

Modeling the Development of Resistance by Stalk-Boring Lepidoptera (Crambidae) in Areas with Irrigated Transgenic Corn

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ABSTRACT The population dynamics and population genetics of two bivoltine species of corn borers are modeled in a hypothetical region of irrigated transgenic and nontransgenic corn. European corn borer, *Ostrinia nubilalis* (Hübner), adults were assumed to disperse throughout the landscape for both mating and oviposition. Southwestern corn borer, *Diatraea grandiosella* Dyar, adults were assumed to have very localized dispersal behaviors. Resistance developed quickly in both species when the allele for resistance to the transgenic toxin was dominant. When the allele for resistance was not dominant and few or none of the heterozygous larvae survived the toxin, the behaviors of adult insects determined the speed of resistance development. With block refuges of 10–40% the European corn borer developed resistance within 15–38 yr, but the southwestern corn borer never developed resistance within the 100 yr simulated. A row-strip refuge configuration did not change the time for resistance to develop in the European corn borer; however, row-strip refuges cannot be recommended for the southwestern corn borer. Uncertainty about adult behaviors in irrigated corn led us to examine the potential impact of behavior on resistance development. Adult behaviors influenced resistance development more than refuge size. For instance, if the first flight of moths exhibit random mating and uniform oviposition throughout the landscape and the second flight exhibits localized mating and oviposition, resistance developed at least five times faster in the southwestern corn borer population and three times slower in the European corn borer population compared with our standard assumptions. We discuss the implications of adult behavior, refuge configuration, refuge placement within the landscape and year-to-year relocation on resistance management plans.

KEY WORDS southwestern corn borer, *Diatraea grandiosella*, European corn borer, *Ostrinia nubilalis*, resistance management, simulation modeling

SINCE THE COMMERCIAL introduction of transgenic corn (*Zea mays* L.) in 1996, there has been concern that resistance to the expressed toxin would develop in targeted lepidopteran pests. To slow the development of resistance to transgenic corn, a high-dose refuge strategy has been implemented. For this strategy to work, the expressed dose of the toxin should be high enough to kill all or almost all heterozygotes carrying a resistance allele and growers should plant a refuge area of nontransgenic corn in close proximity to the transgenic field so susceptible and resistant adults can mix during mating (Gould et al. 1994; USEPA 1998).

In western Kansas and Nebraska, eastern Colorado, and northwestern Oklahoma and Texas, corn is usually irrigated. Because irrigation increases the chances of harvesting a good crop, farmers are often willing to invest in other crop inputs that may help produce higher yields. These farmers have a history of aggressive management of insect pests and have quickly adopted transgenic technology. Rain fed corn production has been increasing in western Kansas and eastern Colorado, and transgenic corn is also being adopted for this use.

There are two important corn borer pests in this region, the European corn borer, *Ostrinia nubilalis* (Hübner) (Calvin and Van Duyn 1999), and the southwestern corn borer, *Diatraea grandiosella* Dyar (Knutson and Davis 1999). The European corn borer causes damage by feeding on leaf and stem tissues of the developing plant, resulting in physiological yield losses. The European corn borer also can cause losses by tunneling within plants and weakening ear shanks, which results in dropped and unharvested ears. There are two main generations each year and both can

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cause yield losses. In this region the damage from the second generation is usually more severe. The southwestern corn borer is another destructive pest of corn from Texas to southern Kansas and Colorado. The life cycle of the southwestern corn borer is similar to that of the European corn borer but the southwestern corn borer can cause much greater harvest loss. In addition to leaf feeding and stalk tunneling, at harvest time the southwestern corn borer burrows to the base of the stalk and girdles the stem causing the plant to lodge. Physiological losses of up to 38% of the expected yield have been recorded (Whitworth et al. 1984), but harvest losses can be even more extensive (L.L.B., and P.E.S., unpublished data).

The percentage of cornfields planted with transgenic corn in the western high plains was initially high; however, growers found that they still had to treat for spider mites and western corn rootworm adults and, therefore, some of these growers returned to applying insecticides to control the wider spectrum of pests. Current adoption of transgenic corn in this region averages between 20 and 50% (USEPA 2000, p. 18).

We developed a simple model to address resistance management of the southwestern corn borer and the European corn borer. These serious corn pests are highly susceptible to current transgenic hybrids; however, major differences in how these insects mate, distribute their eggs, and colonize the host crop will play a central role in resistance management. Here we modeled resistance management for the southwestern corn borer and simulated conditions representing irrigated cornfields in the western and southwestern Corn Belt.

Materials and Methods

This section describes our approach to the problem, the mathematical model, and the analysis of the results. Although two insects are being modeled, there is fundamentally only one model with differences in the parameter values based on the biology of each insect. We describe the model details and indicate differences in parameter values as dictated by the biology of each species listed in Table 1. Finally, the programming, computation, and analysis of the model are described.

Overview of Population Genetics. The problem of predicting or describing the evolution of resistance to transgenic crops must include explicit and implicit assumptions about population genetics. We assume that resistance involves a single locus with two alleles: allele Z is susceptibility to transgenic corn; R is resistance to transgenic corn. The locus is assumed to be autosomal, not sex-linked, with no epistasis or linkage between loci. For heterozygotes, recessive alleles are identified with lower case letters. Mutations do not occur after the start of the 100-yr simulations. No fitness costs are simulated. Sex ratio of eggs is 50:50.

Landscape. The landscape is ≈ 260 ha of continuous corn consisting of transgenic corn and a refuge of nontransgenic corn. This 260 ha area is surrounded by a region of similar units of cornfields. In this paper we

Table 1. Standard parameter values for the model

Parameter	Southwestern corn borer	European corn borer
Sex ratio at eclosion (female/male)		0.5
Initial population (adults/ha)		50,000
Plant population (plants/ha)		67,000
Carrying capacity per plant (C)	1	22
Initial frequency of Resistance allele (B)		0.001
Fecundity (eggs/female):		
First generation	384	288
Second generation	498	288
Dispersal of males to alternate block for second mating	1%	Complete mixing of sexes
Distribution of oviposition in Field of emergence (J)	75%	0%
Proportion to landscape	25%	100%
Overwintering survival (2nd generation)	14%	18%
Normal survival (both generations)	20%	7.7%
Survival to transgenic toxin theoretical high dose		
ZZ		0.0
ZR		0.0
Practical high dose		
ZZ		0.001
ZR		0.01

describe the landscape in terms of both the insect's adult behavior and the planting arrangement for the corn. Thus, we try to describe the landscape both ecologically and agriculturally.

From an agricultural perspective, the landscape consists of either two kinds of blocks (large areas of corn) or two kinds of wide row strips (Fig. 1). Immature insects remain in the block or strip in which they were oviposited. We use the term "natal field" to represent the block or field with both kinds of strips (transgenic and nontransgenic) in which an adult develops and emerges.

From an ecological perspective, we can think of the landscape as patches of habitat with adults moving between patches for mating and/or oviposition. For purposes of mating, the patch is either the natal field, where mating occurs soon after emergence and before dispersal, or the patch is the entire landscape (both blocks or a field with row strips), where dispersal beyond the strip or block occurs before mating.

Natural Density-Independent Survival. Overwintering survival of the southwestern corn borer is density independent and averages 0.14 for the stage in diapause (Davis et al. 1973, Knutson and Gilstrap 1990). Survival of first generation southwestern corn borer is also density independent and ranges from 0.20 to 0.52 per generation (Knutson and Gilstrap 1990). Survival during the second generation ranges from 0.007 to 0.038 in Texas (Knutson and Gilstrap 1990) and ≈ 0.09 in Mississippi (Davis et al. 1972). We chose to use 0.2 as both our first-generation survival value and the density-independent survival before overwintering.

For the European corn borer, we used parameters described by Onstad and Gould (1998). Normal natural survival through the larval stage is 0.077 for both generations. Overwintering survival is 0.18 for dia-

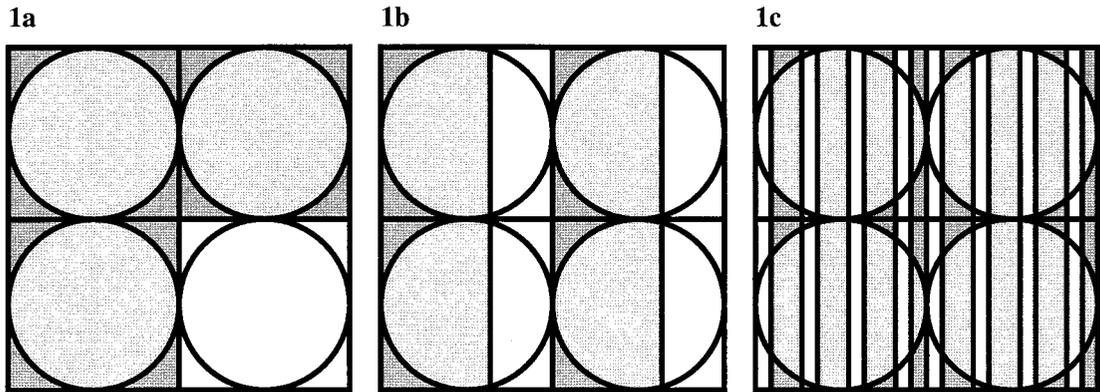


Fig. 1. Three kinds of landscapes with transgenic corn (shaded) and nontransgenic corn refuge (unshaded): (a) block refuge outside the transgenic field, (b) block refuge along side the transgenic field, and (c) wide rows of refuge within the transgenic field.

pausing fifth instars. We ignore the small level of mortality in the egg and pupal stages.

Density-Dependent Survival. According to Knutson and Gilstrap (1990), cannibalism and disease cause the final larval stage of the second generation southwestern corn borer to experience density-dependent mortality. Typically, only one larva survives within a corn plant. Slower maturing larvae kill older larvae and possibly ensure better pupation or overwintering sites (Tarpley et al. 1993). We modeled density-dependent survival in both generations of southwestern corn borer by simply limiting the maximum population density to the number of plants per field (one larva per plant). Uniform distribution of older larvae in fields supports this simplification (Overholt et al. 1990). We use this low carrying capacity for the first generation for two reasons. First, Knutson (1987) observed 0.3–1.8 pupae per plant in the first generation over 3 yr at two sites. Second, in laboratory studies, Breden and Chippendale (1989) and Tarpley et al. (1993) observed extensive cannibalism by southwestern corn borer larvae reared under both diapause-inducing and diapause-averting conditions. Knutson (1987) observed cannibalism in the first generation in 2 out of 5 yr in the field.

Density-dependent survival of European corn borer was modeled on a daily, age-specific basis by Onstad (1988) and Onstad and Guse (1999). For this model, we used a generational time step, and a maximum of 22 larvae per plant (Onstad 1988). We assume that this carrying capacity limits both generations, with the second-generation population reduced before winter mortality occurs.

Survival in Transgenic Corn. For survival in transgenic corn, we used the same approach for both insects. For a theoretical high dose, we assume that susceptibility is functionally dominant or the other extreme, that resistance is dominant. With functional dominance of susceptibility, there is no intermediate expression of resistance and neither the homozygous susceptibles nor the heterozygotes survive (Table 1). For a practical high dose, the survival per stage is 0.001

for ZZ and Zr larvae, whereas the value for ZR larvae with partially recessive expression is 0.01. All homozygous resistant individuals, RR, and all heterozygotes with a dominant R have 100% survival in transgenic corn. (Note that only 75% of RR resistant individuals survive transgenic corn in the model of Onstad and Gould (1998).)

Diapause. We do not include first-generation diapause in the model. This is reasonable for southwestern corn borer because in Texas at least 99% of southwestern corn borer larvae pupate during the first generation and do not enter diapause (Knutson and Gilstrap 1990). In southern areas, the European corn borer is not likely to enter diapause during the first generation; however, at latitudes similar to Colorado and Illinois (40° N), as much as 25% of first-generation larvae enter diapause (Onstad and Brewer 1996, Onstad and Gould 1998). For both species, the proportion entering diapause may be higher in more northern regions or if transgenic corn causes developmental delays. Diapause in the first generation has counteracting effects on the populations. It reduces the annual population growth but it also allows some individuals to escape selection pressures in the second generation. Cannibalism by the second-generation southwestern corn borer population may further reduce the survival of diapausing first-generation larvae.

Mating and Dispersal. Several observations lead us to assume that mating by southwestern corn borer occurs mainly in the cornfield in which the larvae develop (the natal field). First, at least 91% of females are mated within 24 (Rolston 1955) or 48 h of emergence (Langille and Keaster 1973). Rolston (1955) and Davis and Henderson (1967) showed that 66% of females mated on the same night that they emerged. Second, the precopulatory flight of males is mostly within the natal field (Langille and Keaster 1973). Given these observations, the model assumes random mating within the natal field, but not in the entire landscape.

Our standard assumption is that one mating occurs before any dispersal from the natal field. However,

southwestern corn borer males can mate twice (Rolston 1955). We therefore assume that a small percentage of males (1%) disperse and mate outside of the natal field. We varied this parameter from 0 to 10% per generation in a sensitivity analysis.

Rolston (1955) observed dispersal and oviposition by southwestern corn borer in fields 2.4 km from source fields. Thus, postmating dispersal may be significant. Buschman (unpublished) observed very little difference between dispersal of the southwestern corn borer and the European corn borer in mark-recapture studies.

Our standard assumption is that mating and dispersal of European corn borer are completely random throughout the entire landscape (Chiang and Hodson 1959, Legg and Chiang 1985, Shelton et al. 1986, Showers 1993). The vast majority of literature concerning European corn borer is based on data collected in humid, rain fed cornfields surrounded by weedy borders where moths congregate for mating (Showers et al. 1976, Showers et al. 2001, Stockel et al. 1985). However, in a 2-yr, one site/year study, Lee (1988) observed more adults and eggs at field centers than near borders under irrigated conditions in Alberta, Canada. He did not measure beyond field edges to adjacent fields nor did he measure virgin female activity. In a single year and single site study, Hunt et al. (2001) used light traps to measure dispersal of males and females in irrigated and nonirrigated fields in Nebraska. Only 10 virgin females were collected. Their data indicates that movement in and beyond cornfields of all kinds is random in June but more restricted to irrigated fields in August.

Oviposition. For southwestern corn borer we assume that mean fecundity is 384 and 498 eggs per female in the spring and summer periods, respectively (Knutson and Gilstrap 1990). Three-quarters are oviposited on the first night and the remainder on the second and third nights (Rolston 1955). On the first night, eggs are laid near the site of mating (Davis et al. 1933). Female mobility increases as her egg load decreases over the next two to three nights (Davis et al. 1933). Therefore, our standard assumption is that 75% of southwestern corn borer eggs are deposited in the natal field and 25% are distributed uniformly across the entire landscape. In a sensitivity analysis, we varied the percentage initially oviposited in the natal field from 0 to 75%.

Fecundity is the same in both generations for the European corn borer: 288 eggs per female (Onstad and Gould 1998). All European corn borer eggs are distributed proportionally with respect to the cornfields in the landscape; thus, oviposition is completely random in the landscape. In a sensitivity analysis, we varied the percentage initially oviposited in the natal field from 0 to 75%. For both species we assume that all eggs are viable.

Modeling Larvae. The number of larvae, L , developing during each generation t is as follows:

$$L(t,g,p) = E(t,g,p) \times Sh(g,p) \times Si(t,g,p) \times Sn(t,g,p), \quad [1]$$

E is the number of eggs in genotype g and block or strip p , and Sh , Si , and Sn are the proportion of larvae per generation that survive the transgenic host plant, insecticide application and natural factors, respectively. Insecticide applications are the focus of a second manuscript based on this model (Onstad et al. 2002) therefore, Si is 1.0 for all simulations in this paper. Natural survival (Sn) accounts for the mortality from egg through the last larval stadium for both generations. Sn does not include the very limited mortality that occurs during the pupal stage or the overwintering mortality that was covered in density-independent survival (above). For density-dependent survival, we assume a plant population of 67,000/ha. The total number of older larvae T per block in generation t is:

$$T = \sum_{g=1}^{\max g} L(t,g,p)$$

Carrying capacity is as follows:

$$K = 100P(p) \times 67,000C,$$

P is the proportional area of block p , and C is the maximum number of larvae per plant. For $T \leq K$, $L(t,g,p)$ remains as calculated in equation 1. Otherwise, for $T > K$,

$$L(t,g,p) = (K/T) \times L(t,g,p).$$

Modeling Adults and Oviposition with Nonrandom Mating in the Landscape. When mating is not random throughout the landscape, it is random within each block i . The number of adults $A(t,g,i)$ in each genotype g and generation t is as follows:

$$A(t,g,i) = L(t,g,i) \times Sw(t), \quad [2]$$

Sw is survival of the fifth instar and equals 1.0 for the first generation and the overwintering survival for the second generation.

With nonrandom mating across the landscape, the number of males in genotype m that disperse from block j to mate again in block i is as follows:

$$M(t,m,i) = 0.5A(t,g,j) \times D, \quad [3]$$

where D is the proportion dispersing to mate a second time and 0.5 is the sex ratio. The number of females F in genotype g mated with males in genotype m in block i is as follows:

$$F(t,g,m,i) = 0.5A(t,g,i) \times Q [0.5A(t,m,i) + M(t,m,i)], \quad [4]$$

Q is the proportion of genotype m in the male population of block i .

Each generation, t , the number of offspring of genotype g in block p is as follows:

$$E(t,g,p) = \sum_{f=1}^{\max f} \sum_{m=1}^{\max m} [J \times B(t) \times H(g,f,m) \times F(t,f,m,p) + (1 - J) \times B(t) \times P(p) \times \sum_{q=1}^2 H(g,f,m) \times F(t,f,m,q)], \quad [5]$$

where J is the proportion of total oviposition in the natal field and B is fecundity (eggs per female) in each generation. H calculates the Mendelian proportion of eggs in genotype g resulting from the mating of female genotype f and male genotype m . The first term ($\dots J \times B(t) \dots$) represents the oviposition by females before they can disperse. The second term ($\dots (1 - J) \times B(t) \dots$) represents the distribution of eggs by females moving about the landscape. Thus, females from both blocks, q , deposit eggs in proportion, P , to the area of block p in the landscape.

Modeling Adults and Oviposition with Random Mating. When adults treat the cornfields as one area ($i = 1$), regardless of whether the refuge is row strip or block, we assume random mating for the entire population and uniform oviposition throughout the landscape. Therefore, the adult population for both blocks, p , is

$$A(t,g,1) = \sum_{p=1}^2 L(t,g,p) \times Sw(t), \quad [6]$$

Coefficient D (male dispersal before mating) becomes 0.0 therefore, equation 3 becomes

$$M(t,m,i) = 0 \quad [7]$$

also Q becomes the proportion of genotype m in the total male population so, equation 4 becomes

$$F(t,g,m,1) = 0.5A(t,g,1) \times Q[0.5A(t,m,1)] \quad [8]$$

Because adults disperse before mating, oviposition occurs uniformly in the two blocks ($J = 0$) and equation 5 reduces to the following:

$$E(t,g,p) = B(t) \times P(p) \sum_{f=1}^{\max f} \sum_{m=1}^{\max m} H(g,f,m,1) \times F(t,f,m,1). \quad [9]$$

Computation and Analysis. The model is coded in Fortran and calculated on a Dell computer. The time step is one generation and the time horizon is 100 growing seasons. The initial resistance allele frequency is 10^{-3} with genotypes in Hardy-Weinberg equilibrium. The insect species are simulated separately with an initial density of 50,000 adults/ha.

The standard model simulates refuges as blocks near the transgenic blocks, Fig. 1 a and b. We also examined the effect of a row-strip configuration on resistance development, Fig. 1c. Carrying capacity equations were maintained independently for the refuge and

transgenic blocks since the mechanism functions at the level of the individual plant. When mating is random throughout the landscape during the first flight and the resulting oviposition is uniformly distributed (proportional to area of blocks in landscape), then block refuges can be relocated on an annual basis or not. The model results are the same. Row strips can also be moved or not on an annual basis, given our assumption that mating and oviposition are random across the strips in a field. Thus, for the standard European corn borer behavior in the model and for sensitivity analyses considering random mating during the first flight of either species, no assumption needs to be made explicitly about block refuge location being static or dynamic over time. However, for the southwestern corn borer, our standard assumptions about behaviors implicitly assume that block refuges are planted in the same location year after year. In the discussion section, we explain how the sensitivity analyses of adult behaviors might apply to a dynamic refuge location for the southwestern corn borers.

We examined the sensitivity of the model to changes in four assumptions. We evaluate two components that describe the genetic exchange: the dispersal of males for second mating and the distribution of eggs across the landscape. Then, we evaluate the effect of differences in adult behaviors between the two generations. We changed the first flight to random mating and uniform oviposition throughout the landscape and the second flight to nonrandom mating and preferential oviposition in the natal field. Finally, we examined the effect of increased survival of heterozygote larvae in the transgenic crop. Using the scenario for partially recessive expression of the allele for transgenic resistance in a practical high dose crop we increased the survival of heterozygote larvae from 0.01 to 0.05 per generation.

Unless explicitly described otherwise, results are reported for four refuge sizes (10, 20, 30, and 40%), three forms of expression of the R allele (dominant, recessive and partially recessive), and both doses of the transgenic toxin. Note that with the theoretical high dose of the toxin, there is no intermediate expression of the R allele for resistance because it is functionally recessive.

Resistance was evaluated as the number of years required for the resistance allele to reach 3% frequency in the whole population. When the R allele frequency reaches 3% the Hardy-Weinberg relationship indicates that there should be one fully resistant individual (RR) in a population of $\approx 1,000$. We believe this represents the time when growers might initially observe damage in the transgenic field.

Results

Southwestern Corn Borer. *Transgenic Corn and Block Refuge.* Resistance develops to the transgenic corn immediately (1–2 yr) if allele expression is dominant. However, if the expression of the resistance allele is partially recessive or recessive, the frequency of the resistance allele does not develop to the level of

Table 2. Low levels of dispersal by the male southwestern corn borer affect the years to 3% resistance allele frequency when a practical high dose strategy is used and the resistance allele is partially recessive

Refuge	Years to 3% resistance allele frequency					
	Proportion of male population dispersing to mate a second time					
	0.000	0.001	0.002	0.003	0.004	0.005
10%	18	70	>100	>100	>100	>100
20%	18	53	80	>100	>100	>100
30%	19	51	74	92	>100	>100
40%	20	54	77	95	>100	>100

3% within the 100 yr of simulation. Since resistance develops so quickly with dominant expression, the following results refer to recessive or partially recessive expression of the resistance allele, unless indicated otherwise.

Sensitivity to Changes in Behavior. With males mating only in their field of emergence (0% male dispersal), resistance developed to the transgenic corn in 3 yr or less when the crop expressed the theoretical high dose of toxin. With a practical high dose crop, resistance never developed in the 100-yr simulation when the resistance allele was recessive.

Table 2 presents results of simulations when resistance allele expression was partially recessive and male dispersal was 0.5% of the population or less. When no males disperse before mating, resistance developed within 18–20 yr. With no dispersal of males there is no gene flow between the refuge and the transgenic fields, therefore, only resistant individuals are available to mate in the transgenic field. Once the resistance allele frequency in the transgenic field exceeded 3%, the population in the transgenic field increased toward carrying capacity and the resistant population overwhelmed the refuge population. However, a very small level of male dispersal between fields before mating resulted in a significant delay in resistance development (Table 2). For example, if only 0.4% of the refuge population of males disperse to the transgenic field, resistance did not develop within the 100 yr of simulation, regardless of refuge size.

Resistance to transgenic corn developed much sooner when all oviposition was distributed in proportion to the landscape. For example, when the crop expressed a theoretical high dose, resistance developed within 37–52 yr for 10–40% refuges (data not shown). When the crop expressed a practical high dose, resistance developed within 27–37 yr (10–40% refuges) for recessive expression of the resistance allele and within 8–10 yr (10–40% refuges) when resistance allele expression is partially recessive (data not shown).

With 50% of oviposition in the field of emergence and 50% distributed proportional to the landscape, no resistance developed in the theoretical high dose crop within 100 yr. Resistance did not develop when the crop expressed a practical high dose and the insect resistance allele was recessive. With 50:50 oviposition, partially recessive expression of the resistance allele,

Table 3. Transgenic corn resistance development (years to 3% R allele frequency) in southwestern corn borer when a practical high dose strategy is used with a 20% refuge and the resistance allele is partially recessive

% male dispersal ^a	Years to 3% resistance allele frequency			
	% oviposition in proportion to landscape			
	25	50	75	100
1st and 2nd generations-random mating only within a field				
0	18	12	9	6
1	>100	35	14	8
10	>100	56	23	10
1st generation-random mating across the landscape ^b 2nd generation-random mating only within the field				
0	14	10	9	7
1	18	12	10	8
10	21	13	10	9

^a Percentage of males that disperse from the block of emergence to mate a second time in the alternate block.

^b During first generation, oviposition is also random across all corn-fields.

and a practical high dose crop, resistance developed within 33–44 yr for the range of refuge sizes.

When both male dispersal and the distribution of eggs are varied for a 20% refuge, resistance developed more slowly as male dispersal increased and more quickly as eggs were distributed more uniformly across the cornfields (Table 3; top). If the first flight behavior includes random mating and uniform oviposition throughout the landscape, resistance developed much faster (Table 3; bottom). Almost all the numbers in the lower half of Table 3 are less than or equal to those in the upper half of Table 3.

Higher Heterozygote Survival in Transgenic Corn. With a practical high dose in the transgenic corn and partially recessive expression of the R allele in the larvae, a five-fold increase in the survival rate for heterozygotes caused all resistance development times (except one) to drop to 10 yr or less (as low as 3 yr). These periods of evolution are shorter than those simulated with heterozygote survival of 0.01 in the standard model (Table 3). Only when 10% of the males disperse and 25% of the oviposition is proportional to the landscape does the resistance allele frequency reach 3% in as many as 21 yr. Thus, the uncertain level of heterozygote survival has a significant influence on the predictions of resistance development.

Row-Strip Refuge. This spatial configuration, that allows random mating and oviposition 100% proportional to the landscape, caused resistance to develop faster than it did with the standard block scenario. With a theoretical high-dose crop, where the resistance allele was recessive, resistance to transgenic corn developed within 25–33 yr for 10–40% refuges, respectively. A practical high dose with recessive expression of the resistance allele was comparable with resistance developing to the 3% resistance allele frequency within 26–33 yr for 10–40% refuges. However, resistance developed more quickly with partially recessive expression of the resistance allele in a practical

Table 4. Transgenic corn resistance development (years to 3% R allele frequency) in European corn borer when a practical high dose strategy is used with a 20% refuge and the resistance allele is partially recessive

% male dispersal ^a	Years to 3% resistance allele frequency			
	% oviposition in proportion to landscape			
	25	50	75	100
1st and 2nd generations - random mating only within a field				
0	>100	>100	58	23
1	>100	>100	>100	40
10	>100	>100	>100	51
1st generation - random mating across the landscape ^b				
2nd generation - random mating only within the field				
0	>100	68	48	27
1	>100	87	60	35
10	>100	94	64	38

^a Percentage of males that disperse from the block of emergence to mate a second time in the alternate block.

^b During first generation, oviposition is also random across all cornfields.

high dose crop. In this situation the resistance allele frequency reached 3% within 7–9 yr for 10–40% refuges.

European Corn Borer. Transgenic Corn and Block Refuge. With transgenic corn, a refuge, and dominant expression of the resistance allele, resistance to transgenic corn developed immediately (1–2 yr) regardless of the dose of toxin. When the resistance allele was recessive, resistance developed within 56 yr for a 10% refuge and never developed within 100 yr for refuges of 20% or more. With partially recessive expression of the resistance allele and a practical high-dose crop, resistance developed within 15–38 yr. Onstad and Guse (1999) assumed 75% survival of resistant homozygotes in transgenic corn and reported resistance developing in 35 yr with a 20% refuge. In this model, using 100% survival of resistant homozygotes in transgenic corn, resistance developed in 30 yr with a 20% refuge.

Sensitivity to Changes in Behavior. Table 4 shows the sensitivity of European corn borer resistance development to changes in male dispersal and distribution of oviposition with a practical high-dose crop, partially recessive expression of the resistance allele and a 20% refuge. The patterns in times to development of resistance for European corn borer (Table 4) were similar to those for the southwestern corn borer (Table 3). In general, resistance developed faster as more eggs were oviposited throughout all the cornfields (fastest in the 100% column of Table 4) and slower when levels of male dispersal much less than the expected 100%. For comparison, resistance developed in 30 yr with the standard behaviors of adult European corn borer (100% male dispersal and 100% dispersal of oviposition throughout the landscape). Only two cases with 0% male dispersal and 100% of oviposition distributed proportionally in the landscape result in resistance developing faster than the 30 yr calculated with the standard model.

Higher Heterozygote Survival in Transgenic Corn. With a practical high dose in the transgenic corn and

Table 5. Transgenic corn resistance development (years to 3% R allele frequency) in European corn borer when heterozygote survival in transgenic corn is 5%, a practical high dose strategy is used with a 20% refuge and the resistance allele is partially recessive.

% male dispersal ^a	Years to 3% resistance allele frequency			
	% oviposition in proportion to landscape			
	25	50	75	100
1st and 2nd generations - random mating only within a field				
0	11	11	10	6
1	>100	56	24	10
10	>100	72	34	14
1st generation - random mating across the landscape ^b				
2nd generation - random mating only within the field				
0	>100	15	13	8
1	>100	20	16	10
10	>100	24	19	11

^a Percentage of males that disperse from the block of emergence to mate a second time in the alternate block.

^b During first generation, oviposition is also random across all cornfields.

partially recessive expression of the R allele in the larvae, a five-fold increase in survival rate for heterozygotes caused most resistance development times to decrease by $\approx 75\%$ (Table 5 versus Table 4).

Row-Strip Refuge. A row-strip refuge configuration encourages completely random mating and oviposition totally in proportion to the landscape. These are the standard assumptions for adult European corn borer behavior, and consequently, refuge configuration appears to have little effect on resistance development. In our simulations a row strip had no effect on the year when the resistance allele reached 3% frequency. A row-strip refuge was equally as effective as a block refuge in the more detailed model of Onstad and Guse (1999).

Discussion

When the allele for transgenic resistance was dominant, resistance to the transgenic crop developed very quickly in both species. However, when the expression of the resistance allele was not dominant, adult behavioral differences between the species resulted in dramatic differences in the time required for resistance to develop. In the southwestern corn borer, resistance did not develop within 100 yr with a block refuge of nontransgenic corn ranging from 10–40%, regardless of the dose of toxin in the crop. In contrast, resistance developed in the European corn borer within 15–38 yr for the same refuge sizes when the dose of toxin was not high enough to kill all heterozygous larvae.

Our standard assumptions about the behavior of the southwestern corn borer were that both mating and oviposition were very localized in both generations. The combination of limited male dispersal before mating and sedentary females that oviposited 75% of their eggs near the site where they emerged, resulted in a refuge that maintained a very large population independent of the larger area of transgenic corn nearby.

The standard assumptions about the European corn borer behavior included random mating and uniform oviposition throughout the landscape for both generations. This combination resulted in complete mixing of the genetics of the population and puts the majority of eggs in the transgenic field. Consequently, with our standard assumptions of adult behavior, the selection pressure for resistance development in the European corn borer population was much higher than for the southwestern corn borer.

Cultural practices such as refuge size, refuge configuration (block versus row strip) and proximity of the refuge to the transgenic crop may all affect the behavior of these species. For example, the spatial arrangements of irrigated cornfields that moths are likely to encounter are illustrated in Fig. 1. Fig. 1a shows the nontransgenic field as a separate field located within one-half mile of the transgenic field. In this situation we assume that adult borers mated in the natal field and laid 75% of their eggs in the natal field. This is consistent with our assumptions about the behavior of the southwestern corn borer. In landscapes where transgenic and nontransgenic fields are adjacent (Fig. 1b), it is possible our standard assumptions regarding the behavior of the southwestern corn borer may break down. Adult mixing could be more extensive between the transgenic and nontransgenic blocks in such a landscape and it is possible that oviposition could be more random across the blocks. Table 3, columns 3–5 may represent conditions illustrated in Fig. 1b. There was a considerable difference in how quickly resistance developed in these two scenarios. Given limited survival of the heterozygous larvae (a practical high dose crop with a partially recessive resistance allele) and the standard assumption of 1% male dispersal, as illustrated in Fig. 1a, resistance did not develop within 100 yr (Table 3; column 2). With the same male dispersal and larval survival but an adjacent block scenario (Fig. 1b), we might assume more random oviposition across the landscape. In this situation, resistance developed within 8–35 yr. (Table 3; columns 3–5).

Row-strip refuge configurations (Fig. 1c) cannot be recommended for the southwestern corn borer, because resistance developed much faster when compared with a block refuge. Strips of refuge within a field produce uniform distribution of the sexes before mating and uniform distribution of eggs in the landscape. If the resistance allele was recessive, the situation where resistance is least likely to develop because there is no survival of the heterozygous larvae, resistance developed in 33 yr or less. When the resistance allele was partially recessive, resistance developed as soon as 7–9 yr.

Although our assumptions about the standard behaviors of both species are supported by the literature, few studies have examined the behaviors of the first flight of moths. In addition, most studies of adult behaviors have been conducted in geographic areas dominated by rain-fed corn. The work of Lee (1988) and Hunt et al. (2001) are exceptions that suggest the European corn borer may behave like the southwest-

ern corn borer under irrigated conditions. Scenarios presented in Table 4 examine this possibility. Given the standard assumptions about European corn borer behavior and a 20% refuge, resistance developed in 30 yr. Using assumptions about European corn borer behaviors suggested by Lee (1988) and Hunt et al. (2001), resistance developed more slowly (Table 4; bottom). Only anecdotal evidence is available regarding the behavior of the first flight of southwestern corn borer adults. If the first flight involves greater dispersal than the second flight, resistance would develop much faster as indicated in the bottom of Table 3.

The sensitivity analysis for behavioral differences between the first and second flight of moths may also provide some insight into the year-to-year placement of the refuge. The top halves of Tables 3 and 4 represent localized adult behaviors (standard assumptions for the southwestern corn borer) in both flights and would suggest that the refuge should be placed on the same ground each year. If however, first flight behaviors involve greater dispersal (Tables 3 and 4; bottom half) then refuge location is not as critical and there is no reason to require that the refuge be placed on the same ground each year. If we assume a widely dispersing first flight and a more localized second flight then the southwestern corn borer develops resistance at least five times faster than under standard assumptions (Table 3), whereas the European corn borer develops resistance three times slower than the standard assumptions for that species (Table 4). We assume that the decision of where to place refuges should be determined, in part, by the behavior of the insect but it is possible that the relocation of refuge cornfields could cause more uniform mixing of populations.

In this study we have attempted to address the questions of growers and regulators regarding resistance management plans. Refuge size and the expression of the resistance allele (which affects heterozygote survival) are important considerations, however, adult insect behavior may be more significant in determining the speed of resistance development. Cultural practices such as irrigation, refuge configuration and refuge placement may all affect the behavior of these insects. This suggests that cultural practices could eventually be employed as tools for managing insect behavior with respect to resistance management or that resistance management plans might require modifications for different landscapes and cultural practices. Either situation requires more detailed modeling to examine the potential impact and consequences.

To develop more confidence in our predictions, we require more detailed quantification of adult behaviors under a variety of landscape conditions. Overwintering survival of these species may differ from north to south and may also differ between refuge and transgenic fields, especially if cultural practices such as tillage are not similar between fields. Measurements of the proportion of males and females dispersing away from the site of emergence before mating and the distances traveled would be informative. We need

to know what proportion of mating occurs in natal fields and what proportion takes place outside natal fields for each species and for each set of landscape conditions. We need confirmation and quantification of southwestern corn borer oviposition behavior, especially for the first flight. Do females deposit most of their eggs in the field from which they emerge? The first flight of adult females may emerge in fields with an open canopy or the oviposition sites may be inadequate causing females to disperse more actively. Finally, we need to know more about interactions between larvae of the two species. Cannibalism by southwestern corn borer substantially reduces its own population during the second generation. It is possible that the southwestern corn borer also reduces European corn borer populations in the same plants. This information would allow us to model the interspecific competition between these pests. Once the behaviors of the two species are known with more certainty, we will be better able to address specific questions about resistance management relative to cultural practices in specific geographic areas where transgenic corn is grown.

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