

2-2012

Modeling the Impact of Cross-Pollination and Low Toxin Expression in Corn Kernels on Adaptation of European Corn Borer (Lepidoptera: Crambidae) to Transgenic Insecticidal Corn

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Abstract

We used a mathematical model with processes reflecting larval mortality resulting from feeding on cross-pollinated ears or Bt ears of corn to analyze the risk of evolution of Cry-toxin resistance in *Ostrinia nubilalis* (Hübner). In the simulations, evolution of resistance was delayed equally well by both seed mixtures and blocks with the same proportion of refuge. Our results showed that Bt-pollen drift has little impact on the evolution of Bt resistance in *O. nubilalis*. However, low-toxin expression in ears of transgenic corn can reduce the durability of transgenic corn expressing single toxin, whereas durability of pyramided corn hybrids is not significantly reduced. The toxin-survival rate of heterozygous larvae in Bt-corn ears expressing one or two proteins has more impact on evolution of Bt resistance in *O. nubilalis* than the parameters related to larval movement to Bt ears or the toxin-survival rate of the homozygous susceptible larvae in Bt ears. Bt resistance evolves slower when toxin mortality is distributed across the first two larval stadia than when only the first instars are susceptible to Bt toxins. We suggest that stakeholders examine toxin-survival rates for insect pests and take into account that instars may feed on different parts of Bt corn.

Keywords

Bt corn, resistance management, simulation

Disciplines

Entomology | Plant Breeding and Genetics

Comments

This article is from *Environmental Entomology*; 41 (2012); 200-211; doi: [10.1603/EN11133](https://doi.org/10.1603/EN11133)

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Source: Environmental Entomology, 41(1):200-211. 2012.

Published By: Entomological Society of America

DOI: <http://dx.doi.org/10.1603/EN11133>

URL: <http://www.bioone.org/doi/full/10.1603/EN11133>

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Modeling the Impact of Cross-pollination and Low Toxin Expression in Corn Kernels on Adaptation of European Corn Borer (*Lepidoptera: Crambidae*) to Transgenic Insecticidal Corn

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Environ. Entomol. 41(1): 200–211 (2012); DOI: <http://dx.doi.org/10.1603/EN11133>

ABSTRACT We used a mathematical model with processes reflecting larval mortality resulting from feeding on cross-pollinated ears or Bt ears of corn to analyze the risk of evolution of Cry-toxin resistance in *Ostrinia nubilalis* (Hübner). In the simulations, evolution of resistance was delayed equally well by both seed mixtures and blocks with the same proportion of refuge. Our results showed that Bt-pollen drift has little impact on the evolution of Bt resistance in *O. nubilalis*. However, low-toxin expression in ears of transgenic corn can reduce the durability of transgenic corn expressing single toxin, whereas durability of pyramided corn hybrids is not significantly reduced. The toxin-survival rate of heterozygous larvae in Bt-corn ears expressing one or two proteins has more impact on evolution of Bt resistance in *O. nubilalis* than the parameters related to larval movement to Bt ears or the toxin-survival rate of the homozygous susceptible larvae in Bt ears. Bt resistance evolves slower when toxin mortality is distributed across the first two larval stadia than when only the first instars are susceptible to Bt toxins. We suggest that stakeholders examine toxin-survival rates for insect pests and take into account that instars may feed on different parts of Bt corn.

KEY WORDS Bt corn, resistance management, simulation

European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), has been the most widespread insect pest of corn (*Zea mays* L.) in the Corn Belt since the introduction in the United States (Cafrey and Worthley 1927, Mason et al. 1996, Hutchison et al. 2010). Transgenic-insecticidal corn was grown commercially for the first time in 1996, and, by 2009, was grown in 135 million ha in 25 countries (James 2009). Transgenic corn hybrids that express one or more proteins from the bacterium *Bacillus thuringiensis* Berliner (Bt corn), can have high efficacy against lepidopteran and coleopteran pests and comprised over 63% of the total U.S. corn production in 2010

(USDA-ERS 2010). In the absence of field-evolved resistance to Bt, populations of *O. nubilalis* have gradually declined over time in at least five major corn producing states because of Bt corn use (Hutchison et al. 2010). Despite the significant selection pressure on *O. nubilalis* since 1996, no *O. nubilalis* populations with major resistance to Cry toxins have been reported in the field (Carrière et al. 2010).

Chilcutt and Tabashnik (2004) concluded that cross-pollinated corn ears in the refuge can accelerate resistance evolution if fewer susceptible moths are produced in a refuge or if intermediate levels of toxin in cross-pollinated ears in a refuge kill susceptibles but allow heterozygotes to survive. Heuberger et al. (2008) studied the effect of refuge contamination by transgene on Bt-resistance evolution by the pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae), in cotton, *Gossypium hirsutum* L. They concluded that Bt-pollen contamination has negligible effects on resistance evolution as long as contamination does not confer a selective advantage to heterozygotes over homozygous susceptible larvae in the refuge. Reduced mortality of Bt-susceptible-*O. nubilalis* larvae feeding on kernels of Bt corn expressing Cry1Ab was observed by Burkness et al. (2011), and it raised the possibility that the survival rates of heterozygotes and homozygous susceptibles feeding on ears of Bt corn can be greater than those of heterozygotes and homozygous susceptibles feeding

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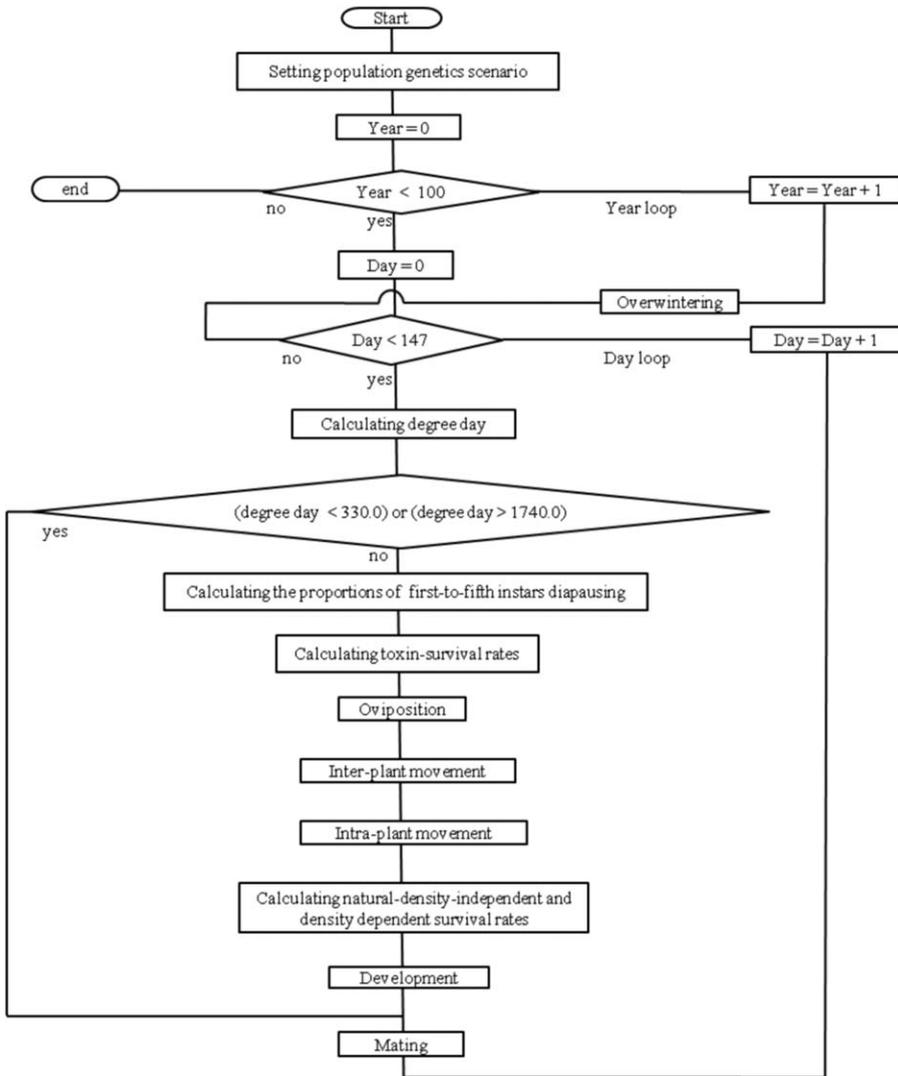


Fig. 1. Processes in *O. nubilalis* Bt resistance management model.

on stems or leaves of Bt corn. If more heterozygotes on ears of Bt corn survive, it can accelerate resistance evolution. We used a mathematical model with processes reflecting larval mortality caused by feeding on 1) cross-pollinated ears in the refuge and 2) ears of Bt corn, to analyze the risk of these factors in the evolution of Bt resistance in *O. nubilalis*.

Methods

The model of Onstad and Gould (1998b) was modified to simulate two plant toxins. For each resistance gene, the initial frequency of the resistant allele is 0.0001. To reflect the current *O. nubilalis* population levels (Hutchison et al. 2010), we start the model with 11.7 million eggs per 100 ha and assume 16.2 larvae per 100 maize plants (Gray 2009). The initial egg genotypic frequencies followed a Hardy-Weinberg distri-

bution. Resistance evolution is defined as the number of years required for the resistance allele frequency to reach 0.5. Many variables and processes can be important, but we have restricted our analysis to factors that relate to larvae in single toxin scenarios with 20% refuge and pyramided, double-toxin scenarios with 5% refuge. The model is programmed in Microsoft Visual C++.

Two discrete generations per year are simulated in a landscape containing Bt-pollen contamination in the refuge and low-toxin expression of Bt ears. The sequence of the processes in the model is described in Fig. 1. Each season lasts 147 d under Illinois conditions, when degree-day is in the range of 330–1,740 with base temperature 10°C (Onstad 1988). Simulations last 100 yr (200 generations) and the same temperature pattern, which is the 30-yr normal maximum and minimum temperatures for Champaign-Urbana, IL, is used

for each year (Onstad and Guse 1999) and the time step is 1 d.

Diapause. We use the following function by Onstad and Brewer (1996) to calculate the proportion of larvae entering diapause (proportion/1 d).

$$Dia_{(l)} = \max DlapInd_{(l)} \times [-384 + (30.8 \times S) + (2.33 \times T) + (5.11 \times L)] \quad [1]$$

$Dia_{(l)}$ is proportion of life stage, l , in diapause. $Dia_{(l)}$ is never >1 or <0 . $\max DlapInd_{(l)}$ is the maximum induction rates of diapause for life stage. The maximum induction rates of diapause for first, second, third, fourth, and fifth stadium are 1, 0.89, 0.685, 0.285, and 0, respectively. Scotophase (S , hours) is calculated by using functions described by Sellers (1965). T is five-day-running-average temperature in degrees Celsius and L is the latitude in decimal degrees. For each time step, Dia is multiplied to output from larval stages to calculate the number of larvae in diapause.

Toxin Mortality of First and Second Instars. Because loci providing major resistance to Cry toxins have not been found in *O. nubilalis* (Carrière et al. 2010), we emphasize scenarios in which resistance confers 100% survival in resistant homozygotes; $stox_{RR} = 1$, where, $stox_{(g)}$ is the toxin-survival rate of a genotype (g). Toxin survival rate for susceptible first instars feeding on Cry1 F corn is calculated as 0.001 before the date of anthesis (Pereira et al. 2008). Crespo et al. (2009) estimated the survival rates of a laboratory-selected Cry1Ab-resistant strain, a susceptible strain and their heterozygous cross on Cry1Ab-expressing vegetative stage corn at 0.0. However, when larvae were reared on Cry1Ab-expressing plants at reproductive stages, the survival rate of the Cry1Ab-resistant strain (18.3%) was greater than those of the cross (11.0%) and the susceptible strain (0.3%). The dominance of a resistance allele is h .

$$stox_{RS} = h \times stox_{RR} + (1 - h) \times stox_{SS} \quad [2]$$

The standard value for h is 0.01 to keep the frequency of major-resistant allele below 1% for 15 yr with the standard simulations of the one-locus-to-one-toxin population genetics scenario (Carrière et al. 2010).

For the pyramided-toxin scenario, the toxin survival rate is either 1) the product of the two single toxin-survival rates (multiplicative-toxin-survival rates), or 2) the smaller value of the two toxin-survival rates (minimum-toxin-survival rates; Onstad and Meinke 2010). Because no studies provide evidence of cross-resistance to Cry1 F and Cry1Ab in *O. nubilalis*, we assume that cross-resistance to Cry1 F and Cry1Ab does not occur.

Recent studies showed that the toxin-survival rates of the first instars feeding on Bt corn at reproductive stages (R1–2 or R1–3) are significantly greater than those on Bt corn at vegetative stages (V6–9; Pereira et al. 2008, Crespo et al. 2009). To simplify this, we used the linear-toxin-titer-decline model (Onstad and Gould 1998a) in which the decline of the titers of two toxins is similar because there is no evidence that the

decline is different for two toxins expressed in pyramided corn. The model uses three time periods to describe the decline in toxin mortality; the first period, $plag$, is the lag between the date of anthesis (50% silking or pollen shed) and the first date of reduced mortality. For this simulation, $plag$ is 0 because survival rates of Bt-resistant strains and F1 hybrids, produced by Bt-susceptible strains and Bt-resistant strains, declined when their host plants were at early reproductive stages (Pereira et al. 2008, Crespo et al. 2009). The date of anthesis is 14 July (Julian dates: 195) to represent the condition in Illinois. The second period, $pdec$, is the duration of the linear decline in toxin mortality from maximum larval mortality to 0%. The survival rates of Bt-susceptible strains are $<10\%$ of those of Cry1 F-or-Cry1Ab-resistant strains on Bt corn at early reproductive stages (Pereira et al. 2008, Crespo et al. 2009). For this reason, we decided that $pdec$ should be longer than the period of R1–3. We chose 42 d for the value of $pdec$ because R5 is 35–42 d after silking in Illinois (Nafziger 2007). The third period, $psep$, is calculated as seven and is defined as the delay between the decline for the genotypes that are heterozygous at any locus and the decline in toxin mortality for wild-type susceptible larvae. Toxin-survival rate after the date of anthesis for genotype is $stoxAnthesis_{(g)}$.

$$stoxAnthesis_{(g)} = stox_{(g)} \text{ if } time < anthesis + plag \quad [3]$$

Julian date is the unit for $time$ and $anthesis$. After anthesis, toxin-survival rates can increase for fully resistant or heterozygous larvae.

$$stoxAnthesis_{(g)} = stox_{(g)} + \frac{(time - anthesis - plag)}{pdec} \text{ if } anthesis + plag \leq time < anthesis + plag + pdec \quad [4]$$

Genotypes not having a resistance allele at any locus are considered completely susceptible,

$$stoxAnthesis_{(g)} = stox_{(g)} \text{ if } time < anthesis + plag + psep \quad [5]$$

$$stoxAnthesis_{(g)} = stox_{(g)} + \frac{(time - anthesis - plag - psep)}{pdec} \text{ if } time \geq anthesis + plag + psep \quad [6]$$

None of the values of $stox_{(g)}$ and $stoxAnthesis_{(g)}$ are allowed to exceed 1.0.

Table 1 describes how mortality factors affect the early larval stages. We incorporate toxin survival rates into the survival rates of first, and possibly, second instars. For first instars after interplant movement, the survival rates are

$$surFst_{(g)} = stox_{(g)}^{TOXW} \quad [7]$$

Table 1. Sequence of mortality factors in early larval stages based on movement and location

Life stage	Location	Mortality factors
Neonate	Bt and non-Bt corn	80% survive on original plant of any kind if they do not move 10% survive during inter-plant movement
First instar	Bt corn	100% survive due to predispersal tasting of Bt corn
	Bt corn	If $TOXW = 1$, low survival due to feeding on vegetative tissue If $TOXW = 0.5$, higher survival due to feeding on vegetative tissue If $PNIMB = 1$ and $PIMB > 0$, Mortality on kernels
Second instar	Non-Bt corn	If $PNIMR = 1$ and $PIMR > 0$, Mortality on cross-pollinated kernels
	Bt corn	Mortality due to natural factors If $TOXW = 0.5$, mortality due to feeding on vegetative tissue If $PNIMB = 0$ and $PIMB > 0$, mortality on kernels
	Non-Bt corn	Mortality due to natural factors If $PNIMR = 0$ and $PIMR > 0$, mortality on cross-pollinated kernels

$TOXW$ is the exponent for the toxin survival rate (distributes the effect over first two stages).
 $PNIMB$ or $PNIMR$ equals 1 for the instar that moves to corn ears.
 $PIMB$ and $PIMR$ are the probabilities of moving to corn kernels given $PNIM = 1$.

$surFst_{(g)}$ is the survival rate for a genotype of first instars. We used $TOXW$ to study the effect of the timing of intra-plant movement and the timing of toxin mortality. We evaluated two scenarios: toxin mortality occurring only for first stadium $TOXW = 1.0$, and toxin mortality occurring for first and second stadia $TOXW$ is 0.5. The survival rates for second instars are

$$surSnd_{(g)} = 0.81 \times (stox_{(g)}^{(1-TOXW)}) \quad [8]$$

$surSnd_{(g)}$ is the survival rate for a genotype of second instars. 0.81 is the natural survival rate for second instars. Table 1 describes how mortality factors affect the early larval stages.

Reproduction by Second-generation Moths. Similar to Onstad and Gould (1998b), we modeled mating and reproduction separately for two subsequent generations. The eggs oviposited in the second generation are distributed over time by the functions used by Onstad (1988).

Reproduction by First Generation Moths. We built a simulation model based upon the data from Royer and McNeil (1993) to calculate the proportion of males mating in age classes. A Monte Carlo method was used for generating a series of simulations for polyandry. Data points from Fig. 1 in the publication of Royer and McNeil (1993) are acquired by using Engauge Digitalizer (<http://digitizer.sourceforge.net>). We assume that males can mate the maximum five-times for the 10-d mating period because the data for matings after the fifth are not available. Within the 10-d-mating period, 84%, 45%, 34%, 20%, and 20% of males mated at least once, twice, three-times, four-times, and five times, respectively. The means and standard errors of lags between the 0-first, first-second, second-third, third-fourth, fourth-fifth mating are (1.71 ± 0.11) , (1.54 ± 0.13) , (1.58 ± 0.17) , (1.67 ± 0.25) , and (1.64 ± 0.20) , respectively. For each replicate, a random deviate between 0 and one is drawn and compared with the probability of the mating for a given sequence. If the random deviate is less than the probability of the mating for a given sequence, a male copulates. When mating occurs, a normal deviate, with the mean and standard error of the lag for the given mating sequence, is drawn to determine timing of

mating. The simulations were run 10,000-times. We calculated, $prob_{(ma)}$, the probability of mating with competition of all ages at an age of male (ma), from 0-d-old male to 9-d-old male as 0, 0.837, 0.037, 0.417, 0.258, 0.098, 0.169, 0.087, 0.124, and 0.003, respectively (Fig. 2).

Anwar and Féron (1971) observed that 70% of pairs mated on the first night after emergence and 30% on the second night. Female moths are known to mate usually once (Caffrey and Worthley 1927, McNeil et al. 1997). For these reasons, we assumed that female moths mate only once in a life time when they are 0 d old. For the first generation, $propP_{(p)}$, which is the proportions of male moths of a genotype, p , in the equation 11 is calculated by using the equation 12.

$$propP_{(p)} = \frac{\sum_{ma=0}^9 nP_{(p)(ma)} \times prob_{(ma)}}{\sum_{p=1}^{gt} \sum_{ma=0}^9 nP_{(p)(ma)} \times prob_{(ma)}} \quad [12]$$

$nP_{(p)(ma)}$ is the number of males of a genotype at an age, ma , p is paternal genotype. gt is the total number of genotypes of *O. nubilalis*. $prob_{(ma)}$ is the probability of mating of males at an age with competition. We assume oviposition declines with the age of female moth (Barber 1925, Vance 1943) according to the linear function developed by Onstad (1988) for first-generation moths.

Interplant Neonate Movement and Survival. Several researchers have studied neonate movement of *O. nubilalis* in different settings (Davis and Coleman 1997, Davis and Onstad 2000, Goldstein et al. 2010, Prasifka et al. 2009 and 2010, S. E. Moser et al., unpublished data). We calculated interplant movement parameters as the proportion of neonates leaving non-Bt corn (0.76) and the proportion of neonates leaving Cry1 F-, Cry1Ab-expressing corn (0.90), or both. The survival rate because of movement is 0.1 (Onstad and Gould 1998b). The survival for those staying on non-Bt corn after the eggs hatched is 0.8 (S. E. Moser et al., unpublished data). The predispersal-tasting survival rate, spd , is defined as the survival of susceptible larvae that taste the Bt corn and then move to a refuge plant (Onstad and Gould 1998b), and

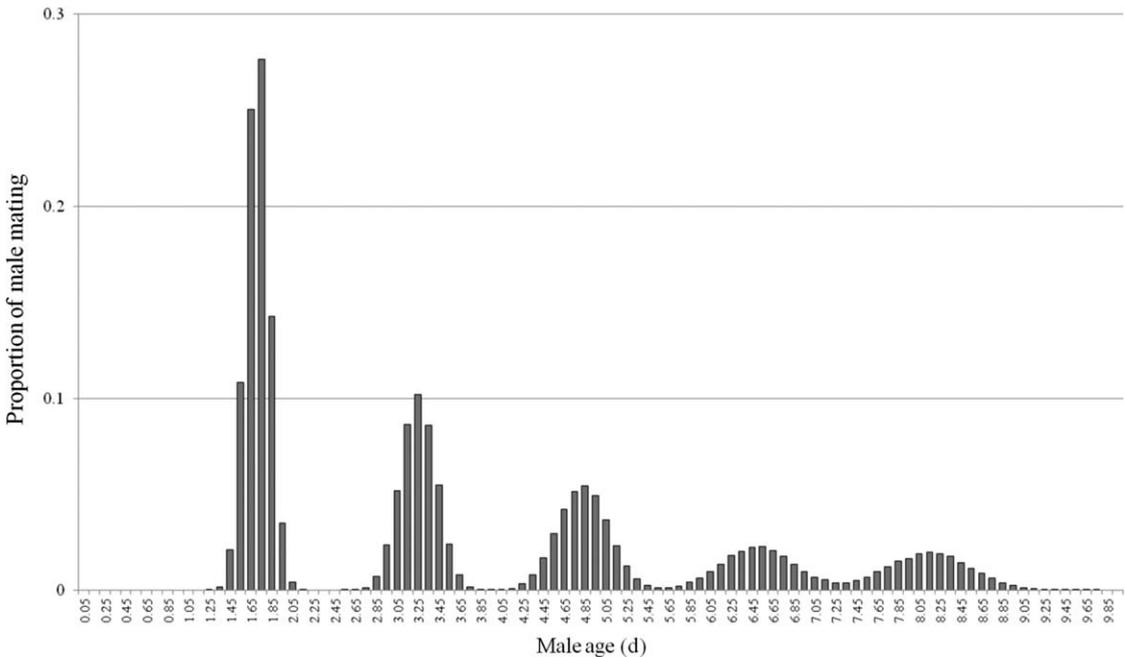


Fig. 2. Proportion of male mating in age classes calculated from the *O. nubilalis* protandry simulation model based upon the study of Royer and McNeil (1993). The width of male-age class is 0.1 d.

in the model this factor is set to 1.0 based on observations of S. E. Moser et al. (unpublished data). For a seed mixture plot, the function to calculate the input rate to first instars is

$$N_{r-ls} = (1 - 0.76) \times hch_n \times 0.80 + 0.76 \times Prop_r \times 0.1 \times hch_n + 0.90 \times (1 - Prop_r) \times 0.1 \times hch_t \times spd \quad [14]$$

$$N_{Bt-ls} = (1 - 0.90) \times hch_t \times 0.80 \times spd + 0.90 \times (1 - Prop_r) \times 0.1 \times hch_t \times spd + 0.76 \times Prop_r \times hch_n \times 0.1 \quad [15]$$

N_{r-ls} and N_{Bt-ls} are the input rates of a given genotype to first instars on stem or leaves in refuge and on those in transgenic plot, respectively. hch_n and hch_t are the number of a given genotype hatching each day. $Prop_r$ is the proportion of non-Bt corn. In each equation, the terms in sequence on the right hand side are the number of neonates staying on the plant upon which they hatch, the number of neonates moving to a plant of the same type, and the number of neonates moving to a plant of a different type (Onstad and Gould 1998b). The input rate for first larval stadium in a block plot is

$$N_{r-ls} = (1 - 0.76) \times hch_n \times 0.80 + 0.76 \times 0.1 \times hch_n \quad [16]$$

$$N_{Bt-ls} = (1 - 0.90) \times hch_t \times 0.80 + 0.90 \times 0.1 \times hch_t \quad [17]$$

For the block plot, we ignore the movement between a non-Bt plant and a Bt plant because the

proportion of neonates moving between a non-Bt plant and a Bt plant in block plot is very small.

Intraplant Larval Movement to Kernels. Shelton et al. (1986, Fig. 2) studied sweet corn and observed 2% of small larvae on ears from midtasseling stage (VT) to late-silking stage (R1) and 42.6% from late-silking stage to dent stage (R5). Batchelder (1949) reported that the mean proportion of larvae on ears from early-silk to roasting-ear (R) stage was 38% on field corn. We assume that larvae move to ears from the day toxin mortality in the vegetative tissues starts to decline. We assume that larvae move from leaves or stems to kernels right after the interplant movement or right before the end of the first stadium. Equations 18–21 are the functions for neonates not moving to non-Bt kernels, moving to non-Bt kernels, not moving to Bt kernels, and moving to Bt kernels, respectively.

$$N_{r-ls} = N_{r-ls} \times (1.0 - PIMR \times PNIMR) \quad [18]$$

$$N_{r-k} = N_{r-ls} \times PIMR \times PNIMR \quad [19]$$

$$N_{Bt-ls} = N_{Bt-ls} \times (1.0 - PIMB \times PNIMB) \quad [20]$$

$$N_{Bt-k} = N_{Bt-ls} \times PIMB \times PNIMB \quad [21]$$

N_{r-k} and N_{Bt-k} are the input rates of a given genotype to first instars moving to non-Bt kernels and to Bt kernels, respectively. $PIMR$ and $PIMB$ are the proportion of larvae moving to kernels on refuge and Bt plants, respectively. For the standard simulations, $PIMR$ and $PIMB$ are 0.2. $PNIMR$ is the proportion of neonates that move to ears of non-Bt corn. $PNIMB$ is the proportion of neonates that move to ears of Bt corn. $PNIMR$ and $PNIMB$ are 0 or 1. The standard value for $PNIMR$ and $PNIMB$ are 1.0. Equations 22–23 are the

functions for larval movement to non-Bt kernels right before the end of the first stadium. The functions for larval movement to Bt kernels right before the end of the first stadium are equations 24–25.

$$First_{r-ls} = First_{r-ls} \times (1 - PIMR \times (1 - PNIMR)) \quad [22]$$

$$First_{r-k} = First_{r-k} + First_{r-ls} \times PIMR \times (1 - PNIMR) \quad [23]$$

$$First_{Bt-ls} = First_{Bt-ls} \times (1 - PIMB \times (1 - PNIMB)) \quad [24]$$

$$First_{Bt-k} = First_{Bt-k} + First_{Bt-ls} \times PIMB \times (1 - PNIMB) \quad [25]$$

$First_{r-ls}$ and $First_{Bt-ls}$ are the input rates of a given genotype to second larval stadium not moving to non-Bt kernels and to Bt kernels, respectively. $First_{r-k}$ and $First_{Bt-k}$ are the input rates of a given genotype to second instars moving to non-Bt kernels and to Bt kernels, respectively. Larvae in kernels, except overwintering fifth instars, are assumed not to leave kernels until pupation. Shelton et al. (1986) and Batchelder (1949) added the proportions of small larvae on kernels and on silks to calculate the proportion of larvae on ears. For this reason, we use 40% as the upper limit in the sensitivity analysis for the proportion of larvae moving to kernels. For the standard simulation, 20% of larvae in refuge and transgenic plots are assumed to move to kernels.

Cross-pollination From Bt to Refuge Corn. Cross-pollination from Bt corn to non-Bt corn, and vice versa, can influence the toxin exposure of kernel-feeding larvae. For example, an ear on a refuge plant can express Bt toxin when it is fertilized by pollen from corn expressing Bt toxin (Chilcutt and Tabashnik 2004, Burkness et al. 2011), and this may increase the Bt-toxin-induced mortality for the individuals developing in ears of non-Bt corn. These changes in toxin exposure may undermine the effect of refuge on Bt-resistance evolution. Chilcutt and Tabashnik (2004) reported that Cry1Ab is detected in kernels of non-Bt corn up to 31 m from a plot of Cry1Ab-expressing corn. The negative correlation of Cry1Ab concentration in kernels of non-Bt corn and the distance between a refuge plot and a transgenic plot was shown by Chilcutt and Tabashnik (2004). Chilcutt and Tabashnik (2004) estimated that the mean Cry1Ab concentration in kernels of non-Bt corn, located 1 m from a plot of Cry1Ab-expressing corn, was 45% of the mean Cry1Ab concentration in kernels of corn expressing Cry1Ab. The configuration of a refuge block and a transgenic-corn block influences the proportion of non-Bt ears in a refuge fertilized by Bt pollen from a transgenic block (Burkness et al. 2011). Burkness et al. (2011) estimated that the survival rates of second instars feeding on non-Bt ear fertilized by Cry1Ab pollen and non-Bt ear fertilized by non-Bt pollen are 0.600 ± 0.066 and 1.000 ± 0.0 , respectively.

For the single-toxin scenario, the toxin-survival rate for susceptible homozygotes on cross-pollinated ref-

uge corn ears is $stoxRK_{SS} = 0.6$. $stoxRK_{(g)}$ is the toxin-survival rate of a genotype, g , on ears in refuge. Resistance confers 100% survival in resistant homozygotes on cross-pollinated corn kernels. The toxin-survival rate for heterozygotes is

$$stoxRK_{RS} = (stoxRK_{RR} \times h_{RK}) + [stoxRK_{SS} \times (1 - h_{RK})] \quad [26]$$

h_{RK} is the dominance of resistance allele for larvae in non-Bt-ear fertilized by Bt pollen. The standard value for h_{RK} is 1.0, which is a conservative estimate that promotes evolution of resistance. We studied a range of $stoxRK_{SS}$ and h_{RK} .

For the pyramided-toxin scenario, the toxin survival rate of larvae feeding on kernels in the refuge is either 1) the product of the two toxin-survival rates determined by the two loci or 2) the smaller value of the two toxin-survival rates (Onstad and Meinke 2010). We evaluated values for $stoxRK_{SS}$ ranging from 0.01 to 0.77 in a sensitivity analysis. Because we found <2% difference in durability, we used 0.77 as our standard value for each toxin per locus.

For larvae on ears in refuge we use function 27 to calculate $stoxAnthesis_{(g)}$, toxin-survival rate after the date of anthesis.

$$stoxAnthesis_{(g)} = PropCr \times stoxRK_{(g)} + (1.0 - PropCr) \times 1.0 \quad [27]$$

$PropCr$ is the proportion of refuge contaminated by Bt pollen. For block-refuge scenarios, the proportion and toxin concentration of non-Bt-corn ears can be influenced by factors including refuge size, refuge shape, distance from the Bt corn plot, wind speed, wind direction, and pollen longevity (Chilcutt and Tabashnik 2004). For our simple model, we study a range of $PropCr$ in block-refuge scenarios to determine how sensitive the model results are to pollen contamination. The standard value for $PropCr$ is 0. For a seed mixture plot, each non-Bt corn plant is expected to be located close to a Bt corn plant, so 100% of non-Bt ears can be fertilized by Bt pollen, which means that some kernels in all ears in a refuge are fertilized by pollen from Bt corn plants. The standard value of $PropCr$ for seed-mixture refuge is 0. To study the effect of cross-pollination in a seed mixture refuge, we compare the result of the simulation with the standard value of $PropCr$ and that with 100% cross-pollination in a seed mixture refuge.

Larvae Feeding on Bt Ear. Burkness et al. (2011) estimated that the survival rates of second instars feeding on Cry1Ab ears fertilized by Cry1Ab pollen and on Cry1Ab ear fertilized by non-Bt pollen are 0.075 ± 0.053 and 0.029 ± 0.029 , respectively, which are not significantly different. For the single-toxin scenario, we assume that the toxin-survival rate of the homozygous susceptible feeding on a Bt ear, $stoxBK_{SS}$, is 0.052 the mean of the two treatments described above. To be conservative, we assume that resistance confers 100% toxin survival ($stoxBK_{RR} = 1$) in resistant homozygotes on Bt ears. The toxin-survival rate for heterozygotes is

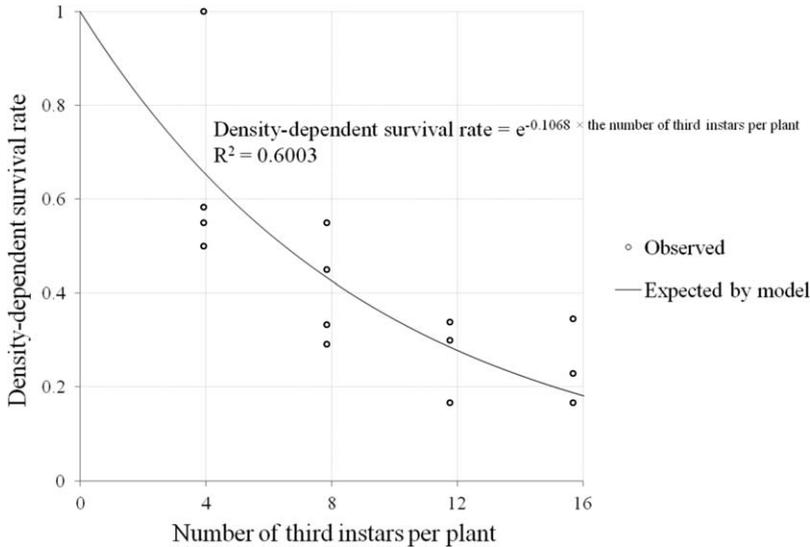


Fig. 3. Density-dependent survival rate function based upon data from Witkowski and Echtenkamp (1987).

$$\text{stoxBK}_{RS} = (\text{stoxBK}_{RR} \times h_{BK}) + [\text{stoxBK}_{SS} \times (1 - h_{BK})] \quad [28]$$

h_{BK} is the dominance of resistance allele for larvae in Bt-ears. The standard value for h_{BK} is 0.25. We studied a range of stoxBK_{SS} and h_{BK} .

For the pyramided-toxin scenario, the toxin survival rate is either the product of the two toxin-survival rates determined by the two loci or the smaller value of the two toxin-survival rates (Onstad and Meinke 2010). In a sensitivity analysis we evaluated a range of values for $\text{stoxAnthesis}_{(g)} = \text{stoxBK}_{(g)}$ from 0.001 to 0.23. Because the difference in durability was $<2\%$, we set the standard value for $\text{stoxBK}_{SS} = 0.23$.

Survival. Onstad and Gould (1998b) estimated that the natural-density-independent survival rates of third instars, fourth instars, fifth instars, pupa, male moths, and female moths are 0.81, 0.81, 0.81, 0.89, 1.0, and 1.0, respectively. The natural-density-independent survival rate of eggs is described in the oviposition section. The survival rates for the first and second instars are the product of the natural-density-independent survival rates, which are 1.0 for the first instars and 0.81 for the second instars, and the toxin-survival rates. The survival rate of third instars is calculated by multiplying the natural-density-independent survival rate of third instars by density-dependent survival rate, which occurs in life stages experiencing significant competition. The survival rates for the fourth instars, the fifth instars, and pupae are the natural-density-independent survival rates.

We assign a certain number of age cells to each life stage. Each day, the number of individuals in one cell in life stage is shifted to the next cell. During that shifting, the number of individuals is multiplied by $B^{(1/C)}$, where B is survival rate per life stage and C is the number of age cells in a life stage. When insects complete one life stage, the number of individuals in

the last age cell in one life stage is shifted to the first age cell in the next life stage.

The survival rates of third instars in the refuge plot and those in the Bt plot are calculated separately. A function for density-dependent survival of third instars is based upon data reported by Witkowski and Echtenkamp (1987). They sampled the number of second-generation egg masses per plant, and dissected corn plants to sample the number of live larvae in the plants 20 d after the egg-mass sampling. To convert the mean numbers of egg masses per plant to the mean numbers of third instars per plant, 3.918 is multiplied by the mean number of egg masses per plant. ($3.918 = 20$ [the number of eggs per egg mass] $\times 0.95$ [the probability of egg fertilized] $\times 0.95$ [the probability of egg hatching] $\times (0.76$ [the probability of neonate moving away from non-Bt corn] $\times 0.1$ [the survival rate because of movement] $+ (0.24$ [the probability of neonate staying on non-Bt corn] $\times 0.8$ [the survival rate because of staying on the natal plant] $\times 0.81$ [the survival rate of second instars])). The data points indicating zero egg masses per plant were not used. The maximum egg-to-larva survival rate for 20 d in the data are 0.3, so 0.3 is assumed to be the density-independent egg-to-third-instar-larval-survival rate for the analysis. Egg-to-larva survival rates for 20 d are divided by 0.3 to calculate density-dependent survival rates. We applied a negative exponential function to describe the density-dependent-survival rate (Fig. 3).

$$DDS = e^{-0.1068 \times \sum_{35}^{D_t}} \quad \text{when } t < 210 \quad [29]$$

$$DDS = e^{-0.1068 \times \sum_{210}^{D_t}} \quad \text{when } t \geq 210 \quad [30]$$

DDS is density-dependent-survival rate, and 158 and 210 are the day simulation starts and the day second-generation eggs first appear, respectively. D_t is the number of individuals completing second stadium on day t . Equations 29–30 are for the first and the

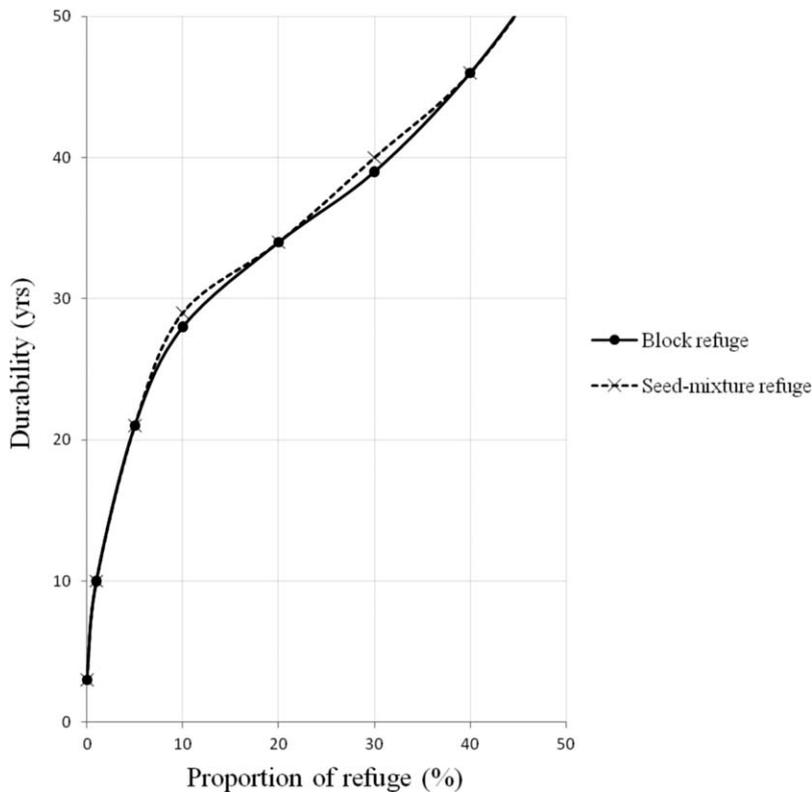


Fig. 4. The number of years required for the resistance allele frequency to increase from 0.0001 to 0.5 in block or seed-mixture refuge under single-toxin scenario without cross-pollination.

second generations, respectively. Because of the lack of data, the first- and second-generations are assumed to be affected by the same limitation in density-dependent survival.

Insect Maturation. Nine life stages (egg, five larval stadia, pupa, male and female adults) with constant rates of maturation are simulated. The developmental period for each life stage was estimated by averaging the first and second generation data collected in Illinois (Calvin et al. 1991, Mason et al. 1996). The pupal period, however, was estimated from Iowa data (7.6 d) (Calvin et al. 1991, Mason et al. 1996) because that in Illinois was not investigated and the development of *O. nubilalis* in Iowa is similar to that in Illinois. Based upon this information and rounding to nearest integer, the duration of egg, first, second, third, fourth, and fifth instars, and pupal stage were determined to be 6, 5, 5, 6, 10, 8, and 8 d, respectively. Female adults pass through a 4-d preoviposition period before the 10-d period for oviposition (Caffrey and Worthley 1927, Huber et al. 1928, Bottger and Kent 1931, Sparks et al. 1966). The time step in the model is 1 d, and the number of insects in each cell in life stages is transferred to the next cell with attrition each day. The first cell in the life stage receives the input to the life stage.

Overwintering. Postdiapause development of larvae, pupae, and adults in the following spring is not modeled explicitly because of the lack of data. Onstad

and Gould (1998b) estimated that the proportion of fifth instars surviving from October to May is 0.18. The number of second-generation adults for a given genotype is the sum of the fifth instars in diapause multiplied by overwintering-survival rate and the survival rate for pupal stage (0.89) (Onstad and Gould 1998b).

Results

Without Cross-pollination. With single-toxin, the model predicts that it takes 38 yr for the resistance allele frequency to exceed 0.5 with the 20% block and 20% seed-mixture refuge (Fig. 4). The comparison of 5% block refuge and 5% seed-mixture refuge for pyramided corn is shown in Fig. 5. For pyramided toxin, when there is no refuge, the frequencies of two resistance alleles exceed 0.5 in 5 yr if multiplicative-toxin-survival rates are used and 12 yr if minimum-toxin-survival rates are used. When the proportion of seed-mixture refuge is 5%, the resistance allele frequencies exceed 0.5 in circa 64 yr. Overall, without cross-pollination the seed-mixture delays the evolution of resistance at least as well as a block refuge when examined with two separate population genetics scenarios.

Cross-pollination in Refuge. When 50% of ears in 20% block refuge are cross-pollinated, the resistance allele frequencies exceed 0.5 2 yr earlier than with 0%

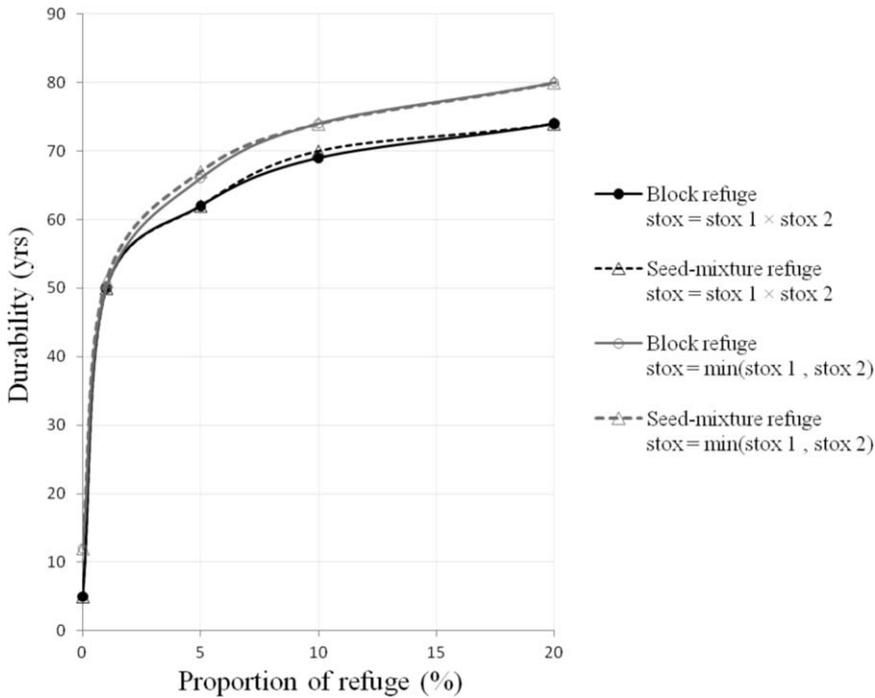


Fig. 5. The number of years required for the resistance allele frequencies to increase from 0.0001 to 0.5 in block or seed-mixture refuge under pyramided-toxin scenario with multiplicative-toxin-survival rates without cross-pollination.

cross-pollination under single-toxin scenario. When 100% of non-Bt ears in a seed-mixture are cross-pollinated, the resistance allele frequencies exceed 0.5 2 yr earlier under single-toxin scenario. Therefore, the proportion of cross-pollinated ears in refuge has little effect on resistance evolution given our standard assumptions.

The timing of toxin mortality during the larval stage is very important for model results (Tables 2 and 3). Generally, resistance evolution is delayed if some of the mortality is avoided with movement. This occurs because there is a high probability susceptible larvae move away from a Bt-corn plant (see *TOXW*, one versus 0.5, in Tables 2 and 3). The proportion of larvae moving to the corn ears (*PIM*) had only a slight influence on the results (Tables 2 and 3). The value of *PNIMR* had no influence on the results. This indicates that the timing of intraplant movement is not very important.

Neither the survival on non-Bt corn kernels nor the dominance of resistance when feeding on these cross-pollinated kernels influences the evolution of resistance. When the toxin survival rate of the homozygous susceptible larvae feeding on cross-pollinated kernels (*stoxRK_{SS}*) is within a range of 0.01–0.6, there is 1–2 yr difference in durability (<5%) for the scenarios with a single-toxin and a 20% block refuge. The same is true for the scenario with pyramided toxin and a 5% seed mixture when *stoxRK_{SS}* is within a range of 0.01–0.77. For both single-toxin and pyramided-toxin scenarios, changing the dominance *h_{RK}* over its entire range had

little impact on results; < 1 yr change compared with values in Tables 2 and 3.

We simulated a worst-case scenario with 100% cross-pollination of ears in seed-mixture refuge, toxin mortality only for first instars, 40% of larvae move to non-Bt ears, only first instars move to non-Bt ears, *stoxRK_{SS}* is 0.01 for single-toxin and pyramided-toxin scenarios, and the dominance of the resistance allele is 1.0. In this case, resistance evolves in 23 yr under single-toxin scenario with 20%-seed-mixture refuge, and 59 yr under pyramided-toxin scenario with 5%-seed-mixture refuge and multiplicative-toxin-survival rates.

Toxin Expression in Bt Corn Ear. Resistance evolves circa 5 yr earlier when neonates move to ears (*PNIMB* = 1) than when movement occurs with first

Table 2. The effect of *PIMR* (the proportion of larvae moving to kernels on refuge plants) and *TOXW* (the proportion of toxin mortality during first-instar stage) on Bt-resistance evolution in *O. nubilalis* under single-toxin scenario with cross-pollination

	<i>PIMR</i>					
	Block refuge			Seed-mixture refuge		
<i>TOXW</i>	0	0.2	0.4	0	0.2	0.4
1	34	32 ^a	31	34	32	29
0.5	37	36	34	38	35	32

Numbers in the table are the no. of years required for resistance allele frequency to exceed 0.5 with a 20% block refuge with 50% cross-pollination or 20% seed-mixture refuge with 100% cross-pollination.

h_{RK} = 1.0 and *PNIMR* = 1.

^a Result with standard version of model.

Table 3. The effect of *PIMR* (the proportion of larvae moving to kernels on refuge plants) and *TOXW* (the proportion of toxin mortality during first-instar stage) on Bt-resistance evolution in *O. nubilalis* under pyramided-toxin scenario with cross-pollination

<i>TOXW</i>	<i>PIMR</i>					
	Multiplicative ^b			Minimum ^c		
	0	0.2	0.4	0	0.2	0.4
1	62	61 ^a	59	67	66	66
0.5	78	75	72	83	83	82

Numbers in the table are the number of years required for resistance allele frequency to exceed 0.5 with a 5% seed-mixture refuge with 100% cross-pollination.

$h_{BK} = 1.0$ and $PNIMR = 1$.

^a Result with standard version of model.

^b Product of two toxin-survival rates.

^c Minimum of two toxin-survival rates.

and second instars ($PNIMB = 0$). When the proportion of larvae moving to ears on Bt corn (*PIMB*) changes from 20% to 40%, the resistance allele frequency exceeds 0.5 5–6 yr faster under single-toxin scenario (Table 4), and 6–11 yr faster under pyramided-toxin scenario (Table 5). Under the pyramided-toxin scenario, with standard dominance h_{BK} is 0.25, the resistance allele frequencies do not exceed 0.5 within 50 yr in any combinations of *PIMB*, *PNIMB*, and *TOXW* (Table 5). As noted above for Tables 2 and 3, when toxin mortality occurs during first and second instars ($TOXW = 0.5$), resistance evolves slower only when neonates move to Bt ears compared with when only second instars move to Bt ears (Tables 4 and 5).

The survival of susceptible larvae on Bt-corn kernels has only a minor influence on results. When the toxin survival rate of the homozygous susceptible larvae feeding on cross-pollinated kernels ($stoxBK_{SS}$) is within a range of 0.001–0.1, there is 1–2 yr difference in durability (<10%) for the scenarios with a single-toxin. With pyramided toxin and a 5% block refuge, varying $stoxRK_{SS}$ over the range 0.01–0.23, the results changed by no >5%.

The dominance of resistance when feeding on these cross-pollinated kernels is much more important for evolution of resistance. For the single-toxin scenario, evolution occurs in circa 23 yr with $h_{BK} = 1$, but requires 41–46 yr with $h_{BK} = 0$, depending on the value of *TOXW*.

Table 4. The effects of *PIMB* (the proportion of larvae moving to Bt ears) and *TOXW* (the proportion of toxin mortality during first-instar stage) on Bt-resistance evolution in *O. nubilalis* under single-toxin scenario

<i>TOXW</i>	<i>PIMB</i>					
	Block refuge			Seed-mixture refuge		
	0	0.2	0.4	0	0.2	0.4
1	39	34 ^a	29	39	34	29
0.5	43	37	31	44	38	32

Numbers in the table are the no. of years required for resistance allele frequency to exceed 0.5 with a 20% block refuge with 0% cross-pollination or 20% seed-mixture refuge with 0% cross-pollination.

$h_{BK} = 0.25$ and $PNIMB = 1$.

^a Result with standard version of model.

Table 5. The effects of *PIMB* (the proportion of larvae moving to Bt ears) and *TOXW* (the proportion of toxin mortality during first-instar stage) on Bt-resistance evolution in *O. nubilalis* under pyramided-toxin scenario

<i>TOXW</i>	<i>PIMB</i>					
	Multiplicative ^b			Minimum ^c		
	0	0.2	0.4	0	0.2	0.4
1	65	59 ^a	53	63	58	51
0.5	83	73	62	79	70	60

Numbers in the table are the number of years required for resistance allele frequency to exceed 0.5 with a 5% block refuge with 0% cross-pollination.

$h_{BK} = 0.25$ and $PNIMB = 1$.

^a Result with standard version of model.

^b Product of two toxin-survival rates.

^c Minimum of two toxin-survival rates.

Thus, recessive expression of resistance to kernel toxicity on Bt corn can double the time to resistance evolution compared with the completely dominant case. For the pyramided Bt corn scenario, the results are generally less sensitive to this parameter (Table 6), but still demonstrate the importance of this parameter.

The worst case scenario of reduced mortality on ears of Bt corn results in resistance evolution in 16 yr under single-toxin scenario with 20% block refuge, and 56 yr under pyramided-toxin scenario with 5% block refuge and multiplicative-toxin-survival rates. The worst-case parameters are $stoxBK_{SS}$ is 0.01 for single-toxin and pyramided-toxin scenarios, toxin mortality occurs only during first-instar stage, 40% of larvae move to Bt ears, only first instars move to Bt ears, and the dominance of the resistance allele for larvae feeding on Bt-ears is 1.0.

Discussion

Our model results indicate that seed mixtures and block refuges of the same proportion can delay resistance by *O. nubilalis* to Bt corn equally well. This occurs because, in this model, *O. nubilalis* mates at random across blocks and the empirical data used in this study did not find differential selection because of larval genotype or movement in single-toxin corn, which is assumed for the pyramided toxin in this model (S. E. Moser et al., unpublished data). The warnings about less effective

Table 6. The effects of h_{BK} (the dominance of resistance allele for larvae feeding on Bt kernels), and *TOXW* (the proportion of toxin mortality during first-instar stage) on Bt-resistance evolution in *O. nubilalis* under pyramided-toxin scenario

<i>TOXW</i>	h_{BK}							
	Multiplicative ^b				Minimum ^c			
	0.0	0.1	0.5	1.0	0.0	0.1	0.5	1.0
1	66	62 ^a	58	50	67	66	65	63
0.5	84	77	70	60	84	82	81	78

Numbers in the table are the number of years required for resistance allele frequency to exceed 0.5 with a 5% block refuge with 0% cross-pollination.

PIMB = 0.2 and *PNIMB* = 1, and $stoxBK_{SS} = 0.23$.

^a Result with standard version of model.

^b Product of two toxin-survival rates.

^c Minimum of two toxin-survival rates.

seed mixtures for Bt corn IRM made by Onstad and Gould (1998) and Davis and Onstad (2000) were based on the unsupported assumption that differential selection did occur because of larval movement by susceptible and heterozygous individuals.

Resistance evolution is not significantly expedited by cross-pollination or *O. nubilalis* larval movement and survival on kernels of non-Bt corn for four reasons: 1) Ears are available only for the second generation of *O. nubilalis* in Illinois; 2) the proportion of refuge is 20% for single-toxin-trait corn or 5% for multiple-toxin-trait corn; 3) only a fraction of the second generation larvae are assumed to move to the ears; and 4) mortality caused by toxins expressed in non-Bt ears, which are fertilized by Bt pollen, is not high.

The two most important processes identified by this modeling exercise are the timing of toxin mortality during the larval stages and the dominance of resistance to toxins expressed in kernels of Bt corn. Additional work on other corn pests also is needed because Bt-pollen drift in seed-mixture plantings may have a significant impact on the evolution of Bt resistance in insects which complete most of their Cry-toxin-susceptible-life stages on corn ears, including *Helicoverpa zea* (Boddie) and *Striacosta albicosta* (Smith) (Burkness et al. 2011, Onstad et al. 2011). More ears in a seed mixture refuge are subject to the fertilization by transgenic pollen than those in block refuge. Therefore, monitoring ear-feeding pests in seed mixtures is important, but plants in a seed-mixture refuge are more difficult to locate and monitor than those in a block refuge (Onstad et al. 2011).

The results of the model partially depend on the knowledge acquired from the experiments of S. E. Moser et al. (unpublished data). They studied movement and survival of three genotypes of larvae on one kind of transgenic corn. Prasifka et al. (2009, 2010) studied two genotypes of larvae under different conditions by using another transgenic insecticidal corn trait. Prasifka et al. (2010) found that Cry1Ab resistant larvae had a higher probability of leaving a Bt corn plant for an adjacent non-Bt plant than did susceptible larvae. Yet S. E. Moser et al. (unpublished data) observed that susceptible larvae disperse from Cry1 F-expressing corn more than Cry1 F-resistant larvae. Similarly, Goldstein et al. (2010) also determined that susceptible neonates frequently move from Bt corn and can establish on neighboring non-Bt corn. In our model, the main factors of concern for insect resistance management are the movement and survival of heterozygotes, measured by S. E. Moser et al. (unpublished data). The variety of observations for resistant larvae demonstrates the need for additional studies of larval movement by all possible genotypes of insect pests controlled by transgenic corn, and in particular larval movement in reproductive stage corn.

Acknowledgments

This research was a joint contribution from the USDA Agricultural Research Service and the Iowa Agriculture and

Home Economics Experiment Station, Ames (Project3025). Research was funded by a grant from the USDA Biotechnology Risk Assessment Research Grants Program (2007-39211-18461). We thank Barry Pittendrigh's lab for the academic and financial support.

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Received 29 May 2011; accepted 25 October 2011.