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Insecticide Resistance and Resistance Management

Evolution of Resistance by *Helicoverpa zea* (Lepidoptera: Noctuidae) Infesting Insecticidal Crops in the Southern United States

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Abstract

We created a deterministic, frequency-based model of the evolution of resistance by corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), to insecticidal traits expressed in crops planted in the heterogeneous landscapes of the southern United States. The model accounts for four generations of selection by insecticidal traits each year. We used the model results to investigate the influence of three factors on insect resistance management (IRM): 1) how does adding a third insecticidal trait to both corn and cotton affect durability of the products, 2) how does unstructured corn refuge influence IRM, and 3) how do block refuges (50% compliance) and blended refuges compare with regard to IRM? When Bt cotton expresses the same number of insecticidal traits, Bt corn with three insecticidal traits provides longer durability than Bt corn with two pyramided traits. Blended refuge provides similar durability for corn products compared with the same level of required block refuge when the rate of refuge compliance by farmers is 50%. Results for Mississippi and Texas are similar, but durabilities for corn traits are surprisingly lower in Georgia, where unstructured corn refuge is the highest of the three states, but refuge for Bt cotton is the lowest of the three states. Thus, unstructured corn refuge can be valuable for IRM but its influence is determined by selection for resistance by Bt cotton.

Key words: corn earworm, corn, cotton, insect resistance management

Helicoverpa zea (Boddie) (Lepidoptera: Noctuidae), the corn earworm, is an insect pest that infests several important agricultural crops in the southern United States (Fitt 1989, Horner et al. 2003b). The moths are strong fliers, and adult females show oviposition preference for ear-stage corn (*Zea mays*) and bloom and fruiting stage cotton (*Gossypium hirsutum*) (Javaid et al. 2005). Newly emerged adults mate 30–60 h after emergence (Callahan 1958, Agee 1969), and there are four to six generations per year in the southern United States. Transgenic insecticidal corn and cotton expressing genes from the bacterium *Bacillus thuringiensis* are used in the southern United States. Both Bt corn and Bt cotton reduce larval survival (Horner et al. 2003a, Chilcutt et al. 2007).

The larvae of *H. zea* feed on a variety of plant tissues across many different crop species, including leaves, corn kernels, cotton bolls, and seeds (Fitt 1989). In a recent field study, Reay-Jones and Reisig (2014) concluded that corn yields were not statistically different among near-isolines with and without Bt traits, despite larval feeding and damage. In addition, yields were not significantly associated with kernel injury based on regression analyses.

Several groups have modeled this pest in the southern United States to predict the evolution of resistance to Bt crops. Storer et al. (2003) focused on the cotton and corn landscape of North Carolina. They used a stochastic, density-based model that included adult dispersal among small fields to explore the temporal and spatial dynamics of the population over many generations on crops with a single insecticidal trait, Cry1A. Gustafson et al. (2006) used a deterministic, frequency-based model to explore evolution to Cry1Ac Bt cotton in Georgia (GA), North Carolina, and Mississippi (MS).

They assumed random mating across the landscapes. Gustafson et al. (2006) considered soybean (*Glycine max*), peanut (*Arachis hypogaea*), and sorghum (*Sorghum bicolor*) as alternative hosts, as well as corn. Tabashnik et al. (2008) used a simple population genetics model to study the influence of refuge levels on evolution of corn earworm to Cry1Ac cotton. Edwards et al. (2013) extended the work of Caprio et al. (2009) and evaluated the risk of evolution of corn earworm in a landscape of pyramided Bt corn and pyramided Bt cotton in MS. Their modeled landscape consisted of cotton, corn, soybean, sorghum, and wild hosts. They simulated a stochastic, density-based model that included an adult dispersal process similar to that developed by Storer et al. (2003). Hutchison and Storer (2010) extended the model of Storer et al. (2003) to simulate pyramided Bt corn and Bt cotton in an MS landscape that included soybean as an alternative host. They did not consider evolution of the pest to insecticidal traits, partly because of the influence of annual migration of susceptible individuals from Mexico, where Bt crops are not grown. Hutchison and Storer (2010) focused on the potential of population suppression.

The primary purpose of this project was to describe the evolution of resistance by corn earworm to insecticidal traits expressed in crops planted in the heterogeneous landscapes of the southern United States. We used the model results to investigate the influence of three factors on insect resistance management (IRM): 1) how does adding a third insecticidal trait to both corn and cotton affect durability of the products, 2) how does unstructured corn refuge influence IRM, and 3) how do block refuges with 50% compliance and blended refuges compare with regard to IRM? In all of these comparisons, relative durability is emphasized because of the uncertainties in the parameter values.

Materials and Methods

We created a deterministic, frequency-based model of the population genetics of corn earworm. This model is a more complex version of the one published by Gustafson et al. (2006). Because it is frequency based, we do not include processes and mathematical functions concerning fecundity, oviposition, and density-dependent survival (carrying capacity). We generally assume that mating is random at the landscape level and eggs are distributed uniformly in each crop species (Jackson et al. 2008).

We modeled corn products sold by DuPont Pioneer that either contain two events (MON810 × DAS-01507-1) expressing Cry1Ab and Cry1F or three events (MON810 × DAS-01507-1 × MIR162) also expressing a third toxin, Vip3A. We also modeled various hypothetical Bt cotton crops. We did not model other insecticidal corn products sold by other seed companies.

The model essentially uses population genetics equations (Onstad and Gassmann 2014) to calculate changes in allele frequencies by calculating fitness (survival) for each genotype in each landscape patch and then determining the contribution of each genotype based on the proportional area of each patch assuming a new Hardy–Weinberg equilibrium. These calculations are made once for each insect generation.

Population Genetics

The model has four di-allelic, autosomal, resistance genes, one for each type of insecticidal traits, including Cry1A, Cry1F, Cry2Ab, and Vip3A, which are designated based on their mode of action and/or site of action. For the purposes of this model, traits Cry1Ab, Cry1Ac, and Cry1A.105 are all considered to have the same mode of action and thus share the same resistance gene. We assumed that there is no linkage between the four resistance genes and that no mutations occur after the start of simulations.

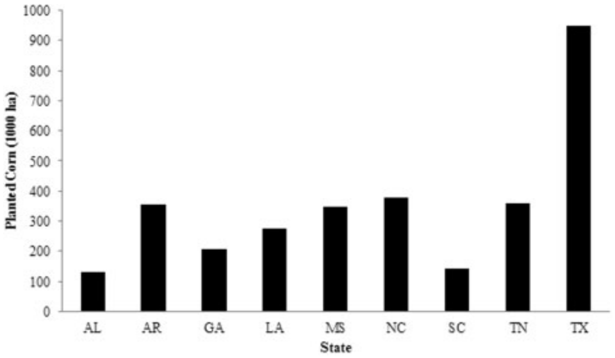

Fig. 1. Hectares planted with corn in 2013 in nine states. http://usda.mannlib.cornell.edu/usda/current/Acre/Acre-06-30-2014.pdf

Landscape

We have attempted to represent the complex, heterogeneous crop landscape in the southern United States consisting of corn, cotton, soybean, peanut, and sorghum (Figs. 1 and 2). The relative proportions of crops in Fig. 2 and information about non-Bt corn refuge (described later in the text) allowed us to categorize the states. In our analysis, we focused on GA, Texas (TX), and MS. Mississippi seemed to be a conservative choice relative to IRM, because it was the state with the lowest predicted durability for Bt cotton, according to Gustafson et al. (2006).

Corn earworm has up to six generations in the southern United States, but we only modeled the generations under Bt selection and did not model the generations in early spring or late fall when corn earworm feeds on wild plants, weeds, and volunteer corn (Babu et al. 2014). During the first two generations of corn earworm under Bt selection, the landscape in the model mainly consists of corn. During Generations 3 and 4, the landscape consists of cotton and unstructured refuge (i.e., non-Bt cotton, soybean, peanut, and sorghum).

Insecticidal cotton has been planted in the southern United States for many years, first with a single trait and now as a pyramid of two traits. No refuge is required for Bt cotton because of the availability of refuge in other crops (Onstad and Knolhoff 2014). According to a 2007 survey of insect control management practices of farmers performed by the U.S. Department of Agriculture (USDA), 96–97% of cotton in GA and MS and 48% of cotton in TX is Bt cotton (USDA 2014). However, more recent estimates from 2012 indicate even higher proportions of Bt cotton in the three states. Based on the report by Williams (2013), we use 0.01, 0.02, and 0.16 as the proportions of non-Bt cotton in GA, MS, and TX, respectively. We assume that a pyramid of two to three insecticidal traits, including Vip3A, will be planted in the future: half with Cry1Ac and Cry2Ab2 and half expressing Cry1Ac and Cry1F. In a second scenario, in which corn has three traits, we included Vip3A in both cotton crops.

Corn Refuge

Corn refuge consists of structured and unstructured refuge. Structured refuge is required by companies and the U.S. Environmental Protection Agency to be planted along with Bt corn. Unstructured refuge consists of non-Bt corn that is planted for any

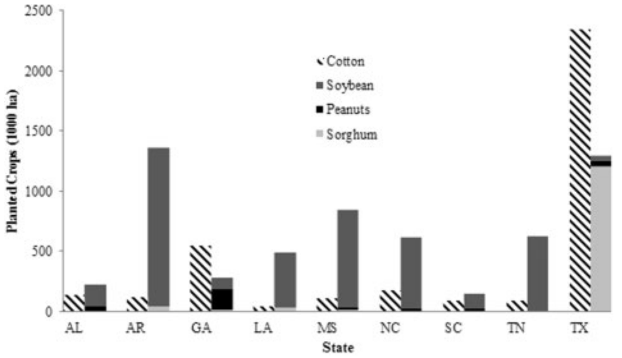

Fig. 2. Hectares planted with cotton, soybean, peanut, and sorghum in 2013 in nine states. http://usda.mannlib.cornell.edu/usda/current/Acre/Acre-06-30-2014.pdf

other reason, including the choice to plant more than that required. (In our model, blended refuge produced by mixing seed in the commercial bags is not unstructured refuge.) The USDA and several states in the southern United States, including MS, do not collect data on area planted with non-Bt or Bt corn (USDA-NASS 2013). Thus, our estimates for the total corn refuge in the southern United States were obtained from two sources.

First, we used observations by Babu (2013) working under the direction of Dr. Fred Musser at Mississippi State University. Of 48 cornfields tested in MS in 2012, 85% (41 fields) were positive for at least one Bt protein: 42% (20 fields) were positive for Cry1A and Cry2A, 42% (20 fields) were positive for Cry1F, and 2% (1 field) was positive for Cry1A alone. Thus, 15% of blocks visited were non-Bt corn.

According to a 2010 USDA survey of insect control management practices of farmers, 13% (SE = 4%) of corn acreage in GA was planted with Bt corn, indicating that 87% of corn is refuge (USDA 2014). For TX and North Carolina, the 2010 survey estimated 35 and 55% corn refuge, respectively (USDA 2014). Another standard USDA survey found 31% non-Bt corn in TX in 2012 (USDA-NASS 2013).

In a 2013 survey of corn growers performed at the request of the Agricultural Biotechnology Stewardship Committee, an industry group, at least 46% of corn growers in cotton-growing regions reported that they complied with the refuge size requirement for corn borers in the southern United States (Smith and Smith 2014). The survey also learned that 25% partially met the size requirement and 29% did not plant a refuge. We assumed 50% compliance with block refuge size requirements in all simulations.

Total refuge was calculated by combining both structured and unstructured corn refuge. First, we assumed that 50% of growers comply with the requirement for a 20% block refuge (the other 50% do not plant a required refuge). Thus, 10% of proportional area of Bt corn should be structured refuge (0.1×0.85, 0.1×0.13 and 0.1×0.65 for MS, GA, and TX, respectively). Unstructured refuge is the total proportional area of non-Bt corn minus the calculated structured refuge. Therefore, unstructured corn refuge is $0.15 - 0.085 = 0.065$, $0.87 - 0.013 = 0.857$, and $0.35 - 0.065 = 0.285$, respectively, for MS, GA, and TX.

Calculation of Refuge Effectiveness

Effective refuge is the percent of refuge (non-Bt crops and non-crop hosts) that effectively contributes to susceptible insect production. The effective refuge value adjusts land area numbers within a given insect generation to account for adoption of Bt crop, insecticide use in the same crop without Bt traits, and amount and quality of alternative hosts.

We found information about insecticide use in cotton, soybean, peanut, and sorghum in various sources. In non-Bt cotton, 64% of the fields are treated and 64.6% of the corn earworm larvae are killed by insecticide (Gustafson et al. 2006). In soybean, 38% of the fields are treated with insecticides lethal to corn earworm (http://quickstats.nass.usda.gov/results/114C2D53-D65E-3B50-A14F-17E81DA4DA99; data for MS accessed May 9, 2014). In treated soybean fields, mortality rate is 86% of larvae 6–7 d after treatment (Akin et al. 2012; Herbert et al. 2012a, b). For sorghum, 10% of the fields are treated, resulting in 82% insecticide-related mortality 4–6 d after treatment (http://quickstats.nass.usda.gov/results/A6E9EBC1-DD9A-3A70-BD9E-D70D701DDAEF; data for TX accessed July 22, 2014; Adams et al. 2013a, b). In GA, 85% of the peanut fields are treated with insecticides lethal to corn earworm and mortality rate is 90% (M. Abney, University of Georgia, personal communication).

The calculation of the proportion of effective refuge for a specific crop is presented in the following equations. First, the proportions of land area planted to Bt, P_B, and non-Bt, P_N, are calculated for each crop:

$$\begin{aligned} P_B &= AD \times (1 - Ref) \\ P_N &= 1 - P_B = 1 - AD + AD \times Ref \end{aligned} \tag{1}$$

where AD is Bt crop adoption level (proportional area) and Ref is the product of the proportion of required refuge and compliance rate.

Gustafson et al. (2006) point out that, when corn earworm feeds on cotton, soybean, sorghum, and peanut in late summer, soybean produces only a tenth as many adults as would a similar area of untreated non-Bt cotton. We assumed that peanut has the same production of corn earworm as soybean. Based on knowledge of corn earworm on sorghum in TX, we assume that sorghum produces one-fifth the number of moths per ha as cotton when neither is treated with insecticide. When we calculate the effective refuge contribution of soybean, peanut, and sorghum for the model, we multiply the landscape proportion by the proportional productivity coefficient. Based on the total acreage for each crop in a state (Fig. 2), we calculated the proportional land area, LP, for each crop. We then calculated the total cotton refuge for Generations 3 and 4 by applying the following equation:

$$Ref_{eff} = \left\{ \sum_{i=1}^{4} A(i) \times LP(i) \times [P_N(i) \times (T(i) \times LS(i) + 1 - T(i)] \right\} / \left\{ \sum_{i=1}^{4} A(i) \times LP(i) \times [P_B(i) + P_N(i) \times [T(i) \times LS(i) + 1 - T(i)] \right\} \tag{2}$$

where i for non-corn crops. A(i) is the productivity relative to cotton (1 for cotton, 0.2 for sorghum, and 0.1 for soybean and peanut). T(i) is the proportion of non-Bt crop treated with insecticide lethal to corn earworm, and LS(i) is the proportion of larvae surviving this treatment. And $P_B(i)$ is 0 for soybean, sorghum, and peanut, as there is no Bt variety of these crops in southern United States. If we do not consider alternative hosts, then the proportion of effective refuge, Ref_{eff}, is calculated by dividing the productive non-Bt part by the productive total crop, which is the special case of equation 2.

$$Ref_{eff} = [P_N (T \times LS + 1 - T)]/[P_B + P_N (T \times LS + 1 - T)] \tag{3}$$

For GA and MS, equation 2 calculated effective refuge levels for Bt cotton of 0.032 and 0.354, respectively. For MS, soybean is important for effective refuge. For GA and MS, P_N for corn is equal to the effective refuge for the first two generations in benchmark simulations, because we assume that no insecticide lethal to corn earworm is used exclusively on non-Bt corn.

We model the system in TX differently from those in GA and MS by making the first two generations of corn earworm in TX infest both corn and sorghum. Thus, we use half the acreage planted to sorghum (Fig. 2) as refuge for Bt corn and half for refuge for Bt cotton (in final two generations).

We assume that sorghum produces equal numbers of corn earworm moths per ha as non-Bt corn that has not been sprayed. The young sorghum crop is not treated with insecticides in either of the first two generations. Non-Bt corn is not sprayed in the first generation, but 20% of the crop is treated in the second generation. For blended corn refuge scenarios, the unstructured refuge is sprayed and none of the blended refuge is sprayed. For block refuge scenarios in TX, the standard simulation assumed 20% of all non-Bt corn is sprayed. (We also modeled the assumption that farmers do not spray any cornfields, which is the same as we modeled for MS and GA.) We assume 30% survival of corn earworm on treated non-Bt corn. Therefore, every ha of non-Bt corn produces $0.8 + 0.2 \times 0.3 = 0.86$ moth for every moth produced by sorghum in the second generation in TX.

The modeling of the third and fourth generations in TX is similar to that for the other two states. The productivity (oviposition preference and immature survival) of sorghum is one-fifth that of cotton, as noted previously. After accounting for insecticide use, the weighted average production of moths is 1 from sorghum for every 3 from non-Bt cotton. For TX, we calculated an effective refuge level for Bt cotton of 0.144 (equation 2).

Insect and Crop Synchrony

For simplicity, we assume that the generations of the corn earworm are discrete in time and that each generation infests a well-defined set of crop species that are available for the entire period of activity for immature stages. Within each of the three most common host plants, corn earworm prefers to oviposit on 1) tasselling and silk stage corn, 2) blooming and early pod set soybean, and 3) flowering cotton, which is preferred over preflowering cotton (squares). The plant species are not equally attractive, as our discussion of effective refuge suggested. In 2002–2003, Jackson et al. (2008) observed no overlap between cotton and corn in five southern states. From 2002 to 2006 in the southern United States, Head et al. (2010) observed that circa 2% of moths flying during “corn” generations had fed as larvae on cotton. The results of Head et al. (2010) also indicate that the natal fields of corn earworm are not soybean until after the generations on corn. Hutchison and Storer (2010) modeled the first two generations on corn only and the third and fourth generations on cotton and soybean. Thus, our benchmark assumption is that corn earworm infests corn for two generations, followed by two generations on cotton, soybean, peanut, and sorghum.

In the sensitivity analysis for MS, we allow corn earworm to exist on both corn and cotton during one generation. When corn is infested for three generations, we make the relative productivity of corn in the third generation equal to one-fifth the production of corn earworm on cotton (Gustafson et al. 2006). When cotton is infested by the second to fourth generations, we make the relative productivity of corn earworm in its second generation on cotton equal to one-fifth the production on corn. For this scenario, soybean, peanut, and sorghum are still only available as refuge for the last two generations. Thus, in this scenario, effective cotton refuge in MS is only 0.012 in the second corn earworm generation (first on cotton).

When we simulate the overlap of one generation of corn earworm on both corn and cotton, corn should be added into equation 2 as a fifth crop. For the third generation, mainly feeding on cotton, A(i) is 0.2, 0.2, 1, and 0.1 for corn, sorghum, cotton, and other crops, respectively.

Calculation of Survival in a Given Patch in Landscape

We used the model to evaluate block refuge and blended refuge (mixture of Bt and non-Bt seed). In both cases, we account for survival before and after a single movement event, probability of larval movement, and survival during movement. We calculate survival for both young and old larvae and assume that survival for entire larval stage is the product of the two. Young larvae are those that do not move off of their natal plant (Burkness et al. 2015). Because of a lack of data, the same rates are used for movement off of Bt and non-Bt plants resulting in symmetrical movement. Burkness et al. (2015) did not observe any early larval movement, but they found that the movement rate of old larvae is 15% with range between 0 and 33%. Thus, our benchmark value for the proportion of old larvae moving off of a corn plant was set at 0.15. For simplicity, we assumed that survival during late larval movement was 1.

The following equations calculate the survival of larvae in genotype g and in patch f for a given generation. Survival of young larvae is simply $S(g,f)^q$, where $S(g,f)^1$ is observed survival for a larva in genotype g that remains on a single plant in patch f for entire larval stage. The exponent q was chosen to cause most of mortality in young larvae ($q > 0.5$). The proportion of older larvae, OL, of genotype g on refuge plant surviving to adulthood i:

$$\begin{aligned} OL(g,r) = {} & S(g,r)^{(1-q)} \times [YL(g,b) \times S(g,b)^q \times M(b) \times P(r) + YL(g,r) \\ & \times S(g,r)^q \times (1 - M(r)) + YL(g,r) \times S(g,r)^q \times M(r) \times P(r)] \end{aligned} \tag{4}$$

Where YL is the young larvae in genotype g in patch f, either refuge, r, or Bt plants, b. Parameter M is the probability of moving off of plant in patch f and P is the proportional area of patch. The terms in the square brackets in equation 4 represent those leaving plants not in patch f and moving to a plant in patch f, those larvae staying on natal plant, and those leaving natal plant but reaching similar plant in patch f. For refuge plants, survival $S(g,r) = 1$, which means survival is 1 for both young and old larvae regardless of q value. For survival on blended refuge corn plants owing to cross-pollination, we used reduced survival owing to cross-pollination for $S(g,r)^{(1\text{-}q)}$ and kept $S(g,b)^q = 1$. The proportion of older larvae on Bt plant surviving to adulthood is:

$$\begin{aligned} OL(g,b) = {} & S(g,b)^{(1-q)} \times [YL(g,r) \times S(g,r)^q \times M(r) \times P(b) + YL(g,b) \\ & \times S(g,b)^q \times (1 - M(b)) + YL(g,b) \times S(g,b)^q \times M(b) \times P(b)] \end{aligned} \tag{5}$$

Again exponent q distributes mortality across two larval stages. A comparison of two studies of young and old larvae by Herbert et al. (2012a, b) leads us to believe that $0.6 < q < 0.65$ when larvae are exposed to insecticides. We used equations 4 and 5 for larvae infesting both corn and cotton. We used the same values of M and q in both Bt corn and Bt cotton, but the S values are different.

Survival on Corn Plants

The relative survival rates on reproductive-stage corn are based on data from field studies performed in three locations in GA and MS (Crespo et al. 2015). The data for each replicate are shown in the Suppl Appendix (online only). We calculated the mean survival by dividing the mean for all replicates for each treatment by the mean for all replicates observed in refuge corn. The means are similar to those used by Edwards et al. (2013), who based their parameters on the opinions of eight entomologists. For the three insecticidal traits acting alone, our means and standard deviations and the estimates of Edwards et al. (2013) are (0.77 ± 0.16, 0.69) for Cry1F, (0.54 ± 0.25, 0.58) for Cry1Ab, and (0.02 ± 0.01, 0.07) for Vip3A, respectively. For corn with MON810 × DAS-01507-1 × MIR162, we observed a mean survival of 0.001 with an SD of 0.003 in 2012 (Suppl Appendix [online only]). Therefore, we use the values observed in the field in 2012 to parameterize survival on reproductive-stage corn. For vegetative-stage corn, productivity is believed to be less than that on reproductive-stage corn (Gustafson et al. 2006, Edwards et al. 2013). Therefore, we use the following survival rates for homozygous susceptible larvae on vegetative corn: 0.49 on Cry1F and 0.36 on Cry1Ab (both based on expert opinions reported by Edwards et al. 2013), and 0.01 on Vip3A, which is less than the 0.02 observed in the field in reproductive-stage corn. We assume redundant killing (multiplicative survival) in all Bt corn products.

For refuge plants, we assume 100% survival unless cross-pollination reduces survival in reproductive stage corn in blended refuge. For refuge plants pollinated by adjacent Bt plants, we base our choice of survival rate for older larvae (those feeding on ears) on several sources of information. We do not model direct mortality caused by Bt pollen consumption by larvae on refuge plants, or at least we do not consider this a separate rate of survival. However, because older corn earworm larvae commonly feed on corn kernels, we do model the influence of cross-pollination from Bt plants on toxicity of refuge-plant kernels. This reduction in survival of older, homozygous susceptible larvae on refuge plants occurs in our model only on corn in the second generation when a seed blend is used for refuge.

Burkness et al. (2015) performed field experiments in Minnesota during 2012–2013 using artificial infestations to determine movement behaviors of corn earworm larvae. In these experiments, only the central plant was initially infested. By comparing the relative number of individuals surviving on the central refuge plant in pure stand refuge plots and blended plots, any negative effects of cross-pollination on survival could be inferred. For blended plots, the central refuge plant was surrounded by corn plants with MON810 × DAS-01507-1 × MIR162. Burkness et al. (2015) observed either no difference in the number of late-instar larvae surviving on central refuge plants or more larvae on the central refuge plant in blended plots than in pure stand refuge plots.

In GA and MS in 2012, D.B. and A.C. (unpublished data) performed field studies with natural infestations in a three-plant cluster planted with refuge corn and corn with MON810 ×DAS-01507-1 × MIR162. Note that in this study, the total number of larvae is the sum of the number collected and the number of exit holes in the corn ears. In MS, the refuge plant in pure stand refuge clusters had higher numbers of larvae on average compared with the blended refuge. The relative survival in blended refuge was 0.61. In GA, the relative survival was 0.95, but there was no significant difference between the number of larvae found on refuge plants in pure stand refuge versus refuge plants in blends.

In TX in 2014, P.P. (unpublished data) performed a field study with natural infestations in a 95:5 seed blend containing corn with MON810 × DAS-01507-1 × MIR162. Porter observed similar numbers of corn earworm adults surviving on seed blend refuge ears and ears in a pure stand of non-Bt corn. The relative survival rate in this study was 1 in the blended refuge.

Thus, in four out of five data sets concerning MON810 × DAS-01507-1 × MIR162 distinguished by year and/or location, there was no evidence of an effect of cross-pollination on corn earworm larvae. Therefore, our benchmark survival rate for older larvae on refuge corn in blends during the second generation is the mean of the five results described previously $(1 + 1 + 1 + 0.95 + 0.61)/5 = 0.91$. This rate is implemented by taking the square or cubed root of 0.91, which is 0.95 or 0.97, respectively, and assigning this as the survival rate for each toxin in model. In a sensitivity analysis, we varied survival owing to cross-pollination from 0 to 1. In all cases, the dominance of resistance for this case is the same as for all other cases, the dominance equals the survival rate for homozygous-susceptible.

It is possible that asymmetrical movement away from Bt corn could cause movement to counteract any losses owing to cross-pollination on refuge plants, but this is a more complicated scenario to model and no data exist to test this hypothesis. In cotton, movement away from Bt and non-Bt plants may be different or the same depending on variety (Jackson et al. 2010).

Survival on Bt Cotton

Edwards et al. (2013) obtained opinions from seven entomologists to select their survival rates on Bt cotton. For Cry1Ac, Cry1Ac x Cry2Ab, Cry1Ab × Cry1F, and Cry1Ab × Vip3A, the estimated survival rates were 0.46, 0.14, 0.20, and 0.09, respectively. Sivasupramaniam et al. (2008) observed survival on Cry1Ac cotton of 0.40 and Cry1Ac × Cry2Ab2 of 0.08. Dividing the Cry2Ab pyramid estimates in both publications by the respective survival on Cry1Ac (0.14/0.46 and 0.08/0.40) allows us to find the Cry2Ab contribution to survival, which is 0.30 and 0.20 in each study. Brévault et al. (2013) measured survival on plant material in the laboratory. They observed survival of 0.70 on non-Bt cotton, 0.04 on Cry1Ac (lower than expected), and 0.01 on Cry1Ac × Cry2Ab. This means that, relative to that observed on non-Bt corn, survival owing to Cry1Ac was 0.057 and that owing to Cry2Ab was 0.25. Thus, the Cry2Ab survival seems to be 0.25 based on all three studies. However, the survival rate of 0.057 on Cry1Ac observed by Brévault et al. (2013) seems similar to that observed by Anilkumar et al. (2009) but much lower than that observed by others (Kurtz 2005, Sivasupramaniam et al. 2008), including the eight experts cited by Edwards et al. (2013). We chose to use the survival rate observed by Sivasupramaniam et al. (2008), which is 0.40 on Cry1Ac cotton. Dividing survival rate of 0.2 on Cry1Ab × Cry1F cotton proposed by experts cited in Edwards et al. (2013) by the 0.4 value just chosen for Cry1A, we obtain a survival rate of 0.5 for Cry1F cotton.

Bommireddy and Leonard (2008) observed 7–28% survival on Vip3A cotton plant tissues after 4 d of exposure. The authors noted that survival would likely be less over a longer period. Therefore, we believe that a benchmark value for survival of the entire larval stage on Vip3A cotton should be 0.07. For the other three survival rates, assuming multiplicative survival on pyramids, we chose the following survival rates to be benchmark values: 0.40 on Cry1Ac, 0.25 on Cry2Ab, and 0.50 on Cry1F.

Brévault et al. (2013) concluded that inheritance of resistance on Cry1Ac cotton was not completely recessive. The value of h was 0.25. Brévault et al. (2013) found no fitness costs for resistant larvae on non-Bt cotton. They concluded that resistant phenotypes of corn earworm have incomplete resistance on at least one insecticidal trait. However, to be conservative in our analysis, we chose to assume complete resistance (100% survival) for homozygous-resistant larvae on all Bt cotton. We also assume redundant killing for larvae on pyramided Bt cotton.

Calculation of Genotypic Survival

We assume there is complete resistance (100% survival) when resistance is homozygous at a single locus. Heterozygote survival is calculated in the typical manner. Given Wss and Wrr survival for the two homozygotes, $Wrs = hWrr + (1\text{-}h)Wss$, where h is dominance of resistance. When $h = 0$, resistance is completely recessive. As demonstrated by Tabashnik et al. (2004), we modeled functional dominance based on dose of insecticide. We assume that dominance, h, equals the proportional survival rate for homozygous-susceptible larvae over the entire larval period. Although Brévault et al. (2013) calculated a value of $h = 0.25$ for expression of the allele conferring resistance to Cry1Ac, we do not use this value because it would actually be lower than all of the values derived from our rule for Cry1Ab and Cry1Ac. We use h to calculate survival of heterozygotes and then use the exponent q to calculate survival for each larval stage (equations 4 and 5).

Our model allows cross-resistance to be modeled as complete or partial and symmetrical or asymmetrical. The parameter $CR_{1\text{-}2}$ is defined as the cross-resistance for the Bt protein 2 when resistance occurs to Bt protein 1. When $CR_{1\text{-}2} = 0$, there is no cross-resistance. When $CR_{1\text{-}2} = \times\ (0 < \times < 1)$, there is partial cross-resistance between Bt proteins 1 and 2. In other words, $CR_{1\text{-}2}$ is a variable that denotes cross-resistance and shows that when 100% survival occurs on *Bt* protein 1, there will be x survival on Bt protein 2. Although there is no evidence of cross-resistance in corn earworm with the common insecticidal traits, we chose a conservative value of 0.1 for cross-resistance for all resistance genes.

The formulas used for calculating survival for two-toxin pyramids are shown in Table 1. Formulas similar to these were created in our computer program (R language in Rx64 V3.02) for a pyramid of three or more *Bt* proteins. For example, given wild-type susceptible alleles A, X, and M and resistance alleles B, Y, and N, we can calculate the survival rate of the genotype ABXYMN on a pyramid of three *Bt* proteins by separately calculating the survival rates of genotype AB on *Bt* protein 1, XY on *Bt* protein 2, and MN on *Bt* protein and multiplying these three survival rates to get the overall survival rate. For example, to calculate the survival rate S_{sr2} of heterozygote AB on *Bt* protein 1, the program performs the following steps. First, it calculates the AB survival rate without cross-resistance, $S_{sr} = (1\text{-}h_1) \times S_1 + h_1 \times s_1$. Then, it calculates the survival rate based on cross-resistance from the Y allele, which is $CR_{2\text{-}1} \times h_2 \times s_{2.}$ The survival rate of AB with cross-resistance of Y is $S_{sr1} = S_{sr}+(1\text{-}S_{sr}) \times (CR_{2\text{-}1} \times h_2 \times s_2)$. Third, it calculates the survival rate based on cross-resistance from the N allele, which is $CR_{3\text{-}1} \times h_3 \times s_{3.}$ The survival rate of AB with cross-resistance caused by Y and N is $S_{sr2} = S_{sr1}+(1\text{-}\ S_{sr1}) \times (CR_{3\text{-}1} \times h_3 \times s_3)$, where s_3 and h_3 are survival rate of NN on *Bt* protein 3 and associated dominance value, and $CR_{3\text{-}1}$ is partial cross-resistance between *Bt* proteins 1 and 3, respectively. Note that the value of $CR_{3\text{-}1}$ is the survival on Bt protein 1 when 100% survival occurs on *Bt* protein 3. This algorithm can be applied to other genotypes. We apply the cross-resistance factor to the separate survival rates for young and old larvae (before and after movement).

Required Corn Refuge

We evaluated IRM strategies that require 5–20% refuge either as a block or blend. We assume that block refuges are planted with a 50% compliance rate.

Calculating Changes in Resistance Allele Frequency

The final step is calculating the landscape-level change in allele frequency by determining mean survival of each genotype. Survival in each part of landscape is multiplied by the proportion of the landscape (after all adjustments described previously) that consists of that plant type. After the proportion of each genotype in the landscape is calculated, the allele frequencies for the adults are determined. The genotypes of the offspring are derived from the adult gene pool by assuming Hardy–Weinberg ratios. Edwards et al. (2013) assumed that initial resistance allele frequencies for Cry1A and Cry2 were 0.002 and 0.0002, respectively. We take a more conservative approach and assume that the benchmark values of initial resistance allele frequencies for all loci are 0.005.

Computer Program Verification

We verified the logic of the computer program (R language in R ×64 V3.02) in several ways. The program was compared with two sets of

Table 1. Example of formulas for survival rates for larvae feeding on a pyramid of *Bt* proteins, with subscripts representing resistance genes

Genotype	Total survival
AAXX	$S_1 \times S_2$
AAXY	$[S_1 + (1 - S_1) \times (CR_{2-1} \times h_2 \times s_2)] \times [(1 - h_2) \times S_2 + h_2 \times s_2]$
AAYY	$[S_1 + (1 - S_1) \times (CR_{2-1} \times s_2)] \times s_2$
ABXX	$[(1 - h_1) \times S_1 + h_1 \times s_1] \times [S_2 + (1 - S_2) \times CR_{1-2} \times h_1 \times s_1]$
ABXY	$\{[(1 - h_1) \times S_1 + h_1 \times s_1] + (1 - [(1\text{-}h_1) \times S_1 + h_1 \times s_1]) \times (CR_{2\text{-}1} \times h_2 \times s_2)\} \times \{[(1 - h_2) \times S_2 + h_2 \times s_2] + (1 - [(1 - h_2) \times S_2 + h_2 \times s_2]) \times (CR_{1\text{-}2} \times h_1 \times s_1)\}$
ABYY	$\{[(1 - h_1) \times S_1 + h_1 \times s_1] + (1 - [(1\text{-}h_1) \times S_1 + h_1 \times s_1]) \times (CR_{2-1} \times s_2)\} \times [s_2 + (1 - s_2) \times (CR_{1-2} \times h_1 \times s_1)]$
BBXX	$s_1 \times [S_2 + (1 - S_2) \times CR_{1-2} \times s_1]$
BBXY	$[s_1 + (1 - s_1) \times (CR_{2-1} \times h_2 \times s_2)] \times \{[(1 - h_2) \times S_2 + h_2 \times s_2] + (1 - [(1 - h_2) \times S_2 + h_2 \times s_2]) \times CR_{1-2} \times s_1\}$
BBYY	$[s_1 + (1 - s_1) \times (CR_{2-1} \times s_2)] \times [s_2 + (1 - s_2) \times (CR_{1-2} \times s_1)]$

In this case, the pyramid includes two toxins related to two resistance genes, where A and X are wild-type susceptible alleles and B and Y confer resistance.

S_1 is defined as the proportion of susceptible insects surviving *Bt* protein 1.

s_1 is defined as the proportion of susceptible insects surviving on non-*Bt* maize or as the proportion of resistant insects surviving *Bt* protein 1. Survival of resistant insects on *Bt* maize was assumed to be the same as survival of susceptible insects on non-*Bt* maize.

$CR_{1\text{-}2}$ is defined as the cross-resistance parameter for the *Bt* proteins 1 and 2.

previous model results. A simple, abstract, two-gene model was used to test two-gene scenarios by eliminating larval movement factors. The results from Mallet and Porter (1992) were also verified by studying one major resistance gene and only incorporating late larval movement. In addition, the genotype survival rates were manually calculated under three-gene scenarios to match the results derived from model. We used a general two-gene model developed in the R programming language to test the effective-refuge and larval-movement algorithms.

Results

The results should be studied for 1) any important differences in durability owing to required refuge level, 2) differences between blended and block refuge, and 3) differences in conclusions across insecticidal products. Note that the large differences in landscapes between the three modeled states make a direct comparison useless without reference to the benchmark values for each state.

Tables 2 and 3 display the results for MS, which was modeled with 6.5% unstructured corn refuge and 35% refuge for cotton. Tables 2 and 3 indicate that durabilities are prolonged with more insecticidal traits. The durability of corn with MON810 × DAS-01507-1 and double-traited Bt cotton products is 8.25 yr with the benchmark parameters and 20% block refuge (Table 2), whereas all the durabilities of corn with MON810 × DAS-01507-1 × MIR162 and the triple-traited Bt cotton are above that number for any required refuge of at least 5% or any unstructured corn refuge of at least 3% (Table 3). Table 2 indicates that blended refuge is slightly superior to blocks with 50% compliance with refuge deployment. With the higher-dose products, such as corn with MON810 × DAS-01507-1 × MIR162, the durabilities are more sensitive to refuge level (Table 3). With 5% required refuge, durabilities are 16.25 and 17.25 yrs, respectively, for block and blend (Table 3). Neither type of refuge is clearly superior to the other with a 50% compliance rate for block refuge.

The "best" insecticidal trait for control may not last longer than the others. In our simulations, the allele conferring resistance to Vip3A evolved faster than the others in MON810 × DAS-01507-1 × MIR162 and Bt cotton. For example, in MS simulations with benchmark parameters, the allele conferring resistance to Vip3A reached 50% frequency 3–5 yr faster than the durabilities for corn with MON810 × DAS-01507-1 × MIR162 with minimum 5% required refuge (Table 3). The difference in durability increases as the refuge size increases.

For GA, our benchmark model included 85.7% unstructured corn refuge and 3.2% refuge for cotton populations. All durabilities for corn with MON810 × DAS-01507-1 and double-traited Bt cotton are less than 10 yr (Table 4). Adding required corn refuge has almost no influence on results. Benchmark durability increases to circa 12 yr for corn with MON810 × DAS-01507-1 × MIR162 and the triple-traited Bt cotton (Table 5). Again, the results are insensitive to amount of required corn refuge. The large amount of unstructured corn refuge in GA overwhelms any contribution from required corn refuge. If we increase non-Bt cotton from 1 to 20% in the model, durabilities for corn with MON810 × DAS-01507-1 × MIR162 increase to 26–31 yr in GA, more than double those shown in Table 5.

Tables 6 and 7 present the results for TX, which we modeled with 54% unstructured refuge (corn and sorghum) for Bt corn and 14% refuge for Bt cotton. Table 6 has results similar to those in Table 3 for MS. Benchmark simulations indicate that durabilities of corn with MON810 × DAS-01507-1 and the double-traited Bt cotton are 10–10.75 yrs (Table 6). The durability of the double-traited products is 10.25 yr with the benchmark parameters and 20% block refuge (Table 6), whereas all the durabilities of corn with MON810 × DAS-01507-1 × MIR162 and the triple-traited Bt cotton are at least double that number (Table 7). Results in both tables are not sensitive to changes in required corn refuge.

In our most complex sensitivity analysis, we allowed corn earworm to infest both corn and cotton for one generation. For MS, durabilities of corn with MON810 × DAS-01507-1 × MIR162 with benchmark unstructured corn refuge declined by no more than 2.25 yr (10%). For GA with three generations on cotton, durabilities decreased by 0.5 yr. However, three generations on corn increased durabilities by 3 yr, or 25%, because the large corn refuge was available during the first cotton generation. For TX, three generations on corn increased durabilities by no more than 2.75 yr (10%). Three generations on cotton changed the results for TX by less than 1 yr.

Because GA and TX have much more unstructured corn refuge than MS, we chose to determine the sensitivity of results for MS simulations. Sensitivity to changes in initial resistance allele frequency (IRAF), dominance of resistance, and cross-resistance will be similar in all simulations. However, the simulations for GA and TX will be less sensitive than those for MS with regard to parameters pertaining to the corn crop. Thus, all of the following sensitivity analyses were performed with benchmark parameters for MS and evaluated the durability of corn with MON810 × DAS-01507-1 × MIR162.

As expected, results are sensitive to IRAF and dominance. For simulations with MS benchmark parameters, durability of corn with MON810 × DAS-01507-1 × MIR162 increased by 7–13 yrs (50%) when IRAF was reduced to 0.001 from 0.005. When the IRAF was increased to 0.02 from 0.005, a reduction in durability of 8–16 yrs occurred. Similar patterns were observed for dominance. When dominance was decreased by half, durability increased by 8–14.5 yrs

Table 2. Years of durability in Mississippi when MON810 × DAS-01507-1 is planted on all Bt cornfields and pyramided Bt cotton does not express Vip3A

Unstructured refuge	No. required refuge	Block refuge proportion, compliance 50%			Blended refuge proportion		
		0.05	0.1	0.2	0.05	0.1	0.2
0	6.25	6.5	7	7.5	7	7.5	8.75
0.03	6.5	7	7.25	8	7.25	8	9.25
0.065	7.25	7.25	7.75	8.25	7.75	8.25	9.5
0.1	7.5	8	8.25	9	8.25	8.75	10
0.2	9	9.25	9.5	10.25	9.5	10.25	11.25

Year in which all alleles conferring resistance to insecticidal corn exceed a frequency of 0.5

Benchmark value of unstructured refuge is 0.065.

Table 3. Years of durability in Mississippi when MON810 × DAS-01507-1 × MIR162 is planted on all Bt cornfields and pyramided Bt cotton expresses Vip3A

Unstructured refuge	No. required refuge	Block refuge proportion, compliance 50%			Blended refuge proportion		
		0.05	0.1	0.2	0.05	0.1	0.2
0	3.25	8.25	11.75	17.75	10.25	14.25	21
0.03	9	12.25	15.25	20.75	13.5	17.25	23.25
0.065	13.5	16.25	19.25	24.25	17.25	20.25	25.5
0.1	17.75	20.25	22.75	27.25	20.75	23.5	28
0.2	28.25	30.25	32	35.5	30	31.75	34.75

Year in which all alleles conferring resistance to insecticidal corn exceed a frequency of 0.5.
Benchmark value of unstructured refuge is 0.065.

Table 4. Years of durability in Georgia when MON810 × DAS-01507-1 is planted on all Bt cornfields and pyramided Bt cotton does not express Vip3A

Unstructured refuge	No. required refuge	Block refuge proportion, compliance 50%			Blended refuge proportion		
		0.05	0.1	0.2	0.05	0.1	0.2
0.5	7.25	7.25	7.5	7.75	7.5	7.75	7.75
0.7	8.25	8.5	8.5	8.75	8.5	8.75	8.75
0.8	9	9	9	9	9	9	9
0.857	9	9	9	9	9	9	9.25
0.9	9.75	9.75	9.75	9.75	9.75	9.75	9.75

Year in which all alleles conferring resistance to insecticidal corn exceed a frequency of 0.5.
Benchmark value of unstructured refuge is 0.857.

Table 5. Years of durability in Georgia when MON810 × DAS-01507-1 × MIR162 is planted on all Bt cornfields and pyramided Bt cotton expresses Vip3A

Unstructured refuge	No. required refuge	Block refuge proportion, compliance 50%			Blended refuge proportion		
		0.05	0.1	0.2	0.05	0.1	0.2
0.5	10.75	10.75	10.75	10.75	10.75	10.75	10.75
0.7	11.25	11.25	11.25	11.5	11.25	11.25	11.5
0.8	11.75	11.75	11.75	11.75	11.75	11.75	11.75
0.857	11.75	11.75	12	12	11.75	12	12
0.9	12	12	12	12	12	12	12

Year in which all alleles conferring resistance to insecticidal corn exceed a frequency of 0.5.
Benchmark value of unstructured refuge is 0.857.

Table 6. Years of durability in Texas when MON810 × DAS-01507-1 is planted on all Bt cornfields and pyramided Bt cotton does not express Vip3A

Unstructured refuge	No. required refuge	Block refuge proportion, compliance 50%			Blended refuge proportion		
		0.05	0.1	0.2	0.05	0.1	0.2
0	8.5	8.75	8.75	9	8.75	9	9.25
0.05	8.75	8.75	9	9	9	9	9.75
0.1	9	9	9	9.25	9	9.25	9.75
0.285	10	10	10	10.25	10	10.25	10.75
0.5	11	11	11	11.25	11	11.25	11.75

Year in which all alleles conferring resistance to insecticidal corn exceed a frequency of 0.5.
Benchmark value of unstructured refuge is 0.285.

(over 50%). When dominance was doubled in the simulations for MS, durability of corn with MON810 × DAS-01507-1 × MIR162 was reduced by 6–12.5 yrs.

Durability of corn with MON810 × DAS-01507-1 × MIR162 was also sensitive to changes in cross-resistance. When we increased cross-resistance for all genes from 0.1 to 0.2, durability declined by 4–7 yrs (25–0% change). When cross-resistance was eliminated from the model, durability was prolonged by 5.5–7.5 yrs (30–40% change). Note that there is no evidence of cross-resistance in corn earworm for the simulated insecticidal traits.

Table 7. Years of durability in Texas when MON810 × DAS-01507-1 × MIR162 is planted on all Bt cornfields and pyramided Bt cotton expresses Vip3A

Unstructured refuge	No. required refuge	Block refuge proportion, compliance 50%			Blended refuge proportion		
		0.05	0.1	0.2	0.05	0.1	0.2
0	22.5	22.75	23	23.75	22.75	23	23.25
0.05	23	23.25	23.75	24	23.25	23.5	24
0.1	23.75	23.75	24	24.5	23.75	24	24.25
0.285	25.25	25.75	25.75	26	25.5	25.75	25.75
0.5	27	27.25	27.25	27.75	27	27.25	27.25

Year in which all alleles conferring resistance to insecticidal corn exceed a frequency of 0.5.
Benchmark value of unstructured refuge is 0.285.

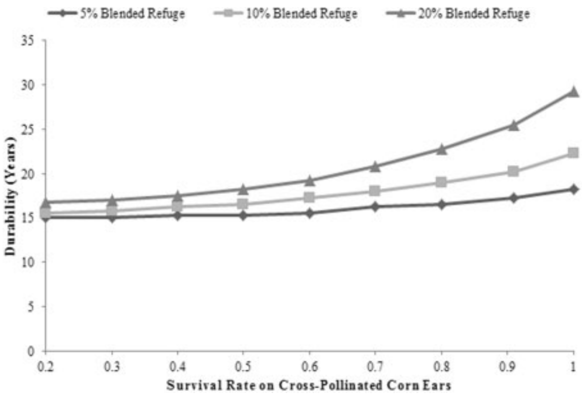

Fig. 3. Influence of cross-pollination effects on corn earworm survival on durability of corn with MON810 × DAS-01507-1 × MIR162 under Mississippi conditions with a benchmark value of 0.91.

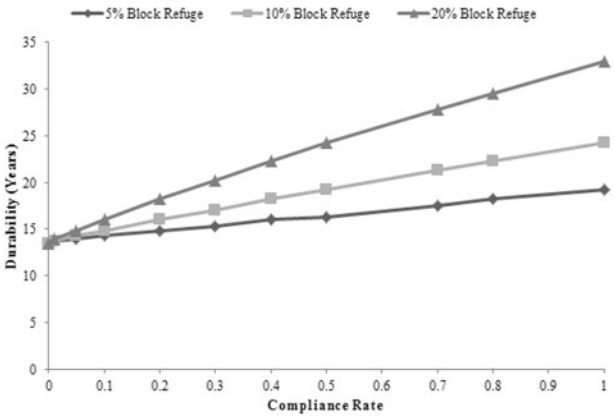

Fig. 4. Influence of farmer compliance rate for planting block refuge on durability of corn with MON810 × DAS-01507-1 × MIR162 under Mississippi conditions with a benchmark value of 0.50.

Larval movement and cross-pollination are two parameters likely influencing the effectiveness of blended refuge. For MS conditions, results are not sensitive to doubling or halving the movement rate. When larval movement was either reduced to 0% or increased to 30%, durability of corn with MON810 × DAS-01507-1 ×MIR162 changed by no more than a 1 or 2.75 yr, for a 5% blend and a 20% blend, respectively. As expected, low rates of movement do not influence IRM for blended refuge and MON810 × DAS-01507-1 × MIR162.

Figs. 3 and 4 indicate the sensitivity of modeling results for MS to changes in survival owing to cross-pollination and compliance rate. Durability of corn with MON810 × DAS-01507-1 × MIR162 with blended refuge is most sensitive to reduction in survival owing to cross-pollination from 1 to 0.7 (Fig. 3). Durability of corn with MON810 × DAS-01507-1 × MIR162 planted with block refuges is sensitive to any changes in farmer compliance with refuge planting requirements (Fig. 4).

We performed a sensitivity analysis on the parameter q, which determines how much of the larval mortality occurs in the young versus old age classes (equations 4 and 5). For blended refuge scenarios under MS conditions, the results changed by no more than 1.5 yr (10% change) when we increased q by 0.15 from its benchmark value of 0.65–0.80. The change was only 0.5 yr when q was reduced by 0.15. In all scenarios, as q increased, causing greater effect on early instars, durability of corn with MON810 × DAS-01507-1 × MIR162 declined.

Discussion

When Bt cotton expresses the same number of insecticidal traits, corn with three insecticidal traits provides longer durability, as expected, than corn pyramided with two. Blended refuge provides similar durability for corn products compared with the same level of required block refuge when the compliance rate is 50%. Results for MS and TX are similar, but durabilities for all traits are lower in GA, where unstructured corn refuge is the highest of the three states and refuge for Bt cotton is 3%, the lowest of the three states.

Cross-pollination from Bt plants to non-Bt plants can be important for IRM of kernel-feeding pests. For corn earworm, we have information from five field trials that measured survival of larvae on refuge plants adjacent to Bt corn. The data indicated that survival can be reduced by cross-pollination and that the survival ranges from 0.61 to 1.0. It is possible that the cross-pollination effect will vary over time and location. However, for a long-term study of evolution, the mean effect (survival reduced to 0.91) will likely give us a reasonable understanding of consequences for evolution and IRM. Note that cross-pollination effect occurs only in second generation and only in one of four generations per year selected by insecticidal traits. As noted above under benchmark conditions for all three states, block and blended refuges contributed equally to IRM when compliance rate was taken into consideration.

Several other field studies of kernel feeding and cross-pollination involving different types of corn demonstrate the variability in experimental techniques applied to this problem. Horner et al. (2003a) compared survival of larvae on ears of field corn expressing Cry1Ab with survival on non-Bt ears in 1996–1997. The feeding patterns suggested that larvae in Bt ears are moving about sampling kernels more frequently than larvae in non-Bt ears. As expected from knowledge of gene expression in kernels, expression of endotoxin was variable in the kernels on Bt corn.

Burkness et al. (2011) demonstrated in several experiments with Cry1Ab sweet corn and natural infestations that survival was 63% on refuge ears pollinated with Bt pollen compared with survival on non-Bt ears not cross-pollinated. Yang et al. (2014b) evaluated a seed blend of Bt corn expressing Cry 1A.105/Cry2Ab2 and Cry1F and non-Bt corn in a field trial with natural infestations in Louisiana. After sampling fourth instars on non-Bt corn, they observed more larvae on the central non-Bt plant in blend than in pure non-Bt refuge at the end of the experiment. However, in a subsequent field study using the same Bt corn, Yang et al. (2014a) observed only 37% survival relative to the pure stand of refuge corn. In this field trial, they placed two neonates from a laboratory colony on the top of each ear after removing all larvae naturally infesting the ears. The ears were then covered with bags (Yang et al. 2014a). In a 2-yr study in MS, Babu (2013) measured survival with artificial infestations of refuge ears hand-pollinated with pollen containing traits for Cry 1A.105 and Cry2Ab2. He calculated relative survival rates of 67 and 64% after 10 d of exposure, respectively, but the latter value was not significantly different from the observed survival on non-Bt corn ears without cross-pollination. Thus, we conclude that the effects of cross-pollination and our ability to observe these effects are likely influenced by many factors, including weather, corn hybrid, cultivation practices, pollination, and corn earworm behavior, that vary from season-to-season and site-to-site.

In 1996, average survival of larvae on Cry1Ab corn was 36% after accounting for survival on non-Bt corn (Horner et al. (2003a). Horner et al. concluded “MON810 corn suppressed the establishment and development of *H. zea* to late instars by at least 75%. This level of control is considered a moderate dose.” Storer et al. (2001) also measured mortality on MON810 in 1997–1998 and concluded that survival could be as much as 40% to the prepupal stage and 35% to adulthood.

Future research should focus on four subjects. First, it will be difficult to create a model of corn earworm population densities for all crops in the landscape unless entomologists measure density-dependent survival (carrying capacity) on crops other than corn. Cannibalism has been well-studied on corn (Horner and Dively 2003, Storer et al. 2003), but we need similar knowledge about density-dependent survival on other crops to abandon the frequency-based approach used in this paper. Second, we did not include fitness costs of resistance in the corn earworm parameters, such as reduced survival or reduced fecundity by resistant phenotypes on non-Bt crops (Gustafson et al. 2006). Third, the influence of annual immigration into these states from corn earworm populations in Mexico was not modeled. The modeling performed by Hutchison and Storer (2010) indicates that this migration could be important to the population dynamics and genetics by adding new susceptible insects each year. Fourth, Edwards et al. (2013) noted that agroecosystems are dynamic. They urged modelers to update their simulated landscapes periodically to account for changes owing to economics and other factors. Furthermore, the landscapes of the southern United States are likely even more complex and heterogeneous than the few landscapes that we represented in our complex model.

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Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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