To validate larval density estimates from the model, we compared field estimates with model predictions for the neonate density on ear-stage corn (region-wide mean = 1.85, SE = 0.04, from 16 runs), and for the number of times cotton fields reach threshold and are sprayed (region-wide mean = 4.6, SE = 0.05, from 16 runs). The number of

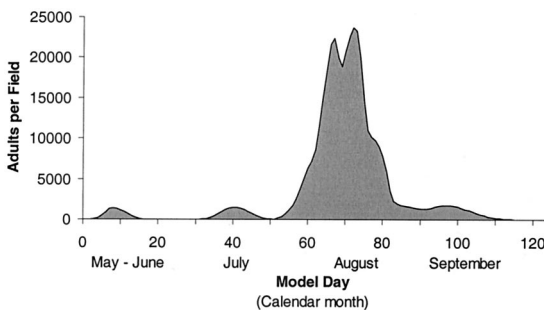


Fig. 7. Number of adults per field, averaged across the entire model region, each day during final year before Bt deployment. Output from a single run. Values for all parameters are the default values shown in Table 1.

neonates per corn ear agrees closely with the number of small larvae found in field tests in North Carolina under moderate infestations (Storer et al. 2001). The number of times cotton fields are sprayed also is close to the average in regions of the North Carolina coastal plain under moderate to heavy insect pressure (J.W.V.D., unpublished data).

Default Runs

Insect Populations. One of the prospects raised by high deployment of insecticidal crops is the potential for area-wide insect suppression. In the model, the population level (overwintering pupae per field before winter mortality) is reduced by the deployment of Bt (Fig. 8, where the population at year 0 is the number of pupae diapaused at the end of the pre-Bt period). The level of population suppression at the highest Bt deployment level (suppressed to a level one-third of the pre-Bt numbers) may be measurable in the field if the effects of the suppression do not interact with the causes of the large inter-annual variation in populations. However, if there are interactions not modeled here, such as compensatory mortality or density-dependence outside of corn ears, such population suppression could be difficult to observe. It seems that the current Bt transgenic varieties of these two crops are likely to play only a minor role in the kind of area-wide population suppression program envisaged by Knippling and Stadlerbacher (1983), especially if there is significant immigration.

A result of population suppression is a reduction in the need to spray non-Bt cotton fields. Sprayed non-Bt cotton fields produce a smaller refuge population than unsprayed non-Bt cotton fields. The reduction in population achieved by high Bt deployment reduces the reliance on insecticide sprays to control the population on non-Bt cotton, but at most only by around 45% (Fig. 9). The reduction in spraying of these fields in turn can increase their contribution to the refuge population.

Population Genetics. Under the high levels of Bt corn and Bt cotton deployment that achieve measurable population suppression, both the population and the need to spray non-Bt cotton start to return toward the pre-Bt levels as the years progress. This is a result of the populations becoming resistant to Bt (Fig. 10). ANOVA for the dependence of the 8-yr region-wide R-allele frequency (q_s , log-transformed) on the proportion of cotton fields that are Bt and the proportion of cornfields that are Bt showed a strong main effect of the proportion of cotton planted to Bt ($F = 1700$; $df = 3,32$; $P < 0.01$), a strong main effect of the proportion of corn planted to Bt ($F = 697$; $df = 3,32$; $P < 0.01$), and a significant interaction effect between these ($F = 4.79$; $df = 9, 32$; $P < 0.01$). This indicates that the more Bt that is deployed, the faster the rate of evolution. The effects of Bt corn are a little smaller than the effects of Bt cotton—for example, the R-allele frequency if 75% of cotton is Bt and 25% of corn is Bt is higher than if 25% of cotton is Bt and 75% of corn is Bt. The greater importance of Bt cotton is mostly

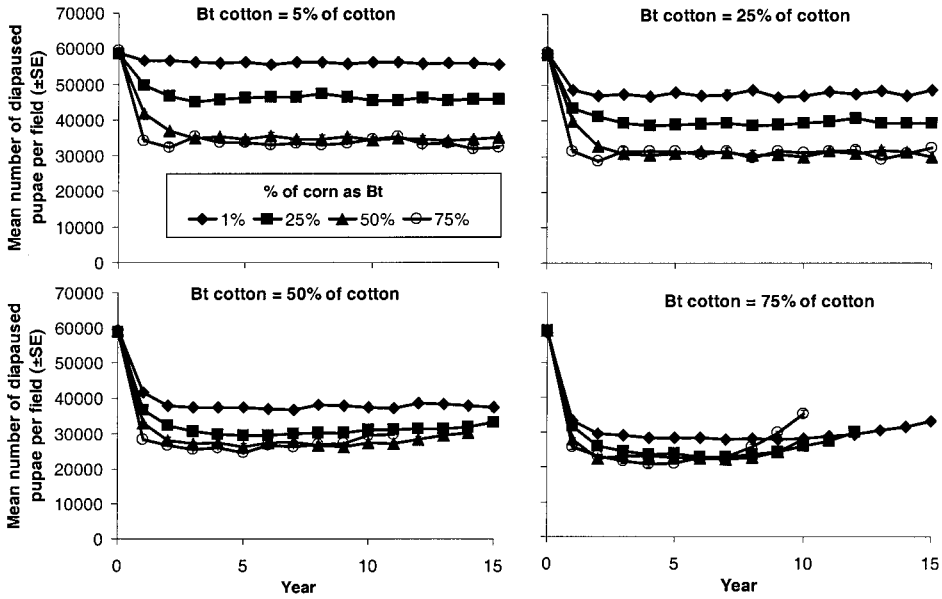


Fig. 8. Reduction in population of diapaused pupae (average per field) over 10–15 yr after deployment of different levels of Bt corn and Bt cotton. Output from default model runs. Each point represents the average of three runs. Values for all parameters are the default values shown in Table 1.

attributable to the spraying of non-Bt cotton fields when they reach threshold, which reduces the effective refuge size. Non-Bt corn fields are not sprayed, so the ratio of adults emerging from non-Bt and Bt corn is higher than the ratio from cotton. The effects of the two crops appear additive over most of the range of values tested, except at high deployment of Bt in both

crops where the rise in R-allele frequency tails off as the frequency approaches one (Fig. 10). This creates the significant interaction between the levels of deployment of Bt in the two crops. In the field, the effects of the levels of the two crops will likely be seen as additive, as Bt would not likely be deployed once resistance reached high levels.

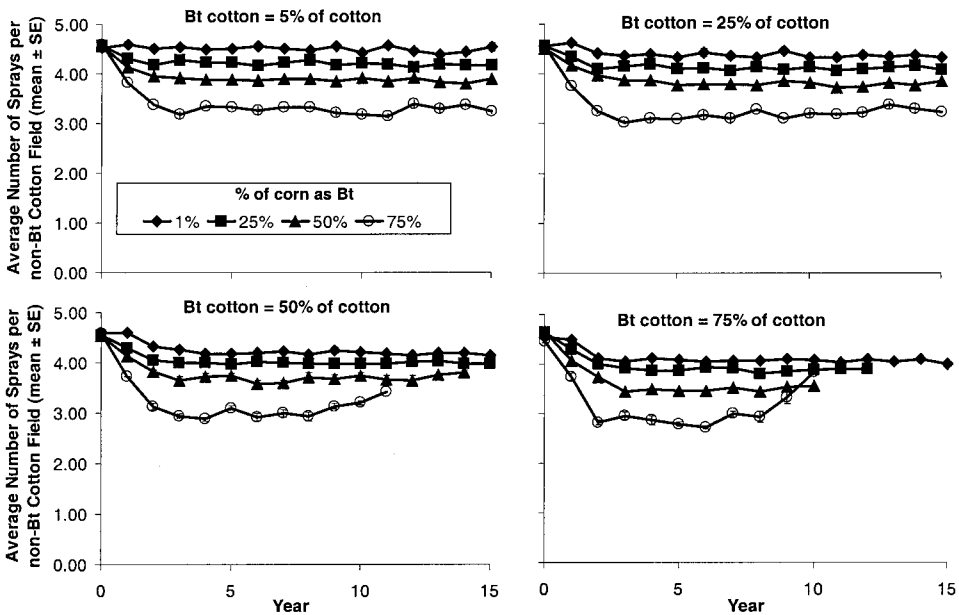


Fig. 9. Average number of times each non-Bt cotton field is sprayed for 10–15 yr after deployment of different levels of Bt corn and Bt cotton. Output from default model runs. Each point represents the average of three runs. Values for all parameters are the default values shown in Table 1.

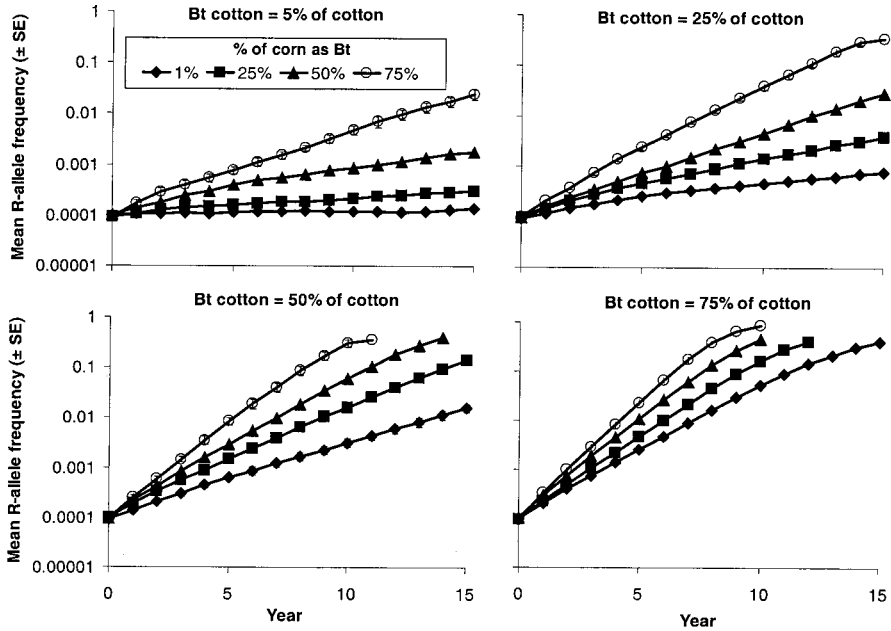


Fig. 10. R-allele frequency changes for 10–15 yr after deployment of different levels of Bt corn and Bt cotton. Output from default model runs. Each point represents the average of three runs. Values for all parameters are the default values shown in Table 1.

Unlike the findings of Peck et al. (1999) using the parent model, extinction of the R-allele never occurred. This difference is a result of the lower toxicity of Bt to *H. zea* in comparison with its toxicity to *H. virescens*, and the associated high survivorship of RS larvae on Bt crops. The R-allele is maintained in all populations.

It is worth noting that in these runs, the standard errors, representing run-to-run variation, are very small, generally smaller than the symbols in the figures. This contrasts with the findings of Peck et al. (1999) who saw great run-to-run variability with a similar model. Thus, the model suggests that for a

nonhigh dose toxin that allows considerable survival of both susceptible insects and heterozygotes, rare events involving rare homozygous resistant insects are less important than they are for the high dose toxin modeled by Peck et al. (1999).

Spatial Distribution of Crops, Pupae, and R-alleles. The spatial aspects of the model output are shown in Fig. 11. Figure 11A shows the overwintering population in each field in a 30 by 30 matrix after 7 yr at 75% Bt cotton and 75% Bt corn. The field layout for this year is that shown in Fig. 2. Fields with the lowest populations (palest squares) are cornfields, from where most insects have left (for cotton) during the

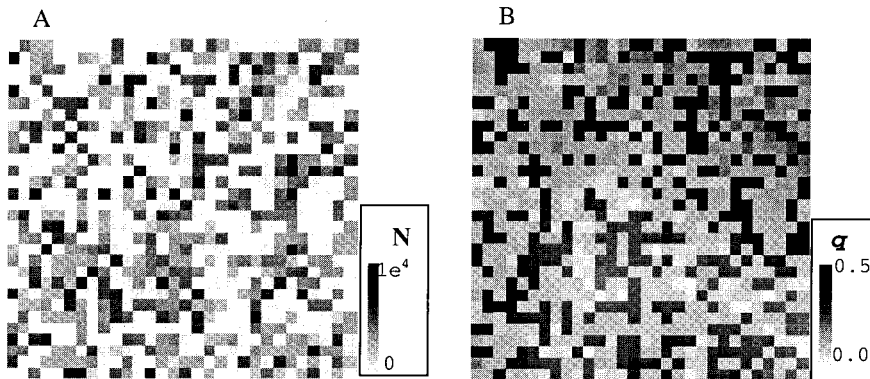


Fig. 11. (A) Distribution of populations of diapaused pupae (N), and (B) distribution of R-allele frequencies (q), when region-wide R-allele frequency is 0.25. Crop layout for the year shown is the same as in Fig. 2. Darker squares represent fields with higher populations or higher gene frequencies. The region size is 30 fields \times 30 fields. 75% of corn is Bt, and 75% of cotton is Bt. All other parameters are set at default values shown in Table 1.

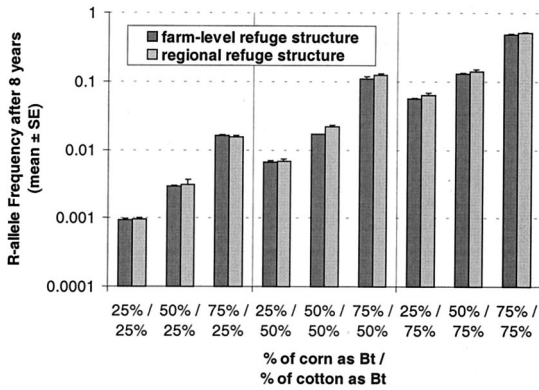


Fig. 12. Comparison between regional refuge structure (as in Fig. 2) and farm-level refuge structure (as in Fig. 5) in their effect on R-allele frequency after 8 yr of deployment of three levels of Bt corn and three levels of Bt cotton. Each point represents the average of three runs. Values for all parameters are the default values shown in Table 1.

summer. Dark squares are cotton fields in which most of the overwintering population is produced. The region-wide R-allele frequency at the time point is 0.26, but the R-allele frequency is variable among fields (Fig. 11B). The lowest frequency is 0.052, and the highest is 0.47. The adjusted coefficient of variation in Fig. 11B is 0.06. Across the region there are several patches of up to 20 contiguous fields, where the R-allele frequency is above 0.4. These small patches are generally in broader areas of the region with above-average gene frequencies. Areas of locally high-Bt deployment can create increased resistance, which then rapidly spread through the region because of the high mobility of the insect.

Spatial Pattern of Crops

Farm-level Refuge. Figure 12 shows the effect of distributing the refuge more uniformly across the region on the region-wide R-allele frequency after 8 yr. In the ANOVA, the main effect of the refuge structure is significant ($F = 3.55$; $df = 1,36$; $P = 0.07$), a farm-level refuge structure slowing resistance evolution because of improved population mixing. The effect of making the refuge structure uniform rather than random is very small and does not interact with the proportion of cotton planted to Bt or the proportion of corn planted to Bt (Fig. 12).

Improving the uniformity of refuge distribution can also reduce the variability in R-allele frequencies among fields. At 75% deployment of Bt varieties in each crop, the adjusted coefficient of variation (equation 2 when the region-wide R-allele frequency reaches 0.25 is reduced from an average of 0.059 (SE = 0.0024) to an average of 0.047 (SE = 0.0022). This difference is significant at the 0.05 level ($t = 3.52$; $df = 4$; $P = 0.02$) (log+1 transformed data). The distribution of R-allele frequencies using a farm-level refuge structure (Fig. 13) is improved when compared with the default regional refuge structure (Fig. 11B).

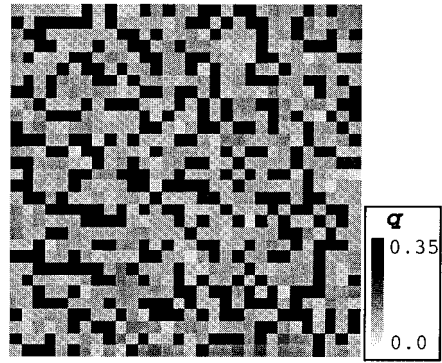


Fig. 13. Distribution of R-allele frequencies using farm-level refuge when region-wide R-allele frequency is 0.25. Region is divided into 25-field farms, each with the same proportion of each crop type. Crop layout for the year shown is in Fig. 5.

Reduced Adoption of Bt Crops. If not all farmers adopt Bt, the region-wide rate of resistance evolution will be slowed. The farmers planting only non-Bt crops provide a nonselected insect population additional to that produced from the refuge planted on Bt-adopting farms. When the on-farm refuge is 50% in each crop (i.e., $BtCR = 0.5$ and $BtCT = 0.5$), the region-wide R-allele frequency after 8 yr is around 0.02 if all farms adopt Bt, but only 0.001 if 50% of farms do not adopt Bt. The effect is significant ($F = 300.8$; $df = 1,4$; $P < 0.01$; Fig. 14A). Similarly, when the on-farm refuge is 25% in each crop ($BtCR = 0.75$ and $BtCT = 0.75$), the region-wide R-allele frequency after 8 yr is 0.5 if all farms adopt Bt, 0.05 if 67% of farms adopt Bt, and 0.001 if 33% of farms adopt Bt. The effect is again significant ($F = 409.6$; $df = 2, 6$; $P < 0.01$; Fig. 14B).

By considering the total refuge across the region, the contribution of farms not adopting Bt to the refuge population for farms that do adopt Bt can be assessed. Having part of the region-wide refuge supplied by farms that do not adopt Bt disrupts the structure of that refuge. In these runs, when the region-wide refuge is 75% of fields, the region-wide R-allele frequency after 8 yr is the same whether all farms adopt Bt (on-farm Bt deployment: $BtCR = 0.25$, $BtCT = 0.25$), 50% of farms adopt Bt (on-farm Bt deployment: $BtCR = 0.5$, $BtCT = 0.5$), or 33% of farms adopt Bt (on-farm Bt deployment: $BtCR = 0.75$, $BtCT = 0.75$) ($F = 0.92$; $df = 2, 6$; Fig. 14C). Hence, the region-wide R-allele frequency is unaffected by disrupting the uniformity of the refuge distribution when the total refuge is large. When the regional refuge is 50% of fields, the region-wide R-allele frequency after 8 yr is lower when all farms adopt Bt (on-farm Bt deployment: $BtCR = 0.5$, $BtCT = 0.5$) than when 67% of farms adopt Bt (on-farm Bt deployment: $BtCR = 0.75$, $BtCT = 0.75$). This difference is significant ($F = 27.5$; $df = 1, 4$; $P < 0.01$; Fig. 14D). The production of susceptible insects from nearby non-Bt farms is insufficient to completely compensate for the local selection on farms that use only a 25% refuge.

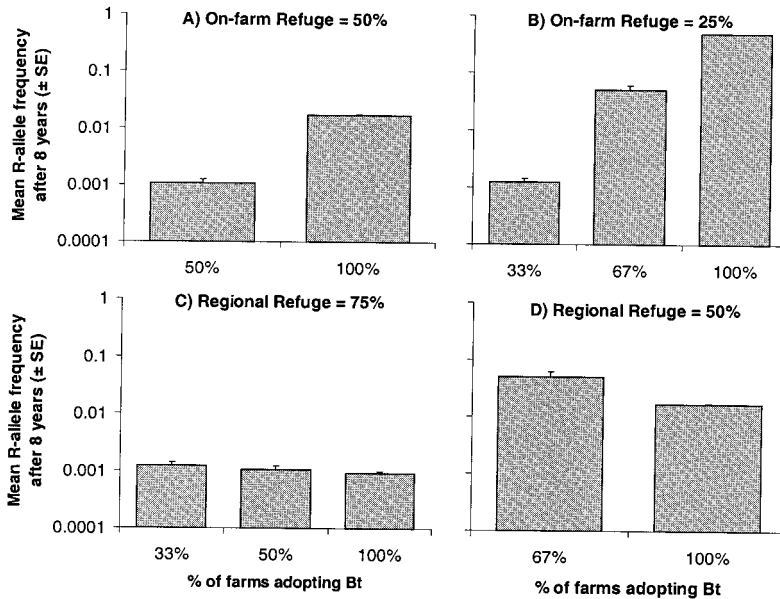


Fig. 14. Effect of incomplete adoption of Bt crops by all farms on R-allele frequency after 8 yr. A) On-farm refuge set at 50% of both cotton and corn fields (i.e., $BtCT = 50\%$, $BtCR = 50\%$); B) on-farm refuge set at 25% of both cotton and corn fields (i.e., $BtCT = 0.75$, $BtCR = 0.75$); C) region-wide refuge set at 75% of both cotton and corn fields (on farm Bt deployment differs among adoption levels); and D) region-wide refuge set at 50% of both cotton and corn fields (on-farm Bt deployment differs between adoption levels). Each column represents the average of three runs. Values for all parameters are the default values shown in Table 1.

More importantly, the local R-allele frequency is not uniform when the refuge distribution is not uniform (Fig. 15). The average adjusted coefficient of variation (equation 2 for the three runs with 67% adoption of Bt crops with a 25% refuge was 0.323, an order of magnitude higher than for the runs with random crop distribution throughout the region. In the run illustrated, the farm layout is as shown in Fig. 6. The R-allele frequencies are far higher in areas where Bt deployment is high than in areas where Bt deployment is low. When the R-allele frequency region-wide is 0.25, in some fields it has reached 0.9 (Fig.

15A). Thus, farms that have deployed Bt varieties in 75% of both corn and cotton fields rapidly create a resistance problem for themselves. Those Bt farms that have a large proportion of their neighbors also deploying Bt (e.g., lower left quadrant) have higher resistance levels than those Bt farms where a larger proportion of neighbors do not deploy Bt (e.g., lower right quadrant). Therefore, Bt farms may not gain much protection from resistance by one or two neighbors who do not deploy Bt.

Even farms that have never deployed Bt crops can end up with populations with elevated R-allele fre-

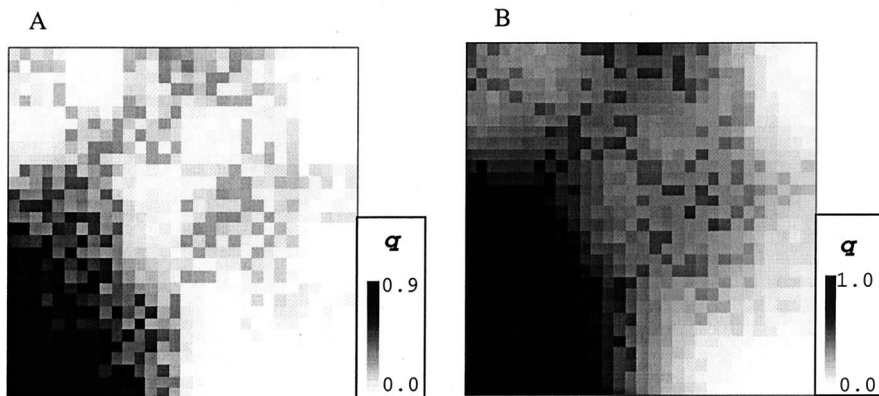


Fig. 15. Distribution of R-allele frequencies (q) with incomplete adoption of Bt crops. Region is divided into 25-field farms, 67% of which adopt Bt technology. Crop layout for the year shown is in Fig. 6. Region-wide R-allele frequency is A) 0.25 after 11 yr; B) 0.5 after 13.5 yr.

quencies. When the R-allele frequency region-wide has reached 0.5, the highly resistant populations have spread through nearly the entire region (Fig. 15B). If refuge requirements on Bt farms are low, the adopting farms create resistance problems for other farms that are slower in adopting the new technology.

These simulation outputs on the spatial distribution of R-alleles under different Bt deployment regimes suggest that it may be very important to monitor R-allele frequencies at several sites in a region to get a complete picture of region-wide resistance, and to be able to track the spread of populations with higher-than-average R-allele frequencies. The spatial variation depends on assumptions about insect movement made in these model runs. When improved empirical data on insect movement and spatial patterns of Bt deployment become available, this model can be used to help guide an effective monitoring scheme. Priority should be placed on sampling populations from areas of historically high Bt crop deployment. Even with allele frequency data from a small number of sites, the among-field variation in R-allele frequency predicted by this model can be used to estimate the highest and lowest local R-allele frequencies likely to be found in a region.

In conclusion, the aim of insect resistance management is to reduce the risk of creating resistant insect populations while deploying the insect control measure. Transgenic insecticidal crops present extremely strong selection pressure for resistance (Gould 1998). The moderately effective dose of toxin in Bt corn and Bt cotton against *H. zea* does not meet the assumptions of conventional resistance management using the high-dose plus refuge strategy. This simulation model has been used to indicate the likely course of resistance evolution under different moderate dose Bt-crop deployment scenarios. Because values for many of the parameters in the model are either unknown or inherently variable, the output cannot be interpreted as predictive. The model can be used as a tool for investigating the effects of varying some of these parameters on model output, and how the parameters interact. Details on these tests are published in a companion paper (Storer et al. 2003).

The biology and life history of *H. zea* differs among regions (Raulston et al. 1990, Bradley 1993). The model presented here was designed to simulate the evolution of resistance in populations of *H. zea* to currently commercialized varieties of Bt corn and Bt cotton in the mixed cropping system of eastern North Carolina. Because the biology and life history of the insects, and their interactions with their hosts, play very important roles in resistance evolution, the results presented here should not be extended to different insect/host systems or the same system in different regions. Rather, the validity of the assumptions should be reassessed, the model should be adjusted to account for differences in the life system and the parameter values should be altered to fit local conditions.

The spatial distribution of transgenic and nontransgenic plantings can affect both the region-wide evo-

lution of resistance, and, especially when the on-farm refuge size is small, the resistance levels in local sub-populations. It is clear that farm-level refuge requirements are important, even for this highly mobile insect pest, if the risk of resistance on farms is to be managed. Because resistant populations can rapidly spread to farms that do not use Bt, all farmers in a region would pay for the high Bt deployment of others. Understanding the spatial aspects of population dynamics and genetics will be crucial to our ability to manage insect resistance to transgenic crops.

As with any model, which is a simplified representation of the real world, key assumptions are made that may not accurately reflect reality. Thorough investigation of the effects of these assumptions on model outputs is required. A companion paper (Storer et al. 2003) presents the results of sensitivity analyses on several of the model parameters. Future work should address the implications of some of the assumptions made in the model architecture on the spatial dynamics of resistance evolution. For example, soybean, an alternate host for *H. zea*, is planted on roughly the same number of hectares as corn and cotton combined in eastern North Carolina. Preliminary work with soybean included in the model showed that in these circumstances soybean can greatly reduce the rate at which resistance evolves (Storer 1999). However, because field data on the production of *H. zea* adults from soybean fields are not currently available for eastern North Carolina, soybean was ignored as a host for the runs presented here. It will be important to conduct field research to obtain such data to understand fully pest adaptation to Bt crops. Other features of the present model that need critical examination include the lack of natural features of the landscape that could inhibit or promote local adult movement among fields, and the lack of insect inter-regional migration.

Appendix

Appendix A—Cannibalism Mortality on Ear Stage Corn

A separate model was created to simulate larval cannibalism in corn ears. This was a stochastic individual-based model, with neonates assigned randomly to ears within a field. Larvae develop through six size classes on non-Bt corn ears. On Bt corn ears, susceptible larvae develop through four size classes to reflect their reduced pupal weight (Storer et al. 2001), resistant larvae develop through six size classes, and heterozygotes develop through five. Development is probabilistic, such that the age-dependent probability of reaching the next size class follows a cumulative normal curve. Development rate is slower for susceptible and heterozygote larvae on Bt than for resistant larvae on Bt or any larvae on non-Bt. In the model, larvae can only cannibalize other larvae of equal or smaller size, and the cannibalism probabilities are size-pair-specific, according to data in Caron (1976). The cannibalism probabilities observed by Caron (1976) are sufficiently high that rarely does more than one

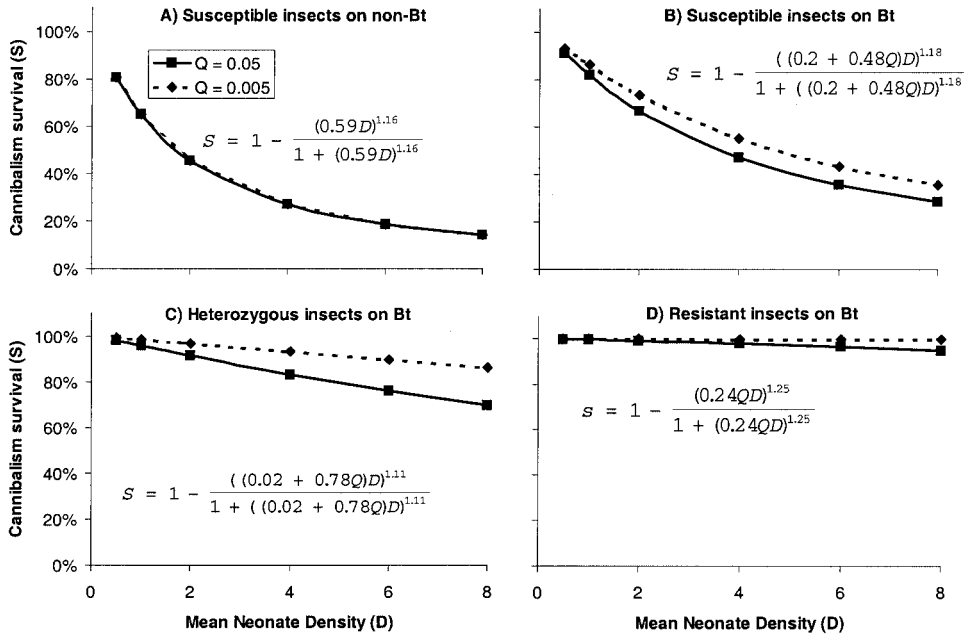


Fig. A1. Percentage of larvae surviving cannibalism, derived from the individual-based model, plotted against neonate density at two levels of R-allele frequency (Q) for different insect genotype/plant-type combinations. A) All insects on non-Bt corn; B) susceptible homozygotes on Bt corn; C) Heterozygous insects on Bt corn; D) resistant insects on Bt corn.

larva complete development in an ear. Cannibalistic aggression (*agro*) is a factor by which the cannibalism probabilities are multiplied to simulate different intensities that may be induced by feeding on a sub-standard diet (Bt corn kernels).

The model predicts the proportion of larvae of each genotype on Bt and non-Bt corn that are cannibalized. Multiple model runs with a range of parameter settings revealed that cannibalism mortality on non-Bt ears is dependent on the neonate density (i.e., the number of neonates produced in a field during the ear-stage (before Bt mortality) divided by the number of ears in the field). Cannibalism survival on Bt ears is dependent on both the neonate density and on the R-allele frequency in the larval population. The model predicts that cannibalism has the largest impact on the genotypes that are most susceptible to Bt as they are already weakened by exposure to Bt. PROC NLIN in SAS (SAS Institute 1990) was used to fit the model output to deterministic equations. The mortality is genotype/plant-type specific, and is approximated by the following equation (see Storer 1999):

$$S(GP) = 1 - \frac{((a + b)D)^y}{1 + ((a + bQ)D)^y}$$

where $S(GP)$ = cannibalism survival of larvae of genotype G on corn type P (Bt or non-Bt); Q = R-allele frequency in the neonate population; D = neonate density (mean number of neonates per ear); and a, b, and y are constants for each insect genotype/corn type combination.

On non-Bt plants, the higher the neonate density,

the higher the cannibalism mortality; all insect genotypes suffer the same level of cannibalism; and there is no effect of R-allele frequency (i.e., $b = 0$). On Bt plants, the density effect on cannibalism is reduced because of Bt mortality of larvae (i.e., a is low). Additionally, the higher the R-allele frequency, the higher the cannibalism mortality of SS larvae, because of increasing interactions with fitter RS and RR larvae (i.e., b is high). The R-allele frequency effect on RR larvae is less strong (b is low), and on RS larvae is intermediate, depending on the value of h. Constant y fine-tunes the mortality rate to fit the output of the cannibalism model. The precise values used for a, b, and y depends on the genetic and behavioral assumptions in effect for any particular run. Figure A1 shows the form of the equations for the default assumptions, illustrated across levels of neonate density and initial R-allele frequency. The constants in the equations are affected by the functional dominance of the R-allele (h), the relative survival of SS larvae on Bt corn (*Scr*), and the relative aggression of susceptible larvae when on Bt corn compared with when on non-Bt corn (*agro*).

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