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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON D.C., 20460

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

May 6, 2009

**MEMORANDUM**

**SUBJECT:** Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held February 23 - 24, 2009 on the Evaluation of the Resistance Risks from Using a Seed Mix Refuge with Pioneer's Optimum<sup>®</sup> AcreMax<sup>™</sup>1 Corn Rootworm-Protected Corn

**TO:** Debbie Edwards, Ph. D.  
Director  
Office of Pesticide Programs

**FROM:** Joseph E. Bailey, Designated Federal Official  
FIFRA Scientific Advisory Panel  
Office of Science Coordination and Policy

Handwritten signature of Joseph E. Bailey in black ink.

**THRU:** Steven Knott, Executive Secretary  
FIFRA Scientific Advisory Panel  
Office of Science Coordination and Policy

Handwritten signature of Steven M. Knott in black ink.

Frank Sanders, Director  
Office of Science Coordination and Policy

Handwritten signature of Frank Sanders in black ink.

Attached, please find the meeting minutes of the FIFRA Scientific Advisory Panel open meeting held in Arlington, Virginia on February 23 - 24, 2009. This report addresses a set of scientific issues being considered by the Environmental Protection Agency pertaining to the Evaluation of the Resistance Risks from Using a Seed Mix Refuge with Pioneer's Optimum<sup>®</sup> AcreMax<sup>™</sup>1 Corn Rootworm-Protected Corn.

Attachment

**cc:**

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Michael McDavit  
Jeannette Martinez  
Alan Reynolds  
Enesta Jones  
Douglas Parsons  
Vanessa Vu (SAB)  
OPP Docket

**FQPA Science Review Board Members**

Steven G. Heeringa, Ph.D. (FIFRA SAP Chair)  
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**FQPA Science Review Board Members**

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Richard T. Roush, Ph.D.  
Douglas V. Sumerford, Ph.D.  
John C. Schneider, Ph.D.  
Jon J. Tollefson, Ph.D.

**SAP Minutes No. 2009-03**

**A Set of Scientific Issues Being Considered by the  
Environmental Protection Agency Regarding:**

**Evaluation of the Resistance Risks from Using a Seed  
Mix Refuge with Pioneer's Optimum<sup>®</sup> AcreMax<sup>™</sup> 1  
Corn Rootworm-Protected Corn**

**February 23 – 24, 2009  
FIFRA Scientific Advisory Panel Meeting  
held at the Environmental Protection Agency  
Conference Center  
Arlington, VA**

## NOTICE

These meeting minutes have been written as part of the activities of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). The meeting minutes represent the views and recommendations of the FIFRA SAP, not the United States Environmental Protection Agency (Agency). The content of the meeting minutes does not represent information approved or disseminated by the Agency. The meeting minutes have not been reviewed for approval by the Agency and, hence, the contents of these meeting minutes do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal Government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and established under the provisions of FIFRA as amended by the Food Quality Protection Act (FQPA) of 1996. The FIFRA SAP provides advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the Environmental Protection Agency, Office of Pesticide Programs (OPP), and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. FQPA Science Review Board members serve the FIFRA SAP on an ad hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at <http://www.epa.gov/scipoly/sap/> or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Joseph E. Bailey, SAP Designated Federal Official, via e-mail at [bailey.joseph@epa.gov](mailto:bailey.joseph@epa.gov).

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by EPA, as well as information presented by public commenters. This document addresses the information provided and presented by EPA within the structure of the charge.

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**SAP Minutes No. 2009-03**

**A Set of Scientific Issues Being Considered by the  
Environmental Protection Agency Regarding:**

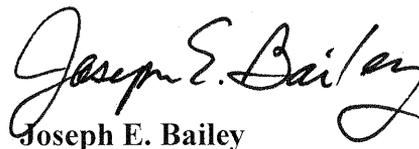
**Evaluation of the Resistance Risks from Using a Seed  
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Corn Rootworm-Protected Corn**

**February 23 – 24, 2009**

**FIFRA Scientific Advisory Panel Meeting  
held at the Environmental Protection Agency  
Conference Center  
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**Steven G. Heeringa, Ph.D.**  
FIFRA SAP Chair  
FIFRA Scientific Advisory Panel  
Date: May 6, 2009



**Joseph E. Bailey**  
Designated Federal Official  
FIFRA Scientific Advisory Panel  
Date: May 6, 2009

**Federal Insecticide, Fungicide and Rodenticide Act**  
**Scientific Advisory Panel Meeting**  
**February 23 – 24, 2009**

**PARTICIPANTS**

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**Designated Federal Official**

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**FQPA Science Review Board Members**

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**Anthony R. Ives, Ph.D.**, Professor, Department of Zoology, University of Wisconsin, Madison, WI

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**John C. Schneider, Ph.D.**, Professor and Research Entomologist, Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS

**Jon J. Tollefson, Ph.D.**, Professor, Department of Entomology, Iowa State University, Ames, IA

## INTRODUCTION

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the Evaluation of the Resistance Risks from Using a Seed Mix Refuge with Pioneer's Optimum® AcreMax™ 1 Corn Rootworm-Protected Corn. Advance notice of the meeting was published in the *Federal Register* on December 10, 2008. The review was conducted in an open Panel meeting held in Arlington, Virginia, from February 23 – 24, 2009. Dr. Steven G. Heeringa chaired the meeting. Joseph E. Bailey served as the Designated Federal Official.

EPA's Office of Pesticide Programs (OPP) received an application of registration from Pioneer Hi-Bred International, Inc. for registration of Optimum® AcreMax™ 1 Corn Rootworm-Protected Corn, which is a corn seed blend containing seeds that express the Bt toxins Cry34Ab1, Cry35Ab1, and Cry1F in a stack for corn rootworm (CRW) and lepidopteran protection mixed with seeds that express only Cry1F for lepidopteran protection (i.e. "refuge in the bag" for CRW). Pioneer proposes to use a seed blend mixture containing  $\geq 2\%$  refuge seeds. OPP has reviewed the submitted studies and modeling chapter and identified several areas of uncertainties with respect to CRW biology, population genetics, and modeling. EPA sought the assistance of the FIFRA SAP to address scientific issues associated with the seed mix proposal for CRW resistance management and to provide the Agency with guidance as to the implications of the uncertainties. Areas of uncertainty included: (1) mode of action of Cry34/35Ab1 (i.e. toxic or repellent) to exposed CRW and implications for a seed mix, (2) aspects of corn rootworm pest biology including the effects of delayed emergence and uneven sex ratios on random mating and ultimately on the rate of resistance evolution in a seed blend environment; (3) assumptions about initial resistance gene frequency to Cry34/35Ab1; (4) contributions of tolerance (minor) and resistance (major) genes and selection consequences in corn rootworm exposed to Cry34/35Ab1 in a seed blend environment; (5) mortality for individuals being heterozygous (XY) and susceptible (XX) for the tolerance (minor) gene; (6) the overall simulation model (i.e., is it adequate to evaluate the proposed seed mix for Cry34/35Ab1?); and (7) the mechanics of mixing refuge seed with Cry34/35Ab1 seed into a single bag and the potential distribution (i.e. random or non-random) of refuge plants within planted fields.

Steven Bradbury, Ph.D., (Deputy Office Director for Programs, Office of Pesticide Programs) and Janet Andersen, Ph.D., (Director, Biopesticides and Pollution Prevention Division) provided opening remarks at the meeting. The agenda for the meeting included presentations by Jeannette Martinez, M.S., and Alan Reynolds, M.S., (Biopesticides and Pollution Prevention Division, Office of Pesticide Programs) as well as public comments.

## **PUBLIC COMMENTERS**

### **Oral statements were presented as follows:**

David Fremark, farmer, South Dakota  
Richard Glass, Ph.D., on behalf of the National Corn Growers Association  
Ron Heck, farmer, Iowa  
Laura Higgins, M.S., Tim Nowatzki, Ph.D., J. Lindsey Flexner, Ph.D., and Raymond J. Layton, Ph.D., on behalf of Pioneer Hi-Bred International, Inc.  
David Onstad, Ph.D., University of Illinois  
Kenneth Ostlie, Ph.D., University of Minnesota  
David Smith, farmer, Illinois  
Nicholas P. Storer, Ph.D. on behalf of Dow AgroSciences LLC

### **Written statements were provided by:**

Anonymous public commenters  
Neil J. Carman, Ph.D., Sierra Club  
Chris Collett, farmer, Ohio  
Rob Elliott on behalf of Illinois Corn Growers Association  
A. Fisher  
David Fremark, farmer, South Dakota  
Bart Ginther, farmer, Colorado  
Richard Glass, Ph.D., on behalf of the National Corn Growers Association  
Ron Heck, farmer, Iowa  
Matthew H. Howe, Pine Ridge Farm Services, Michigan  
Gene Hugoson, Commissioner, Minnesota Department of Agriculture  
Gregory A. Ibach, Director, Nebraska Department of Agriculture  
Rex Johnson, farmer, Illinois  
Christian Krupke, Ph.D., on behalf of Alex Murphy  
George Lukach, farmer, Illinois  
Michael Merten, farmer/Pioneer Sales Representative  
Bill Northey, Secretary of Agriculture, Iowa  
David Onstad, Ph.D., University of Illinois  
Tracy Rood, on behalf of Pioneer Hi-Bred International, Inc.  
Anthony Shelton, Ph.D., Cornell University, NYSAES Entomology  
David Smith, Pioneer Sales Representative, Illinois  
Philip Smith, farmer, Minnesota  
Nicholas P. Storer, Ph.D., on behalf of Dow AgroSciences LLC  
Bruce Tabashnik  
Jeff Theis, Pioneer Sales Representative, Nebraska  
Ron Wegleitner, farmer/Northland Feeders Inc.  
Lawrence Zeph, Syngenta Biotechnology Inc.

## SUMMARY OF PANEL DISCUSSIONS AND RECOMMENDATIONS

Overall, the Panel concluded that there are uncertainties with the scientific data supporting Pioneer's proposed seed blend, Optimum® AcreMax™1, and clear problems with reducing the refuge size. The Panel generally agreed that data presented by Pioneer and data found in the public literature provide no compelling evidence to reduce the proportion of non-Bt plants (either as a seed blend or spatial refuge) from 20% and there was strong concern with the request for any reduction in the refuge size with a seed blend of 5% or less. Data were not presented that supported a claim that potential yield losses justify a seed blend of no greater than 5%. The Panel supported the recommendation to conduct additional research with various percentages of seed mixtures to determine any effects on yield. Therefore, the Panel concluded that, based on the current science, it would be reasonable to commercially use 20% seed blend refuges while research suggested by the committee and other research projects are conducted to examine the performance of the seed blend strategy. The recommendation for use of a 20% seed blend is based on characteristics unique to products targeting CRW (corn rootworm) that, like Optimum® AcreMax™ 1 Corn Rootworm-Protected Corn, have low- or medium-dose effects on the target pest. The Panel wants to be clear, however, that this recommendation should not set precedence for other Bt crop products targeting other pests.

The Panel agreed with EPA that there is uncertainty with regard to whether the mode of action of Cry 34/35Ab1 is through a toxic or repellent mechanism. Pioneer (MRID 47356701) and Lefko et al. (2008) suggest a level of tolerance to 59122 maize (maize expressing Cry34Ab1 and Cry35Ab1) that needs to be better clarified. The Panel indicated that scientific literature documents the ability for lepidopteran larvae exposed to Bt (*Bacillus thuringiensis*) toxin to suffer damage to the midgut, but when exposure to Bt is eliminated, the midgut lining heals or is replaced and the lepidopteran larvae recovers. Therefore, the Panel questioned whether the recovery of CRW from the Cry 34/35Ab1 Bt toxin involves a similar mechanism. The CRW response to 59122 maize could initially be physiological and could then manifest as behavior allowing rapid recovery from toxin induced midgut deterioration. The Panel was not provided any histological data for CRW and 59122 maize. If CRW response to 59122 maize has a physiological underpinning, the Panel questioned if there are multiple modes of action; these types of responses in lepidopteran species and the Colorado potato beetle represent a single gene trait. Deterrence could be a heritable trait at the level of midgut physiology through an insensitivity target receptor site. The Panel also noted that CRW avoidance of Bt plants could be based on physiological lesions that cause a behavioral mode of action; the larvae feed on Bt plants, experience damage to their midguts, stop feeding, and then move around until they find another source of food. Lefko and Binning (MRID 47356701) demonstrated all instars are capable of recovering from feeding on 59122 maize and completing development.

The Panel examined the details of the Lefko et al. (2008) article and found that there was no rigorous evidence in the reported data that the frequency of major genes for resistance was very low. There were a number of problems with the design of the experiments reported in the Lefko et al. (2008) paper, but the most important was that there was no way of determining how many wild CRW haplotypes were subjected to selection for Bt resistance. The Panel was concerned that there could have been very strong selection for the laboratory-adapted phenotype between the first hybridization and the beginning of the Bt selection regime. The Panel, while not stating that the resistance found by Lefko et al. (2008) came from the laboratory colony, nonetheless found no evidence to the contrary. More importantly, they believed that even if the resistance traits found in Lefko et al. (2008) came from the field insects, there was no evidence that there was not a severe bottleneck in the number of field haplotypes in the hybrid population when the selection experiment was begun. They concluded that the Lefko et al. (2008) study cannot be used to establish the initial frequency of major resistance alleles and further concluded that the Lefko et al. (2008) study indicates the presence of partial resistance. Even with this finding, the Panel was concerned that the researchers should have conducted reciprocal crosses to conclusively prove the genetic component of the partial resistance. They also noted that in the Lefko et al (2008) paper, the authors computed realized heritability and degree of resistance in each generation, but that they did this without presenting data on the fitness of the control population on Bt corn in each generation.

The Panel was asked if different selection intensities could select different genes, and possibly a major resistance gene. They noted that different selection intensities in the field, whether in seed blends or pure stands (with structured refuges), could surely select different genes than might be found in laboratory trials, including “major” resistance genes (ones that confer high magnitudes of resistance typically 10-fold or more at an LC50). However, even relatively minor genes could be important in this case since some insects believed not to carry resistance genes still survive. There is a long history of comparison of selection in laboratory and field, with the general conclusion being that laboratory selection tends to produce resistance with complex or at least undetermined genetics, whereas analysis of resistance that evolves in the field almost always finds major genes.

While it is possible that the “tolerance” described in Lefko et al. (2008) could be due to an interaction between two tolerance genes, there is no evidence in the paper for the number of genes involved. It is possible that the relative rate of adaptation could be affected by including more than one minor gene in the model. The survival parameters used by Pioneer are not necessarily unrealistic but, especially because the Panel was not confident about the estimates produced by Lefko et al. (2008), a much wider range of parameters and assumptions about the resistance/tolerance genes should have been tested in a sensitivity analysis. Such an analysis should have included that survival was not just additive, but that the two genes could act synergistically (i.e., multiplicatively, Raymond et al., 1989). When viewed as a dose-response, rather than as survival, pesticide resistance genes tend to act in a multiplicative way.

Regarding uncertainties associated with the model Pioneer used to support its proposal, Pioneer calculated an upper bound constraint on the frequency of a resistance

allele of major effect ( $q^*=0,00037$ ) on the questionably designed study by Lefko et al. (2008). Without additional information, and given the limitations of Lefko et al. (2008), the Panel believed that it is not possible to set a reasonable upper bound estimate for  $q^*$ . Further, Pioneer assumed a value of 0.05 for the dominance parameter ( $h$ ) in their model. Based on data regarding the genotypic fitness for various Bt resistance genes, no upper bound estimate for  $h$  of less than 1.0 (corresponding to complete dominance) can be assumed. Regarding emergence delay data, Pioneer pooled males and females in their analysis of date of emergence (DOE); and, although this is statistically justifiable because of the absence of an interaction between gender and blend proportions, it tends to obscure the issue of whether a delay in DOE of males from blend refuge fields reduces their effectiveness in resistance management compared to males produced in 100% HX1 (Herculex I CRW-susceptible refuge corn) refuge fields. Refuge effectiveness is more likely to be reduced by the dramatic reductions in numbers of males produced in 5% blend plots which averaged less than 5% of production of males from HX1 plots. The Panel concluded that the lack of inclusion of uneven sex ratios does not appear sufficient to appreciably affect resistance management. Regarding the lack of inclusion of density-dependent larval dispersal, the Panel concluded that even if positively density-dependent dispersal of WCR occurs, it may have little effect on the rate of resistance development.

The Panel was asked to comment on whether it is realistic to assume that the minor tolerance trait might drive the delay of major resistance in a seed blend environment. The uncertainties of the dominance value and allele frequency for the major gene in the Pioneer model do not allow a realistic assessment of whether the “minor” gene described by Lefko et al. (2008) will slow the evolution of a major resistance gene. To best address the potential for minor genes to slow the evolution of a major gene, information on how the genes interact with one another is necessary. The Panel agreed that more exploratory modeling varying the dominance and allele frequencies for minor and major genes is warranted. The Panel also agreed that polygenic models using “tolerance” genes may be a more realistic scenario to assess the risk of resistance in seed blends.

In summary, most Panel members believed that corn rootworm biology seems to lend itself to the seed blend concept and that while the seed blend refuge concept has merit, the Panel had concerns regarding the reduction in refuge size. However, the Panel also believed that it is vital to preserve the Bt CRW biology and was significantly concerned about the proposal to move to both a seed blend refuge and a drastic reduction in refuge at the same time.

## PANEL DELIBERATIONS and RESPONSE to CHARGE

**Charge Question 1** - Pioneer suggested based on their host search behavior results (MRID 473567-07) that Cry34/35Ab1 protects against corn rootworm (CRW) through deterrence and sub-lethal toxicity leading to death by starvation. Pioneer's larval recovery study demonstrated high survival rate of CRW that were exposed to 59122 maize at any stage during their development but allowed to recover on non-Bt corn (MRID 473567-01). Conversely, larval exposure to 59122 maize without a recovery phase resulted in a mortality rate of 0.05 for neonates and 0.01 for 2nd and 3rd instars. BPPD concluded that it cannot be determined whether mortality or survival occurred due to deterrence or some other mechanism.

BPPD would like the panel to discuss whether there is evidence that repellency or deterrence by 59122 maize could also be described as a realistic mode of action, specifically for a non-high dose toxin such as Cry34/35Ab1, and any implications for a seed blend refuge strategy.

**Panel Response** - The Panel agreed with BPPD that there is uncertainty regarding the mode of action of Cry 34/35Ab1 (i.e. toxic and/or repellent). Whalon and Wingerd (2003) described the mode of action of Bt and impact on the target pest as follows: "most insects are not killed directly by the effects of the proteins, but die as a result of rapidly induced gut paralysis and feeding inhibition, and subsequent starvation or septicemia." While studies suggest 59122 maize has a repellency or deterrence effect on neonates and later instars (MRID 47356707), a description of the mode of action of Cry 34/35Ab1 was not provided to this Panel.

Pioneer (MRID 47356701) and Lefko et al. (2008) suggest a level of tolerance to 59122 maize that needs to be better clarified. A written comment submitted regarding this meeting raised the issue of "incomplete resistance" which is described as survival of a "resistant strain" on a Bt crop with significantly greater survival than a susceptible strain, but less survival than a "resistant strain" on a non-Bt crop (Carriere and Tabashnik 2001, Tabashnik et al. 2003, 2005). Even with an understanding of the mode of action it will be hard to evaluate the impact on the utility of the blend, and it is difficult to determine how the tolerance trait described by Pioneer will be affected by the proposed seed blend refuge.

The Panel indicated that scientific literature documents the ability for lepidopteran larvae exposed to Bt toxin to suffer damage to the midgut, but when exposure to Bt is eliminated, the midgut lining heals or is replaced and the lepidopteran larvae recovers (Ferre and Van Rie, 2002). Similarly, a strain of Colorado potato beetle that is resistant to Bt sprays, but not Bt transgenic crops, recovers from exposure to Bt toxins, presumably restoring its midgut (Wierenga et al. 1996, Tabashnik et al. 2003). The Panel questioned whether the recovery of CRW from the Cry 34/35Ab1 Bt toxin involves a similar mechanism.

The response to 59122 maize by CRW could initially be physiological and then manifested through behavior, thereby allowing rapid recovery from toxin-induced midgut deterioration. The Panel was not provided any histological data for CRW and 59122 maize. If CRW response to 59122 maize has a physiological underpinning, the Panel questioned if there are multiple modes of action since these types of response in lepidopteran species and the Colorado potato beetle represent a single gene trait. Deterrence could be a heritable trait at the level of midgut physiology through an insensitivity target receptor site.

In both Lepidoptera (e.g. *Heliothis*, *Plutella*) and the Colorado potato beetle, susceptible strains provided with both Bt and non-Bt food (plants or artificial diet) show a choice of normal plants or the artificial diet without the Bt. This gives the appearance that Bt has a behavioral mode of action. In the case of Lepidoptera, strains with physiological resistance to the Bt do not have avoidance behavior. This clearly shows that the behavior is a manifestation of a physiological lesion. When CRW avoid Bt plants, it is likely the same basis; the larvae feed on Bt plants, experience damage to their midguts, stop feeding, and move around until they find another source of food.

One Panel member noted that 59122 maize has suffered little injury from corn rootworm larval feeding, which appears to indicate that the hybrids are being protected from larval attack. The Panel member considered this a genetically-based form of resistance, tolerance. It is possible that the gut membrane is recovering after the Bt exposure, but mortality is low even when there is no Bt recovery period. Consequently the Panel member favored the tolerance premise.

Routinely rotating corn with another crop annually over relatively large areas has selected for a behavioral change in the western corn rootworm, i.e., the laying of eggs in the non-corn crops. The variant that lays eggs in soybeans as well as corn has developed resistance to the cultural practice of crop rotation. Intense selection pressure will be exerted on CRW by growing 59122 maize over large areas and could result in a similar behavioral 'adjustment' so that 59122 maize is no longer 'resistant' to CRW.

While the seed blend refuge concept has merit, the Panel had concerns regarding the reduction of refuge. The reduction in refuge for this product may have broader implications than just acres planted in 59122 maize. A seed blend at the reduced rate could become an industry standard. In light of the low to moderate dose characteristic of this product, and other corn rootworm Bt products, together with the demonstrated ability to feed and survive on 59122 maize, an extensive landscape planted to reduced refuge (block or seed blend) would be ill advised based on current scientific data. While the Panel understands growers' desire to simplify planting, should 59122 maize fail on a broad scale, many growers will be forced to return to soil insecticides or other control measures on 100% of the corn crop. The Panel understands growers' economic interest in increased yield, but has not seen any economic/yield data presented with regard to a seed blend versus separate refuge. With the prophylactic use of Bt corn rootworm and Bt stacked products, due to the perceived (and in some cases documented) increased yield from Bt products compared to isolines protected by soil insecticides, additional selection pressures are being placed on CRW.

**Charge Question 2** – Lefko et al. (2008) collected WCR individuals from two geographically distinct and susceptible populations and introgressed a non-diapausing trait from a WCR lab strain. The resulting offspring were selected on 59122 maize for 11 generations. A number of measurements were taken during the selection experiment to establish the mean survival rate, fecundity, fertility, and percentage of females produced for all cohorts of the 11 generations. Multiple greenhouse experiments were conducted with 4 generations per selected lines to determine if injury potential changed on 59122 roots. In addition, the realized heritability ( $h^2 = R/S$ ) of the tolerance trait was estimated. Pioneer speculates that CRW tolerance for 59122 and apparent rarity of resistance genes in the CRW population may support Lande's (1983) less popular hypothesis that "pest adaptation via a major resistance gene can be prevented or delayed by a minor resistance gene despite strong selection pressure; rarity of major resistance is an essential condition for this interaction".

The Panel is asked to discuss the concept of major vs. minor gene as hypothesized by Lefko et al. and the implications for selection of resistance to 59122 and implications for a seed blend strategy.

**Panel Response** - There are two aspects to this charge from EPA. The first is about the evidence in the Lefko et al. (2008) paper itself about the presence of a minor gene/major gene system in the CRW. The second issue is about the potential that existence of such a system would slow the evolution of resistance to plant incorporated Bt toxins.

Lefko et al. (2008) describe a very labor-intensive selection experiment which initially aimed at determining if resistance to Bt could evolve in a lab system. The result was that a low level of resistance evolved, but that full resistance, in which the insects were unaffected by the Bt corn, did not. These results were interpreted as indicating that the initial frequency of minor genes for resistance (referred to as "tolerance" by Pioneer) was relatively high, but that genes for full resistance were extremely rare. This led to further speculation that the presence of a single minor gene for resistance would slow down the rate at which a major gene for full resistance would evolve. The Panel examined the details of the Lefko et al. (2008) article and found that there was no rigorous evidence from the reported data that the frequency of major genes for resistance was very low.

There were a number of problems with the design of the experiments reported in the Lefko et al. (2008) paper, but the most important was that there was no way of determining how many wild CRW haplotypes were subjected to selection for Bt resistance. Because wild CRW have obligate diapause, the researchers started their experiment by crossing a large number of wild CRW from two geographic areas with a lab population of CRW that has a non-diapause, genetic trait. This is a reasonable approach. After the two independent crossings of the two geographic strains, the two hybrid populations were reared in the lab for a number of generations before a selection regime was established in which the populations were selected on Bt corn roots. Several questions are raised. Were the laboratory beetles ever exposed to 59122 maize? If not and they had been selected for laboratory survival, did their genetics affect the genotype of

the offspring, i.e., were feral genetics lost during the subsequent 11 generations that the progeny were lab-selected on 59122 maize? Because the beetles were held in the laboratory and not screened to determine their genotype, it is not possible to attest to their genetics. Consequently, little can be concluded about the inheritance of the tolerance trait, assuming that it exists.

The Panel was concerned that in the time between the first hybridization and the beginning of the Bt selection regime, there could have been very strong selection for the laboratory-adapted phenotype. This strong selection is expected based on many studies of the fitness of lab versus wild-type insects in a laboratory rearing system. Selection for the lab phenotype could have continued even after selection was begun. Because the non-diapause trait (and presumably other lab-selected traits) appear to be polygenic, the genes involved are likely to have been spread across the genome, thus all wildtype genes at loci linked to loci where genes for lab adaptation reside could have been lost in a selective sweep. If this occurred, it is very possible that the selection for Bt resistance was mostly selection for genetic variation in resistance traits found in the laboratory colony. While the Panel did not state that the resistance found by Lefko et al. (2008) came from the lab colony, it found no evidence to the contrary. More importantly, the Panel believed that even if the resistance traits found in Lefko et al. (2008) came from the field insects, there was no evidence that there was not a severe bottleneck in the number of field haplotypes in the hybrid population when the selection experiment began.

The Panel concluded that the Lefko et al. (2008) study cannot be used to establish the initial frequency of major resistance alleles. They also concluded that the Lefko et al. (2008) study indicates the presence of partial resistance (or “tolerance”). However, even with this finding, they were concerned that the researchers should have conducted reciprocal crosses to conclusively prove the genetic component of the partial resistance. They suggested that it would be possible for the applicants to conduct a single nucleotide polymorphism (SNP) analysis of the beetles that were saved from the initial wild populations and the selection populations to determine how many wild haplotypes were actually sampled in the selection experiment.

The Panel also noted that in the Lefko et al. (2008) paper, the authors computed realized heritability and degree of resistance in each generation, but did this without presenting data on the fitness of the control population on Bt corn in each generation. Given the general variation in time seen in other resistance bioassays, coupled with the fact that the Bt corn varieties used for selection were changed during the experiment, the lack of parallel control strain data in each generation is problematic. While recognizing that these selection experiments are very labor intensive, they suggested that future experiments should give importance to attention to the control strain fitness.

Regarding the more theoretical issue of Lande’s 1983 claim that the presence of minor genes for resistance could slow down the rate at which major genes for resistance are selected, the Panel concluded that this is a possible outcome, but not always expected. This will be described in more detail later in the report, but in brief, minor genes could either slow down or speed up the rate at which a major gene for resistance increases in frequency. Two examples follow: 1) the minor gene starts at a higher frequency than the major gene and therefore becomes fixed or nearly fixed very quickly. This could lower

the selection pressure on the major gene significantly so that the rate of increase in frequency of the major gene is slowed down. 2) The minor gene becomes fixed quickly and the interaction between the minor gene and the major gene makes the inheritance of the major gene more dominant. This increase in dominance results in the major gene being selected more quickly from a low frequency because there is more difference in the survival of heterozygotes and homozygotes for the susceptible allele at the major resistance gene locus.

Examination of Pioneer's model (Appendix A: Modeling) shows that the minor gene fixes very rapidly, and therefore the effect of the minor gene on the evolution of the major resistance gene is the simple result of changes in selection on and dominance of the major resistance gene in the presence of a fixed minor gene. Pioneer provided no strong justification for the strength of selection on a major resistance gene in the presence of a purported minor resistance gene. Therefore, it is impossible to assess the impact of the purported minor resistance gene on the rate of evolution of a major resistance gene.

One Panel member noted that what Lefko et al. (2008) observed may not be genetic, pointing out work done by Rahman et al. (2004) that has demonstrated induction and maternal transmission of *Bacillus thuringiensis* tolerance in the flour moth *Ephesia kuehniella*. As such, the key test is to use reciprocal crosses between resistant (R) and susceptible (S) strains, where crosses of males from the S strain with females from the R strain give resistant offspring because mothers pass along their maternal cytoplasm, whereas the reciprocal cross using S females gives only susceptible cytoplasm and susceptible offspring.

**Charge Question 3** – In BPPD's review it was concluded that varying the selection pressure could have identified different resistance genes. In corn fields across the country the selection pressure will likely be different and more variable than in the lab because of, for example, variability in host-pest interactions and environmental conditions.

Please comment on BPPD's conclusion that different selection intensities could possibly select different genes, maybe a major resistance gene. If different selection intensities could possibly select different genes, maybe a major resistance gene, what does the Panel think are the implications for resistance evolution to 59122 maize in a seed blend environment?

**Panel Response** - Both the Western Corn Rootworm (WCR) and the Northern Corn Rootworm (NCR) seem to be highly genetically diverse and have adapted to both chemical and non-chemical controls. The WCR genome in molecular genetics studies appears to be highly complex and one public commenter at the meeting stated that the NCR are even less sensitive to Bt transgenic corn than WCR.

Corn rootworm, both northern and western species, independently evolved resistance to crop rotation with separate mechanisms (extended diapause (NCR) and shift in oviposition choice (WCR)), as well as resistance to chemical insecticides. Current information for WCR suggests a very complex genome with 25,000 estimated genes, few microsatellites, and numerous genes with unknown functions. The WCR "variant"

(oviposition choice shift) is highly adaptable, and behavioral studies described this population as more “aggressive” than forms encountered previously. Maize is a crop grown across a large landscape and the proposed seed blend refuge will have broad exposure to NCR and WCR in both continuous corn and intense rotation patterns. This highly adaptable ability and potential selection to broad, continuous exposure must be considered when evaluating the seed blend refuge and reduction in refuge size.

Given these factors, different selection intensities in the field, whether in seed blends or pure stands (with structured refuges), could surely select different genes than might be found in laboratory trials. Such genes could include “major” resistance genes (ones that confer high magnitudes of resistance typically 10-fold or more at an LC50), although even relatively minor genes could be important in this case since some insects believed not to carry resistance genes still survive. Because at least 0.4% of susceptible WCR larvae survive on the 59122 maize (and even higher percentages in NCR as pointed out in public comments during the meeting), a gene or genes conferring low resistance (say 2 to 3-fold at the LC50) could be selected and contribute to poor control in the field.

There is a long history of comparing selection in laboratory and field. The general conclusion is that laboratory selection tends to produce resistance with complex or at least undetermined genetics, whereas analysis of resistance that evolves in the field almost always finds major genes. The difference is likely because 1) laboratory populations inevitably derive from relatively small populations and are, therefore, unlikely to include rare genes and 2) laboratory selection allows the close control of dose that more effectively screens for genes of individually small effect (Roush and McKenzie, 1987).

The large populations of rootworm (750,000 CRW per acre) would increase the chances that very rare resistance genes could be found in the populations. Resistance appears to evolve more easily in populations with high densities, such as whiteflies, due to the great capacity to carry rare resistant genes.

In contrast to our traditional considerations for selection, density-dependent selection might operate in this system. The larvae that win the competition for food in the face of Bt may be those that have a slight fitness advantage from resistance genes. Such selection can be very strong even when a similar number of insects successfully develop from a seed mix as in a pure stand of non-Bt corn. Examples of such selection have been documented for the sheep blowfly (McKenzie and Whitten, 1982 and 1984; McKenzie, 1996) and the potential for this type of density-dependent advantage has been noted in several studies involving corn earworm larvae (Stinner et al., 1977); Dial and Adler, 1990). High densities of CRW may drive resistance if a higher level of fitness is evident in heterozygotes or resistant homozygotes.

**Charge Question 4** – In their selection experiment (Lefko et al. 2008), Pioneer found that heritability of the tolerance trait first increased and then decreased again. The tolerance trait could not get fixed by generation 11; Lefko et al. hypothesized that this could be due to an interaction between two tolerance genes. Pioneer did not address the potential presence of more than one tolerance gene in the modeling submission. Furthermore, CRW survival rate due to the tolerance trait and off-type seed rate

(0.00075) was assumed to be half of the survival rate observed by Nowatzki et al. (2008, MRID 473567-05).

Please comment on whether there is reason to believe that the relative rate of adaptation could be affected by including more than one minor gene in the model and whether the assumption of equal survival of susceptible (AA) individuals with genotype XX (0.0125) and genotypes XY and YY (0.0125) is realistic.

**Panel Response** - While it is possible that the “tolerance” described in Lefko et al. (2008) could be due to an interaction between two tolerance genes, there is no evidence in the paper for the number of genes involved. It is possible that the relative rate of adaptation could be affected by including more than one minor gene in the model. The survival parameters used by Pioneer are not necessarily unrealistic, but especially because the Panel cannot be very confident about the estimates produced by Lefko et al. (2008), a much wider range of parameters and assumptions about the resistance/tolerance genes should have been tested in a sensitivity analysis. Such parameters and assumptions should have included that survival was not just additive, but that the two genes could act synergistically; i.e., multiplicatively. (Raymond et al., 1989). When viewed as a dose-response, rather than as survival, pesticide resistance genes tend to act in a multiplicative way.

**Charge Question 5** – After having completed the review of the modeling submission, BPPD has identified several uncertainties and/or weaknesses of the model. Please comment on which of these uncertainties and/or modeling weaknesses are important to explore the risk of resistance evolution to 59122 maize in a seed blend environment.

A. Initial frequency of major resistance gene (0.005) and dominance (0.05): Pioneer determined initial gene frequency based on the selection results and modeling comparison conducted in Lefko et al. (2008). Major resistance was assumed to be almost completely recessive despite the fact that Cry34/35 does not express a high-dose against CRW.

B. Lack of inclusion of emergence delays: delays of up to 13 days in initial emergence and eight days in median emergence were observed between adults from 5% seed blends and 59122 maize. Delays of up to 19 days in initial emergence and 12 days in median emergence were observed between adults from 100% refuge fields and 59122 maize (MRID 473567-05).

C. Lack of inclusion of uneven sex ratios: the field study (MRID 473567-05) showed that the production of female adult CRW was favored over the production of male adults, while in the lab study, the production of males was favored (MRID 473567-01). An inequality in sex ratios results in a special sort of bottleneck in which random genetic drift is enhanced and the effective population size is reduced. For example, if the number of males emerging from Bt plants decreases, then females emerging early from refuge plants could be more likely to mate with refuge males rather than 59122 maize males, especially when one considers that females have been shown to mate once only.

D. Lack of inclusion of density-dependent dispersal: Hibbard et al. (2004) reported that in their experiment conducted from 2000-2002, CRW larval movement was dependent upon egg density per plant (independent of Bt plants).

E. Lack of inclusion of non-random mating: delayed emergence and uneven sex ratios are likely to affect the random mating between adult CRW emerging from refuge maize and adults emerging from 59122 maize.

F. Lack of exploratory modeling: Pioneer's model did not explore the production of different percentages of males and females and non-random mating and, ultimately, their effects on the relative rate of adaptation to 59122 maize.

### **Panel Response**

#### *A. Uncertainty in estimates of initial frequency and degree of allelic dominance of potential resistance alleles of major effect.*

The Applicant, Pioneer, calculated an upper bound constraint on the frequency of a resistance allele of major effect ( $q^*$ ) on the questionably designed study by Lefko et al. (2008):  $q^* = 0.00037$ . The Applicant suggested that  $q^* = 0.0005$  can be taken as a generous, upper bound estimate. However, under the most generous of assumptions concerning suitability of the data generated by Lefko et al. (2008) and with proper application of the formulae for estimation of  $q^*$  for the F2 screen (Andow and Alstad 1998, Schneider 1999), one can show  $q^* = 0.0015$ . A public commenter presented this same value in public comments to the Panel. However, given the limitations of Lefko et al. (2008), even  $q^* = 0.0015$  cannot be considered a generous, upper bound estimate. Without additional information, it is not possible to set a reasonable, upper bound estimate for  $q^*$ .

The Applicant assumed a value of 0.05 for the dominance parameter  $h$  in their model. A compilation of genotypic fitness for 17 *Bt* resistance genes (Caprio et al. 2000) suggests that  $h = 0.05$  may be a reasonable upper bound estimate for genes that confer  $\geq 300$ -fold resistance, i.e.,  $(\text{fitness RR genotype})/(\text{fitness SS genotype}) \geq 300$  in the presence of the toxin. However, the currently available evidence for the CRW/corn production system suggests that resistance may be based on genes of relatively minor effect ( $< 50$ -fold resistance). For genes of this level of effect, the compilation by Caprio et al. (2000) suggests that no upper bound estimate for  $h$  of less than 1.0, corresponding to complete dominance, can be assumed.

#### *B. Lack of inclusion of emergence delays.*

An analysis of average date of emergence (DOE) of WCR by blend, gender, and field location (see Appendix B - Table B1) gives results similar to the Applicant's analysis of median DOE for blend and location: (1) DOE from the 5, 10, and 20% HX1 plots was delayed by ca. 3-4 d relative to DOE from 100% HX1 plots and (2) the observed delay varied within a range of 2-6 d among the four locations studied. The similarity of results for average DOE and median DOE is consistent with the slight

positive skew generally apparent in the plots of rate of emergence over time (see Appendix B - Figures B1-B8).

The Applicant pooled males and females in their analysis of DOE. Although this is statistically justifiable because of the absence of an interaction between gender and blend proportions, it tends to obscure the issue of whether a delay in DOE of males from blend refuge fields reduces their effectiveness compared to males produced in 100% HX1 refuge fields. Males emerged 5-8 d earlier on average than females with no obvious pattern among treatments (Table B1). If one assumes that DOE of resistant females from blend fields is similar to DOE of susceptible females from 100% HX1 plots, then, because the magnitude of the delay in DOE of both males and females from blend plots was 3-4 d (Table B1), average DOE of susceptible males from blend fields is expected to precede DOE of resistant females by around 3 d and be about 90% complete by the median DOE of resistant females (Figure B9). DOE of susceptible males from 100% HX1 refuge plots is expected to be about 100% completed at that time. Thus, approximately a 10% penalty due to emergence delay is incurred in switching from a 100% HX1 refuge to a seed blend refuge. Results using a version of Pioneer's model suggest that this difference is unlikely to have a significant effect on resistance evolution (see Appendix A).

In the view of the Panel, refuge effectiveness is more likely to be reduced by the dramatic reductions in numbers of males produced in blend plots. Production of males from 5% blend plots averaged less than 5% of production from HX1 plots (see Section D below).

#### *C. Lack of inclusion of uneven sex ratios.*

The Applicant analyzed the sex ratio ( $SR$ , M:F) of adult WCR adults emerging from field plots and reported the results in tabular form. Here, the fraction of adults that are male ( $FAM$ ) is analyzed and the results presented graphically (Figure B10). Although analysis of  $FAM$  results in somewhat higher coefficients of variation of the estimate than does  $SR$ , more of the data at low emergence rates can be included ( $M+F \geq F$  so  $M+F > 0$  is more frequently satisfied than is  $F > 0$ ).

Two patterns are immediately apparent in Figure B10: (1) male fraction varies among locations and (2) more males tend to be produced at higher levels of fraction HX1 ( $FSB$ ). BPPD's concern is rightfully directed toward the obverse of the latter effect; however, the magnitude of the observed effect does not appear sufficient to appreciably affect resistance management. For example, regression analysis of the Janesville, WI, data, results in statistically significant estimates for both model parameters (Figure B10); but the fraction of adults that are male is reduced by only about 20%:  $FAM = 0.49$  at  $FSB = 1$  and  $0.39$  at  $FSB = 0$ .

#### *D. Lack of inclusion of density-dependent larval dispersal.*

There is very little data to bring to bear on this issue. Hibbard et al. (2004) report that significantly greater numbers of WCR larvae were recovered on some occasions from corn plants near plants artificially infested with greater numbers of WCR, but they

do not determine whether the fraction of larvae recovered was significantly greater at higher infestation levels. It is the probability of movement or the probability distribution of distance moved that is of interest in determining whether movement is density dependent. In addition, as pointed out by Onstad (2006), the ratio of larvae recovered from nearby plants to the number recovered from the infested plant in Hibbard et al. (2004) actually declined with increase in infestation level.

Even if positively density-dependent dispersal of WCR larvae does occur, it may have little effect on the rate of resistance development. If one assumes that dispersal of larvae encountering HXX (59122 CRW-resistant corn) roots in an Optimum AcreMax1 blend results in an increase in the numbers of larvae around HX1 plants [a supposition only inconsistently supported by the available data (Hibbard et al. 2005, Onstad 2006)], then density-dependent dispersal of larvae should result in the establishment of a local equilibrium density around HX1 plants. It is turnover within this population of larvae that could facilitate the development of resistance (Mallet and Porter 1992). However, it would appear unlikely that larvae initially present or early arriving larvae, which have progressed in growth and development, would be displaced by later arriving larvae, which have not. Thus, it is unclear that resistant heterozygotes would enjoy an increased advantage over susceptible homozygotes in a seed blend compared to 100% HXX.

Closely related to density-dependent dispersal is the effect of seed blend proportions on probability of larval dispersal, an issue that was raised but not fully explored during the Panel's discussion. One Panel member developed a model relating the probability of dispersal to seed blend proportions and the consequences of this dispersal on expected emergence of adults in the face of dispersal-dependent mortality (see Appendix C). Emergence data for the WCR presented by the Applicant (Nowatzki et al. 2009) is fitted to the model and is consistent with the following conclusions: (1) probability of larval dispersal is very high and nearly independent of seed blend proportion over the range of 0-20% HX1 and (2) the probability of dispersal-related mortality varied considerably among study locations, with generally lower but variable levels at the York, NE, and Mankato, MN, study locations and higher levels at the Johnston, IA, and Janesville, WI, locations.

An overview of the model follows. Assume that a randomly selected neonate WCR larva is nearest an HX1 plant with probability  $p$  in a field planted to OAM1 corn with an HX1 blend proportion of  $p$ . With probabilities  $p^2$ ,  $2pq$ , and  $q^2$  ( $q \equiv 1-p$ ) there will be 0, 1, or 2 HXX plants, respectively, adjacent to the focus plant. Assume that the larva has corresponding probabilities of emigrating of 0,  $\frac{1}{2}$ , or 1. [One might envision the roots of adjacent plants partially interpenetrating the root zone of the focus plant; and an encounter with an HXX root causing the larva to disperse even though HX1 roots constitute the majority of local roots.] In the face of probability of dispersal-related mortality,  $d$ , a proportion  $(1-d)$  survive to encounter an HX1 plant with probability  $p$  and an HXX plant with probability  $(1-p)$ . Similarly, a neonate WCR larva initially associated with an HXX plant is assumed to disperse with probability 1 and to suffer a dispersal-related probability of mortality  $d$ . The cycle of dispersal and associated mortality could be repeated, but a single cycle appears to be sufficient for current purposes.

Under this model (see Appendix C for additional details and implementation of the model in MathCad), the probability of dispersal,  $\text{Pr}[\text{disp}]$ , is equal to  $1-p^2$ , so  $\text{Pr}[\text{disp}] = 1$  at  $p = 0$ , 0 at  $p = 1$ , and varies little with  $p$  for  $p < \approx 0.20$  (Figure B11). Consequently, seed blends may dramatically increase the probability of larval dispersal; so, to the extent that larval dispersal promotes resistance (see, however, the discussion of density-dependent dispersal above), seed blends may hasten development of resistance.

Lacking any direct measures of dispersal and mortality, their indirect consequences may be deduced from a model and compared with observation. As presented in Appendix C, one can define a standardized measure of emergence as the ratio of the difference between observed emergence and emergence from 100% HXX ( $E_{(0:100)}$ ) to the difference in emergence from 100% HX1 ( $E_{(100:0)}$ ) and 100% HXX. Expressions for expected standardized emergence (ESE) are derived for both the null hypothesis of no movement and for the alternative hypothesis of movement and mortality as assumed in the above-described model. Under  $H_0$ , ESE is a function of  $p$  only while under  $H_A$  it is a function of  $d$ , ( $E_{(0:100)}/E_{(100:0)}$ ), and  $p$  (Equations 3 and 7, respectively, Appendix C). The effects of all three of these parameters on predicted WCR emergence are considered.

ESE is very insensitive to ( $E_{(0:100)}/E_{(100:0)}$ ) for ( $E_{(0:100)}/E_{(100:0)}) < 1/10$ . Observed values are  $< 1/25$  (Table B2), so the effects of ( $E_{(0:100)}/E_{(100:0)}$ ) will not be considered further.

ESE is plotted as a function of  $p$  for various values of probability of dispersal-associated mortality,  $d$ , in Figure B12. For low values of  $d$ , dispersal from HXX to HX1 plants results in higher ESE than under the assumption of no dispersal. At higher values of  $d$ , dispersal results in a net decline in ESE relative to the assumption of no dispersal.

ESE is compared with observed standardized emergence (OSE) calculated from the data reported by Nowatzki et al. (2009) at their Johnston, IA, and Janesville, WI, study sites (Figure B13) and their York, NE, and Mankato, MN sites (Figure B14). Data from males and females have been pooled because separate analyses by gender showed that this factor had little effect on the observed relationships. OSE fit ESE well under  $H_A$  at Johnston, IA, and Janesville, WI, for  $d = 0.70$  and  $0.45$ , respectively. SEE's were higher at York and Mankato than at Johnston and Janesville, and fit of OSE to ESE was not as good: four of the six values of OSE fall close to the ESE curve for  $d = 0.1$ ; but at both sites, OSE was much lower at one blend level consistent with  $d \approx 0.7-0.9$ . Consequently, high, unexplained variation in probability of dispersal-associated mortality introduces an additional source of uncertainty in estimating the effectiveness of resistance management strategies.

*E. Lack of inclusion of non-random mating.*

Please see Section B above.

*F. Lack of exploratory modeling.*

Please see Sections B-D above.

**Charge Question 6** – The delay in evolution of resistance in Pioneer’s model when the tolerance gene, major resistance gene, and off-type seed rate were included was driven by the rapid increase in frequency of the susceptible genotypes carrying the Y-allele (mostly AAYY). The proportion of susceptible individuals heterozygous for the tolerance trait (AAXY) peaked at 0.62 after generation 2; the proportion of susceptible individuals homozygous for the tolerance trait (AAYY) reached 0.5 by generation 3. The population was 90% homozygous for the Y-allele after generation 7 with an interaction emerging between the Y and B allele around generation 9 when AAYY genotypes declined at a similar rate to its earlier rate of increase. The proportion of population homozygous for both the major resistance gene and the tolerance (BBYY) gene reached 0.5 and 0.9 around generation 17 and 19, respectively.

Please comment on whether it is realistic to assume that the minor tolerance trait, specifically the AAYY genotype, might drive the delay of major resistance to 59122 maize in a seed blend environment.

**Panel Response** - The uncertainties of the dominance value and allele frequency for the major gene in the Pioneer model do not allow a realistic assessment of whether the “minor” gene described by Lefko et al. (2008) will slow the evolution of a major resistance gene. To best address the potential for minor genes to slow the evolution of a major gene, information on how the genes interact with one another is necessary. In the Pioneer model, a simple and non-standard assumption was made that a resistance and a tolerance gene interact in an “either-or” fashion, yet there is no empirical or logical justification for this assumption. The moderate dose that rootworm populations encounter in the field is likely to yield a greater range of possibilities than the single-value genetic parameters used in the Pioneer model. The Panel agreed that more exploratory modeling varying the dominance and allele frequencies for minor and major genes is warranted. The Panel also agreed that polygenic models using “tolerance” genes may be a more realistic scenario to assess the risk of resistance in seed blends. Even while these models would be useful, absence of empirical evidence makes it difficult to justify any specific assumption about the underlying genetic determinants of resistance.

The tolerance trait was not clearly defined and Lefko et al. (2008) states “Only homozygous resistant individuals are assumed to be able to develop on 59122 maize.” The populations used in Lefko et al. (2008) survived on 59122 maize, yet the study described the ability to survive as a tolerance trait rather than classify the populations as resistant. With the genetic data currently available, it is not possible to ascertain if a minor gene (tolerance) exists. Consequently, it is not feasible to determine the realistic ability for a hypothetical trait impact on delaying resistance.

**Charge Question 7** - BPPD has reviewed Pioneer’s request to register a Bt maize seed blend ( $\geq 2\%$ ) for corn rootworm, all the supporting studies, and the model with its general assumptions, parameters and their values, as well as modeling analyses and concludes the following: At this point, BPPD believes that the proposed seed blend of  $\geq 2\%$  for Optimum® AcreMax™ 1 Insect Protection has many uncertainties with respect to biological and genetic parameters and lack of exploratory modeling. BPPD believes these uncertainties must be resolved before a seed blend refuge is a viable long-term

alternative to a structured refuge for corn rootworm. In the interim, BPPD has concluded that no less than 10% refuge seed blend is appropriate. BPPD bases its conclusion on: 1) the Relative Rate of Adaptation (RRA) at the 10% refuge proportion (value ~ 0.7) compared to the higher RRA values at lower proportions of refuge as estimated in Pioneer's model (MRID 473567-08), 2) root protection results for the 10% seed blend (Nowatzki and Meinke, 2008, MRID 473567-06), 3) efficacy results (Davis and MacIntosh, 2008, MRID 473567-10), 4) CRW emergence curve similarities for the 10% seed blend and 100% refuge maize (Nowatzki et al., 2008, MRID 473567-05), 5) similarity in fitness results between females emerging from the 10% seed blend and the 100% refuge maize, and 6) modeling uncertainties.

Please comment on whether the scientific evidence submitted by Pioneer and available in public literature supports a seed blend strategy for CRW protected maize given the uncertainties and what is currently known about CRW biology, ecology, and genetics.

**Panel Response** - In general, the Panel agreed with BPPD that data presented by Pioneer and data found in the public literature provide no compelling evidence for the reduction in the proportion of non-Bt plants (either as a seed blend or spatial refuge) that Pioneer requested; in fact, the Panel recommended that the proportion of non-Bt plants remain at 20% rather than be reduced to 10% as proposed by BPPD. The recommendation from a previous FIFRA Scientific Advisory Panel was for a 50% refuge. In the documentation to support Pioneer's request, there was very little, if any, commercial-size field data presented concerning the yield losses that might occur over a range of infestation and species mixes. Pioneer was not able to provide data on yields to justify claims that 10% (or higher) susceptible seed mixes actually caused yield losses that could be detected on farms, thereby demonstrating that a 5% susceptible seed mix was the most that could be allowed. To the contrary, the data summarized by one Panel member on adult emergence suggested that the effects on yield are low. This weakens the practical arguments in favor of the seed mix "refuge in a bag". There may well be a two-fold increase in loss and lodging in moving from a 5% to 10% or 20% refuge, but it was noted by another Panel member that from data available, that loss might be 1, 2, and 4% and there was concern about the potential for density-dependent selection that wasn't explicitly modeled. Therefore, the Panel supported maintaining the refuge size at the current 20% while the applied data are obtained. Additional data could be obtained using seed mixes (with protection applied to the non-Bt seed) for limited public testing of various mixtures of 5 to 20% refuge and planted at multiple locations (environments) to determine the cost in potential yield reduction under actual field conditions of the blends. The Panel suggested these data also include root injury to the Bt plants in a blended environment, and attempts should be made to determine where mating is occurring.

Panel members' opinions about the utility of seed blend refuges versus spatial refuges varied. One Panel member stated that dispersal and mating characteristics of *Diabrotica virgifera virgifera* (WCR) and lepidopterans, such as the European corn borer (ECB), appear to be quite different. The WCR males will emerge earlier than the females and then move close to the soil, waiting for potential mates. The females are usually mated soon after emergence and therefore, are likely mated in the field where they have emerged. Other than in irrigated corn, ECB will often leave the corn field and call for

mates from weedy habitats. This dispersal to breeding sites encourages mating of adults from several fields. WCR mating within the field from which they emerged will have less chance of mating with individuals from other corn fields. Because of this, rootworm scientists differed with the ECB scientists and the spatial refuge for rootworms was recommended and required to be closer than the refuge for the ECB Bt field. This Panel member believed that a blended refuge within the PIP would accomplish random mating even better than the current block refuge within the same field.

The Panel member then questioned how to protect the non-Bt plants blended within the Bt field. Unlike ECB, there is a seed treatment that can be applied to control CRW. Root protection from use of neonicotinoids has not been great, but the yields have not suffered much unless there is drought stress. As far as chemically treated carry-over seed, it was assumed the Bt seed would be treated with a low rate of the neonicotinoid to improve the spectrum of pest control. Disposal would therefore be the same; the blend would contain neonicotinoid treatments on all the seed in the bag. The difficulty seemed to be that Pioneer is reluctant or unwilling to adjust the assembly process to apply different rates of the seed treatment prior to blending the seed. The Panel member believed that this was a poor reason to ignore the non-Bt plants in a field likely to be infested with rootworms.

This Panel member believed that a second advantage of the seed blend was that the phenology of adult emergence seemed to be closer to the Bt planting than that from the spatially separate refuge. The better synchrony would cause there to be a greater likelihood that those that survived the Bt and non-Bt plants would intermix and mate.

Another Panel member believed there are known ways in which a seed blend could speed resistance evolution, especially involving the reduction in recessiveness caused by larvae moving among plants. However, simulations this Panel member ran (see Appendix A) show that this effect is not large, at least for the Pioneer benchmark parameter values.

While one Panel member believed that the low movement of male WCR among fields is a reason to prefer seed blends, because this ensures greater mixing of the adult population, another Panel member pointed out that models, in fact, show the opposite, that reduced movement of males from natal fields slows resistance evolution (see Appendix A). Thus, model results would argue for maintaining spatial refuges even when male dispersal is low. Nonetheless, the Panel member acknowledged the benefits of spatial refuges are limited and could be outweighed by the benefit of seed blends for easing the burden of resistance management to farmers, under the assumption that Pioneer provides protection (e.g., neonicotinoid treatments) to non-Bt seeds used within a blend.

Overall, the Panel members agreed that corn rootworm biology seems to lend itself to the seed blend concept. However, they also believed that it is vital to preserve Bt CRW technology, and are concerned about the move to both a seed blend refuge and a drastic concurrent reduction in actual refuge size. There are limited genetic data available on CRW, which is a highly adaptable organism with geographic populations that vary in their development of resistance to previous and existing control measures.

Areas with the variant WCR (oviposition preference shift) often have populations well above the economic threshold and exert intense pressure on management technology. The consequences to corn producers of losing resistance to CRW could be severe; therefore the Panel believed that the durability of components of a resistance management plan should be carefully considered when making resistance management decisions..

Significant gaps exist in the information available to confidently predict the durability of Pioneer's Optimum AcreMax 1 corn relative to other refuge options. However, based on the best available information, modeling indicates that durability of 59122 CRW-resistant (HXX) corn may be similar whether 1507 CRW-susceptible refuge (HX1) corn is planted at a given percentage in a blend refuge or the same percentage of HX1 corn is planted in a strip or block spatial refuge. Consequently, (1) the same 20% spatial refuge for Pioneer's Optimum AcreMax1 corn should be required as is required for HXX corn unless it is marketed with no less than 20% HX1 seed in the blend; and (2) Pioneer — or any other applicant for a PIP registration of CRW-resistant corn — should be required to follow the recommendations in the 2002 SAP report for basic studies that would generate the information necessary to rationally evaluate such applications. In general, the Panel concluded that there are uncertainties with the use of a seed blend and clear problems with reducing the refuge size. The Panel recommended additional research that would more completely examine the performance of the seed mix strategy.

#### Additional Comments by Panel Members

Our challenge is always one of trying to reach the optimal balance of short-term gains for growers and society, while delaying resistance until even better or at least newer traits or tools become available. The problem in this case is that rootworms are not highly sensitive to this Bt corn, which tests any resistance management strategy, and is of particular importance in these pests with very high population numbers and an almost unique history of evolving resistance to both chemical and non-chemical controls, in this case, crop rotation. This makes both the scientific questions and implementation of any resistance management strategy very challenging. Furthermore, it means that strategies developed for the management of resistance in CRW will be very different from those developed for ECB and pests of other crops for which Bt causes high mortality; CRW with its low- to medium-dose response to Bt toxins is not a representative pest. Therefore, recommendations from the Panel about the use of seed blends should in no way be used to inform decisions regarding the use of seed blends for pests other than CRW.

A key consideration in Pioneer's presentation was implementation. One Panel member was concerned with Pioneer's view that corn can move to seed mix with all Bt traits, including for corn borers. This Panel member suspected this was not a robust conclusion, but withheld judgment until more of the arguments and data were available. In response to some of the public comments, this Panel member also noted that EPA and the Panel were acutely aware of, and sympathetic to, the costs and complexities of refuges. For example, EPA recently reduced the refuge requirements for cotton carrying two genes compared to a single gene after more than 15 years of effort were devoted to developing and promoting a two-Bt gene toxin strategy for managing resistance, with the primary aim being to reduce refuge requirements. The Panel member felt that the 20% refuge requirement common in corn was based on input from independent scientists and

the best scientific evidence at the time. Finally, in response to two public comments, several Panel members believed that access to transgenic plants by qualified researchers must be more open and transparent to maximally foster resistance management research. It does not reflect well on the biotech industry, nor does it help researchers achieve further advances in resistance management, to restrict research by public sector researchers to plant varieties that can be purchased on the open market.

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## Appendix A: Modeling

This appendix presents results from a model derived independently but following the general structure and assumptions of the Pioneer model (Study ID PHI-2007-144).

The Pioneer request involves two separate components: using seed blends rather than spatial refuges, and reducing the proportion of non-Bt seeds from 20% to 3.5%. The consequences of these two changes differ; therefore, they should be considered separately. The model is first used to compare a seed blend strategy with a spatial refuge strategy. It is then used to address the consequences of reducing the proportion of non-Bt corn on the landscape (distributed either in blends or in spatial refuges). Finally, the model is used to address the issue of limited male movement and whether this increases or decreases the rate of resistance evolution when using a spatial refuge.

Two caveats are needed. First, although the model is built around the same assumptions as the Pioneer model, adopting these assumptions is not an endorsement of the assumptions. Instead, the objective of this appendix is to assess the conclusions that Pioneer draws from their own model. Second, the model used in the appendix has not been carefully and quantitatively compared to the Pioneer model to determine how they might differ, either due to slight differences in assumptions or coding errors. Nonetheless, although the quantitative results between models might differ, it is unlikely that large qualitative differences between the conclusions drawn from the models will occur.

### Model

The model is very similar to the Pioneer model, except in the following ways. The Pioneer model allows some larvae to move twice between plants (Study ID PHI-2007-144, Eq. 7); those that start on a non-Bt plant and move to a Bt plant can move again to a non-Bt plant. Other larvae can move only once; those larvae that start on a Bt plant and move to another Bt plant cannot move again. In the SAP version, all larvae can move once. This is a very minor difference, however, that does not seem to affect the results. In order to address the Charge Questions, the SAP model allows non-random mating due to delayed emergence of susceptible insects on Bt corn.

The code was checked against a single-locus model that had been built independently. The output from the 1-locus model was compared to the 2-loci model with the resistance/tolerance frequency at one locus either set to 0 or 1. The model matches the output from the Pioneer model (Study ID PHI-2007-144, Figs. 1, 8, and 9). Both gave time to resistance (when the resistance allele frequency is 0.5) of 15 years for the same benchmark parameters (without off-type seeds).

The model used here was built independently of the model used in the presentation by Dr. Ives at the SAP meeting on 24 February, 2009. It differs slightly in the manner in which it handles susceptible larvae moving among plants in Bt blends, and the fecundity of susceptible adults exposed as larvae to Bt toxins. Also, the cases considered (i.e., the proportion seed blends) and the initial conditions (i.e., when the resistance alleles are introduced into the population) are changed slightly to conform

more closely to those used by Pioneer. Finally, an error was corrected; the former version did not decrease the population size to account for non-reproducing males in the population. Despite these minor changes to the model, the general qualitative results presented in the SAP meeting are unchanged.

## **Seed Blends vs. Spatial Refuge**

*1. Under Pioneer's assumptions, seed blends and spatial refuges are almost equivalent.*

Figures A1 and A2 give the case using Pioneer's benchmark parameters for WCR in the absence of off-type seeds. In Figure A1, there is 3.5% non-Bt seed in Bt fields and essentially no refuge, and in Figure A2 there is essentially no non-Bt seed in Bt fields and 3.5% spatial refuge. The results are almost identical, with resistance (50% resistance allele) reached in 16 and 17 years for the blend vs. spatial refuge cases, respectively.

This result is not surprising, because there are few differences between these two cases. The only differences that will affect resistance evolution are:

(i) Larvae can move between Bt and non-Bt plants in blended fields. This will only make a difference for resistance evolution, however, if susceptible larvae are more or less likely to settle on Bt plants as a result. For the Pioneer benchmark parameter values, the difference in susceptible larval dispersal from non-Bt and Bt plants is only 25%, which is not enough to have a significant effect on resistance evolution; reducing this to 0% causes almost no change in the rate of resistance evolution.

(ii) Males have limited movement from natal fields which produces population-level non-random mating when there are spatial refuges. If the movement of males from their natal fields is increased from 25% to 100%, the results from the two cases (Figures A1 and A2) are almost identical.

Summary: Pioneer's request to use a blend of 3.5% non-Bt corn is almost equivalent to reducing the spatial refuge from the current requirement of 20% to 3.5%. In the model, the only significant (but slight) difference between seed blends and spatial refuges is caused by non-random mating due to limited dispersal of males from natal fields; this slows resistance evolution in the case of spatial refuges relative to seed blends.

*2. The tolerance trait simply reduces the selection for a purported resistance trait.*

The tolerance trait evolves very rapidly, and the time required for the resistance trait to reach a frequency of 0.5 is changed little whether the initial frequency of the tolerance trait is 0.1 (the Pioneer benchmark value) or 1. Specifically, for the benchmark parameter values, the time to resistance failure is increased by 3 years for 3.5% seed blend and 2 years for a 3.5% spatial refuge when the initial frequency of the tolerance trait is 1. Table A1 gives the selection coefficients for the resistance trait in the presence and absence of the tolerance trait. It is no surprise that the resistance trait evolves more slowly in the presence of the tolerance trait, because selection is much weaker.

Table A1: Survivals of homozygous susceptible (sSS), heterozygotes (sRS), and homozygous resistant (sRR) larvae for the resistance trait in the absence (frequency = 0) and presence (frequency = 1) of the tolerance trait.

	Tolerance trait absent	Tolerance trait present
sSS	0.0125	0.2
sRS	0.0619	0.24
sRR	1	1

This begs the question of whether one would expect the resistance trait to be largely recessive ( $h = 0.05$  is the Pioneer benchmark) when there is a tolerance trait. In the presence of the tolerance trait, the resistance trait is effectively a low-dose trait, and therefore it might be more appropriate to assume higher dominance than  $h = 0.05$ ; this will lead to more rapid evolution. Alternatively, it might be more appropriate to treat resistance as a quantitative (polygenic) trait.

An important point is that the consequences of the tolerance trait on the evolution of the resistance trait depends on how the survivals associated with the two traits are combined. The standard assumption for combining survivals for toxins that act independently is that individuals only survive if they can survive both toxins. Thus, if  $s_1$  and  $s_2$  are the survivals from toxins 1 and 2, then the survival from both toxins in combination is  $s_1 * s_2$ . The Pioneer model assumes that survivals are combined in an “either-or” fashion: the survival of individuals is given by  $1 - (1 - s_1) * (1 - s_2)$ . If the tolerance and resistance traits were considered as conferring resistance to two separate toxins (or sources of mortality), then individuals would be resistant to both toxins if they were resistant to either one or the other. This can be seen by considering the case of high sensitivity to toxin 1,  $s_1 = 0$ , and resistance to toxin 2,  $s_2 = 1$ . In this case, the survival from exposure to both toxins is  $1 - (1 - s_1) * (1 - s_2) = 1 - (1 * 0) = 1$ . In other words, if an individual is resistant to toxin 2, then it has survival equaling one regardless of its sensitivity to toxin 1.

Summary: The effect of the tolerance trait on the rate of evolution of the resistance trait does not involve complex gene-gene interactions. Instead, this effect can be understood by simply fixing the tolerance trait at a frequency of 1 and considering the resulting survivals of the resistance genotypes. This ignores what are reasonable values for genotype survivals once a tolerance trait is fixed; Pioneer did not present any data relevant for determining this.

### 3. *The tolerance trait allows high larval emergence.*

Panels B in Figures A1 and A2 give the relative densities of adults emerging per plant scaled such that the density of emerging adults before the introduction of Bt plants is 1. Panels C give the relative densities of larvae after selection has occurred but before density-dependent mortality.

The results in Figures A1 and A2 suggest that the tolerance trait could cause considerable loss of protection provided by Bt crops. The reduction of adult densities

emerging per Bt plant is less than 75%. Therefore, if plant damage is proportional to the number of adults emerging per plant (panel B), then the tolerance trait will allow considerable damage to Bt plants. If instead, damage is determined by the number of neonate larvae per plant (panel C), Bt provides considerable control even after the tolerance trait is fixed in the population.

Summary: The existence of a tolerance trait claimed by Pioneer could by itself cause considerable loss of efficacy of the Bt product.

*4. Seed blends may slightly speed resistance evolution if they increase relative survival of heterozygotes.*

Mallet and Porter (1992) show that, in the case when there are spatial refuges, introducing seed blends into Bt fields can speed the rate of resistance evolution. This is caused by the outcome of two competing forces. First, the seed blend decreases the force of selection against resistance in the Bt fields and therefore acts to slow resistance evolution. Second, if larvae move among plants in the Bt fields, sampling and potentially being killed on each plant they visit, then seed blends have the effect of reducing the recessiveness of the resistance trait; this in turn speeds resistance evolution. For the case of high-dose effects of Bt (low survivorship of susceptibles and high recessiveness), the latter effect may outweigh the former, so that seed blends speed resistance evolution.

The scenario considered by Pioneer is different. In the Mallet and Porter (1992) scenario, the spatial refuge is always present. Pioneer wants to diminish the size of or remove the spatial refuge. This will tend to increase the rate of resistance evolution relative to the scenario considered by Mallet and Porter. In other words, Pioneer has the more difficult case to show that seed blends do not just slow resistance evolution, but that they slow resistance evolution enough to compensate for the loss of a spatial refuge.

Figure A3 considers the case of a 20% seed blend in which larvae are allowed to sample plants and potentially die before moving to another plant. Because the Pioneer benchmark parameter values have essentially no “sampling mortality” ( $spdt = 1$  or  $0.99$ ), mortality due to Bt occurs almost entirely on a single plant. To compare this with the case of sampling mortality (essentially setting  $spdt = 0$ ), larval growth was divided into two stages corresponding to the first and possibly second plant they feed upon. If  $s$  is the survival of a given larval genotype on Bt or non-Bt plants in the Pioneer benchmark parameter set, then the survival of this genotype was set to  $s^{1/2}$  for the larvae of each genotype in the first and second larval stages. Therefore, if a larva remains on the same plant for both its first and second stage, the total larval survival will be  $s^{1/2} * s^{1/2} = s$ , as in the Pioneer model with  $spdt = 1$ . Differences with the Pioneer model will occur, however, when larvae move between Bt and non-Bt plants.

Figure A3 shows that sampling mortality reduces the time to resistance failure from 12 to 11 generations for the case of a 20% seed blend and in the absence of the purported tolerance trait. This is caused by two consequences of sampling mortality. First, the effective dominance of resistance increases from 0.046 to 0.057. Second, sampling mortality leads to slightly stronger selection for resistance, as suggested by the decrease in larval densities with sampling mortality (Figure A3, panels B, C).

Summary: Larval movement among Bt and non-Bt plants has the potential to speed resistance evolution in seed blends. However, this effect appears to be small for the Pioneer benchmark parameters in the absence of the tolerance trait.

*5. Non-random mating caused by delayed emergence has little impact on resistance evolution.*

The SAP model separates adults into those emerging from Bt and non-Bt plants. To implicitly include non-random mating due to a delay in emergence of susceptible insects from Bt plants, a parameter  $p_m$  was introduced that gives the proportion of males emerging from Bt plants that mate with females emerging from non-Bt plants, and vice versa. Thus, if  $p_m = 1$ , there is effectively no delayed emergence and random mating. If  $p_m < 1$ , there is non-random mating due to delayed emergence.

Figures A4 and A5 show the consequences of non-random mating due to delayed emergence for the cases of 20% blended Bt fields and 20% spatial refuges, respectively, assuming that the tolerance trait is absent. Delayed emergence ( $p_m = 0.1$ ) slightly slows resistance evolution in both cases, with the effect larger for the case of 20% blended fields. Note that this result differs from that presented at the SAP meeting on 24 February, 2009, for the case in which the tolerance allele was present; in the reported results, there was a slight increase in the rate of resistance evolution.

Summary: Delayed emergence of susceptible insects that feed on Bt plants can cause non-random mating. However, the effect of this non-random mating on resistance evolution appears to be small for the Pioneer benchmark parameters in the absence of the tolerance allele. Furthermore, this effect is similar between seed blends and spatial refuges.

*6. Spatial refuges may allow more flexible control options.*

If practical considerations confine insecticide treatment to fields or spatially separated areas, then spatial refuges may present control options that are not available for seed blends. To illustrate this potential, Figure A6 shows the case of a 20% spatial refuge that is treated with an insecticide killing 75% of the neonate larvae. Although the time to resistance failure is diminished, this does provide protection of the refuge corn. The cumulative value of the control without insecticide treatment is  $V_{\text{damage}} = 13.6$ , while with insecticide control it is  $V_{\text{damage}} = 14.6$  (see below for a definition of  $V_{\text{damage}}$ ). Therefore, the benefits of insecticide control in reducing insect abundance may outweigh the costs in terms of reduced durability against resistance evolution.

Summary: Spatial refuges might allow options for the non-Bt control of insects. In the simulation, treating spatial refuges with insecticide reduced insect abundance in the refuge with only a moderate cost to resistance evolution. However, if non-Bt seeds in seed blends can be treated effectively with insecticide, a similar approach can be used for seed blends.

## Proportion of Bt plants

The second component of Pioneer's request is to decrease the proportion of non-Bt plants in the landscape from 20% to 3.5%. As described above, this effect of decreasing the percent non-Bt plants as seed blends will be similar to decreasing the spatial refuge by the same amount. Here, we will consider only the case without the tolerance trait, because the SAP had serious concerns about the applicability of lab studies showing the existence of a tolerance trait for the field situation.

Reducing the proportion of non-Bt plants involves a trade-off between protecting more of the crop by using Bt plants and increasing the rate of resistance evolution. Weighing these benefits and costs is complex because it involves translating control of insect densities into reductions in crop damage. There are additional complications for a formal economic analysis, including the rate at which future benefits are discounted and the time horizon for development of new products (thereby reducing the costs of resistance should it arise). We will not consider these complications here.

We use two simple metrics for weighing the benefits and costs of the proportion of non-Bt plants deployed in either seed blends or spatial refuges:

(i)  $V_{\text{field}}$  – the value of the Bt product measured by the cumulative proportion of plants protected (i.e., Bt plants) per year until resistance occurs. This measure assumes that economic loss is proportional to the relative abundance of protected (Bt) crops relative to unprotected (non-Bt) crops. For this metric, the benefit of the Bt product is measured until the frequency of the resistance allele reaches 0.5. This measure equals the number of years to resistance failure times the proportion of crops that are Bt.

(ii)  $V_{\text{damage}}$  – the value of the Bt product measured by the cumulative reduction of insect density (emerging adults per plant per year) until resistance occurs. This measure assumes that economic loss is proportional to the number of adults emerging per plant. For this metric, the benefit of the Bt product is measured until the density of insects reaches 99% of the value occurring before the introduction of Bt plants.

Figures A7 and A8 give the consequences of reducing the percentage of non-Bt plants from 20% to 3.5% for seed blends and spatial refuges, respectively, using the Pioneer benchmark parameter values. Two things should be noted. First, the value of Bt crops is higher for the 20% blend and 20% refuge than for the 3.5% blend and 3.5% refuge, respectively, using either  $V_{\text{field}}$  or  $V_{\text{damage}}$ . Therefore, the benefits of reducing the proportion of non-Bt plants are outweighed by the costs of resistance evolution. Second, the value (either  $V_{\text{field}}$  or  $V_{\text{damage}}$ ) of a 20% spatial refuge is greater than the value of a 20% seed blend.

Summary: Using simple metrics of the cumulative value of Bt crops and the Pioneer benchmark parameter values in the absence of a tolerance trait, reducing the amount of refuge (either as non-Bt plants within a seed blend or a spatial refuge) reduces the net benefit of the Bt product. Furthermore, the spatial refuge provides greater net benefit than the same amount of non-Bt corn in a seed blend. These conclusions must be

qualified by acknowledging that the measures of cumulative value do not include factors that would be necessary for a thorough analysis of benefits and costs, for example the relationship between insect abundance and crop loss, and the economic discount rate of future benefits.

### **Limited male dispersal**

In the Panel discussion of Question 7, the issue of limited male movement from a spatial refuge was raised, and whether this limited dispersal was an argument for using seed blends. In fact, the SAP model shows that limited male dispersal slows rather than speeds resistance evolution; this effect has also been shown in the models by Ives and Andow (2002) and Carriere et al. (2004). Figure A9 shows this effect for the SAP model in the absence of the tolerance trait. This model assumes that there is no crop rotation or rotation between Bt and refuge fields. Nonetheless, a similar effect also occurs in a spatially explicit model with yearly crop rotation (results not presented).

This result may seem unintuitive. The common explanation for refuges slowing resistance evolution is that refuges provide SS males to mate with RR females from Bt fields, producing heterozygotes that are susceptible to Bt toxins. In fact (at least in models such as the Pioneer model), the role of refuges is more complicated and subtle.

Mathematically, refuges do three things, and only three things, that together sum to give the effect of refuges on resistance evolution (Ives and Andow 2002). First, refuges reduce the number of larvae killed by Bt and hence reduce the strength of selection for resistance. This effect is governed by the movement of females that in turn dictates the distribution of eggs (and hence Bt-caused mortality). Greater movement rates of females actually increases the rate of resistance evolution, because more females move from the refuge and have their offspring killed by Bt. (Note, though, that this gives the benefit of reducing the total insect population size.) Second, as is commonly understood, the movement of males (for the case when females mate before leaving their natal fields) does increase the proportion of RS offspring due to males from refuges mating with females in Bt fields. This slows resistance evolution. These two effects are generally understood.

The third effect is not well appreciated. Reduced male movement (assuming the movement rate of males from natal fields is the same for Bt and refuge fields) reduces the mating success of males from Bt fields, which are the males that are more likely to carry the R allele. To explain this, consider a male emerging and staying in a Bt field. Even though male movement from natal fields is low, the majority of males in Bt fields will be those that move in from the refuge (at least for the high-dose case) simply because few males will survive as larvae in the Bt field. Furthermore, there will be few females in Bt fields because few females survive as larvae in the Bt field. Thus, there are few males from the Bt fields (that more likely carry R alleles) competing with a larger number of males from the refuge for the few females emerging in Bt fields. Thus, these males have reduced mating success. The effect of this on resistance evolution can be large, because it affects the success of heterozygous RS males as well as RR males, and at low resistance frequencies the vast majority of R alleles occur in RS heterozygotes. In contrast, the effect of SS males mating with RR females (i.e., the effect that is commonly used to

explain how the high dose/refuge strategy works) is confined to only those R alleles in the very rare RR females.

Figure A9 shows the net outcome of these three effects of movement on resistance evolution; a reduction in the proportion of males leaving natal fields from 100% to 10% slows resistance evolution by roughly 3 years. While this is not a large effect for such a severe reduction in male movement, it nonetheless repudiates the argument that limited male movement will speed resistance evolution.

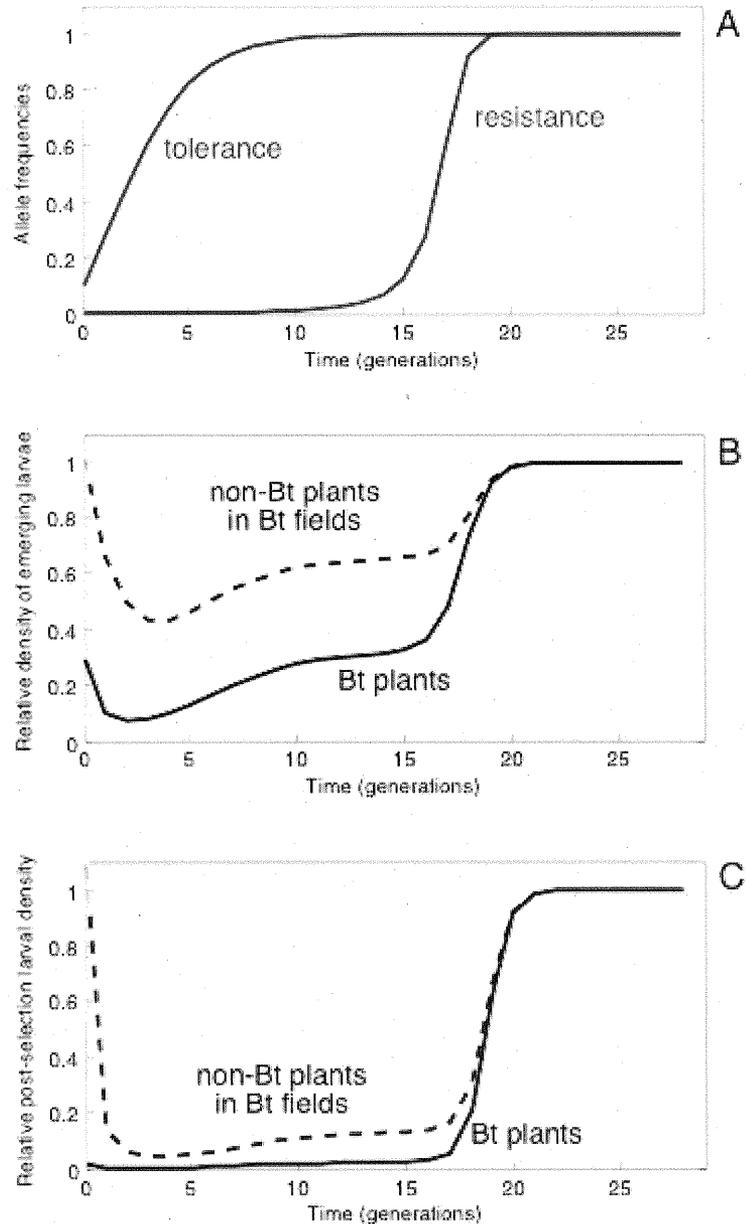


Figure A1: Resistance dynamics for the Pioneer benchmark parameter values with 3.5% seed blend (3.5% of seeds are non-Bt) and no spatial non-Bt refuge. (A) The tolerance and resistance allele frequencies. (B) The density of larvae emerging from Bt plants (black line) and non-Bt plants (dashed line). (C) The density of larvae after selection but before density-dependent mortality in Bt plants (black line), and non-Bt plants (dashed black line). Tolerance and resistance alleles are added at time 0.

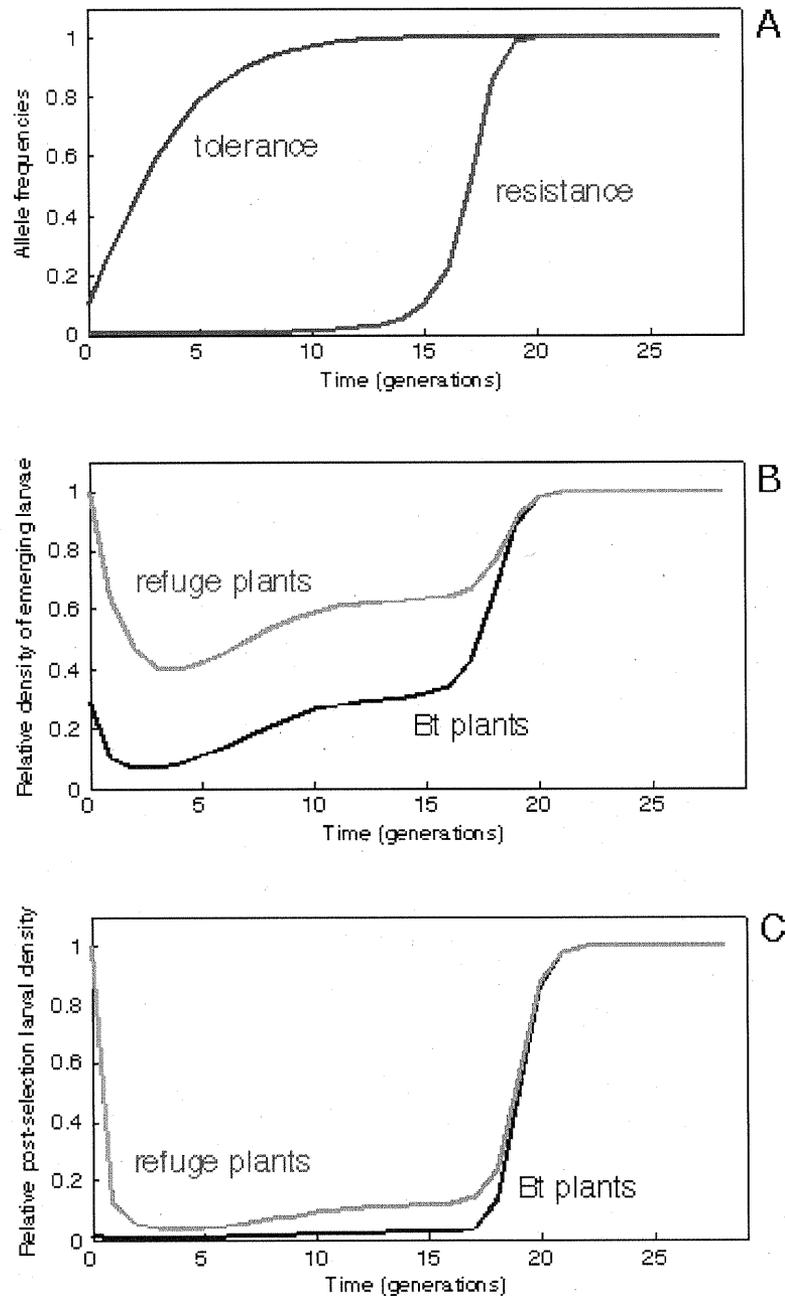


Figure A2: Resistance dynamics for the Pioneer benchmark parameter values with 0% seed blend and 3.5% spatial non-Bt refuge. (A) The tolerance and resistance allele frequencies. (B) The density of larvae emerging from Bt plants (black line) and non-Bt plants in the spatial refuge (green line). (C) The density of larvae after selection but before density-dependent mortality on Bt and non-Bt (refuge) plants.

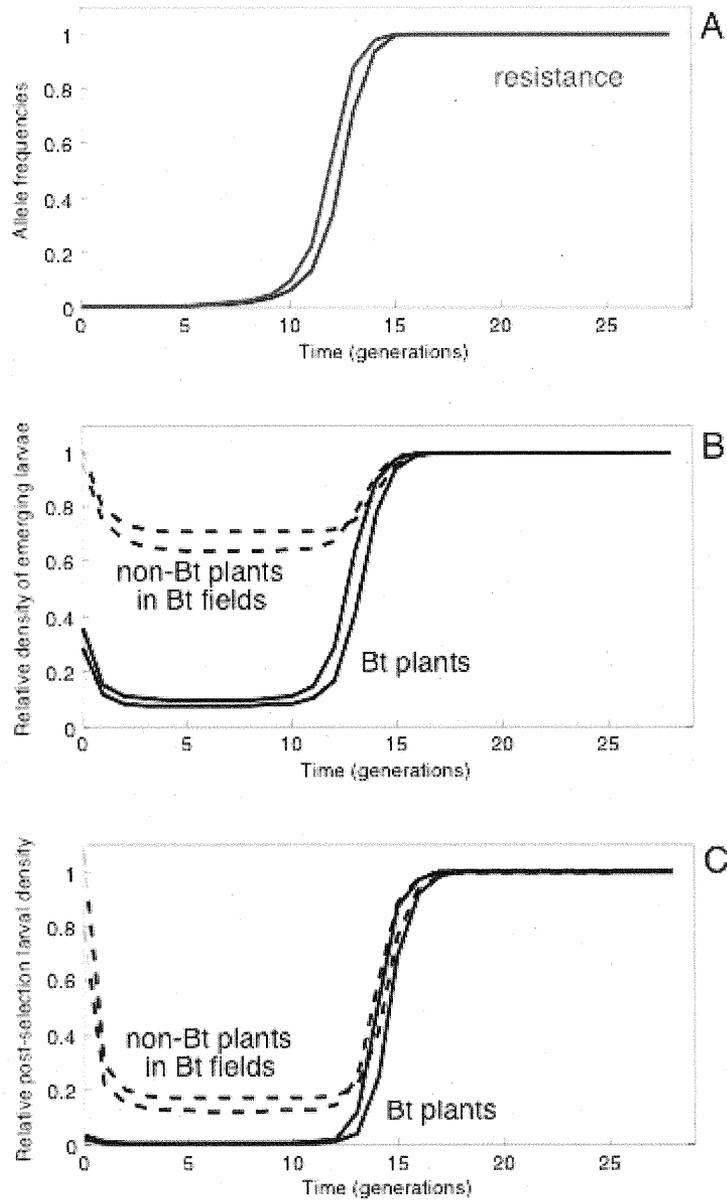


Figure A3: Resistance dynamics when there is “sampling mortality” (death due to biting a host, not caused by sampling) of susceptible larvae on Bt plants for the case with 20% seed blend (20% of seeds are non-Bt) and no spatial refuge. The tolerance allele frequency is assumed to be zero. (A) The resistance allele frequencies without sampling mortality (red) and with sampling mortality (green). (B) The density of larvae emerging per Bt plant (black line) and non-Bt plant (dashed black line). (C) The density of larvae after selection but before density-dependent mortality. Sampling mortality is included by assuming that the larval life stage was divided in half, with survival for each half given by  $s^{1/2}$  where  $s$  is the survival for that stage given by the Pioneer benchmark parameters. The effective expression of dominance,  $h$ , for resistance by larvae increased from 0.0457 to 0.0569.

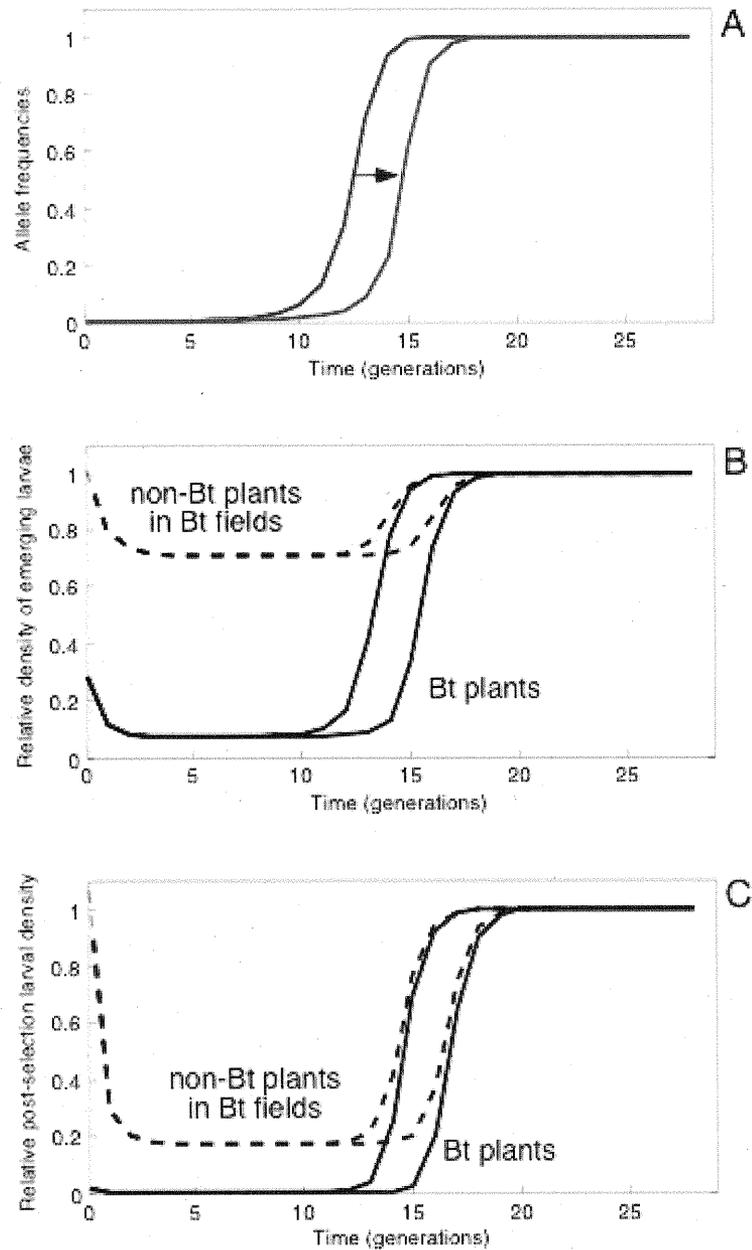


Figure A4: Resistance dynamics when there is delayed emergence of susceptible insects from Bt plants that causes non-random mating for the case with 20% seed blend and no spatial refuge. The tolerance allele frequency is assumed to be zero. (A) The resistance allele frequencies without (red) and with (green) non-random mating. (B) The density of adults emerging per Bt plant (black line) and per non-Bt plant (dashed line). (C) The density of larvae after selection but before density-dependent mortality. The effect of delayed emergence on non-random mating is incorporated by assuming that a fraction  $p_m$  of early emerging males mix with late-emerging males. In the panels, the value of  $p_m$  is reduced from 1 to 0.1.

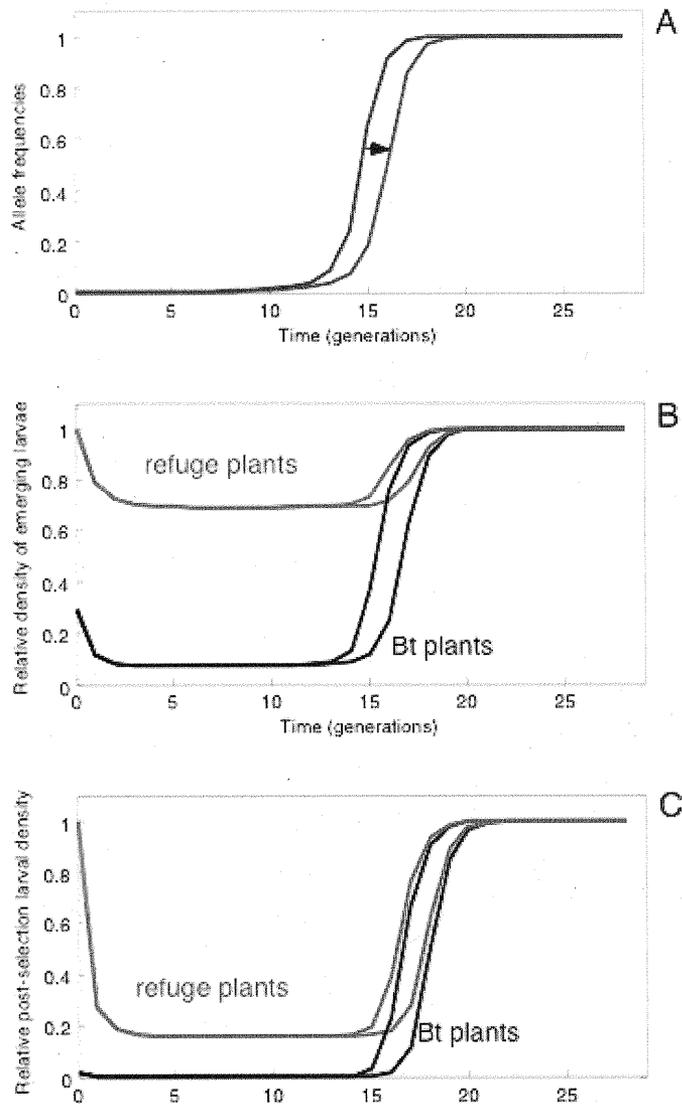


Figure A5: Resistance dynamics when there is delayed emergence of susceptible insects from Bt plants that causes non-random mating for the case without seed blends and a 20% spatial refuge. The tolerance allele frequency is assumed to be zero. (A) The resistance allele frequencies without (red) and with (green) non-random mating. (B) The density of larvae emerging per Bt plant in the Bt fields (black line) and non-Bt plant in the spatial refuge (green line). (C) The density of larvae after selection but before density-dependent mortality. The effect of delayed emergence on non-random mating is incorporated by assuming that a fraction  $p_m$  of early emerging males mix with late-emerging males. In the panels, the value of  $p_m$  is reduced from 1 to 0.1.

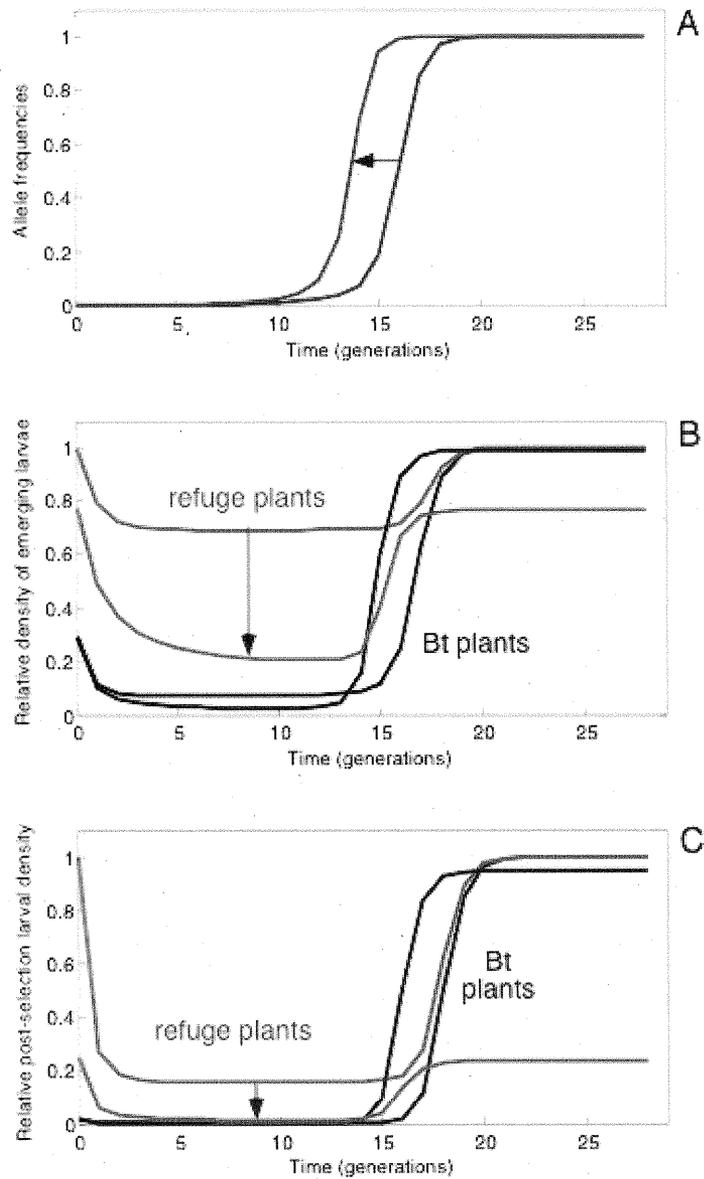


Figure A6: Resistance dynamics when a non-Bt insecticide is applied and causes 75% mortality of neonate larvae in a 20% spatial refuge. The tolerance allele frequency is assumed to be zero. (A) The resistance allele frequencies without (red) and with (green) insecticide application to refuge fields. (B) The density of larvae emerging from Bt plants (black line) and non-Bt plants in the spatial refuge (green). (C) The density of larvae after selection but before density-dependent mortality. The arrows show the reduction in insect densities in refuge fields caused by insecticide application.

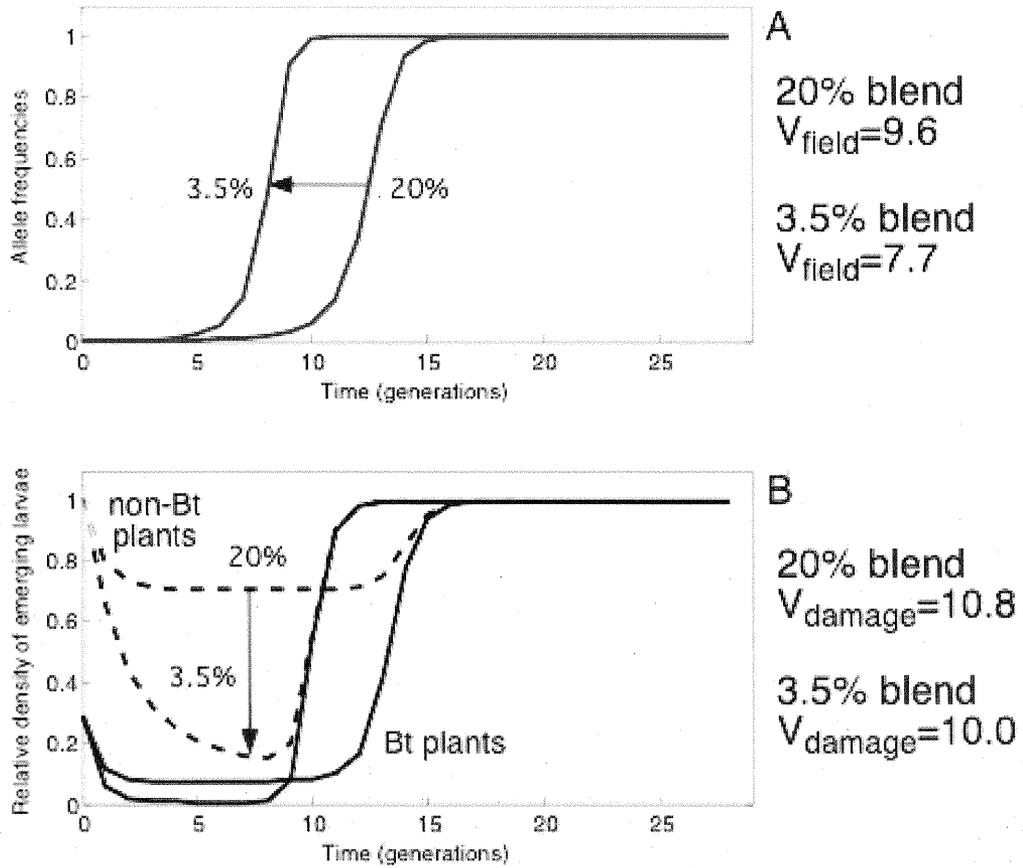


Figure A7: Consequences of reducing a 20% blended refuge to a 3.5% blended refuge for the case of a resistance trait but no tolerance trait. (A) Frequencies of the resistance allele. (B) The density of adults emerging per Bt plant (black line) and per non-Bt plant (dashed line). Definitions of  $V_{\text{field}}$  and  $V_{\text{damage}}$  are given in the appendix text.

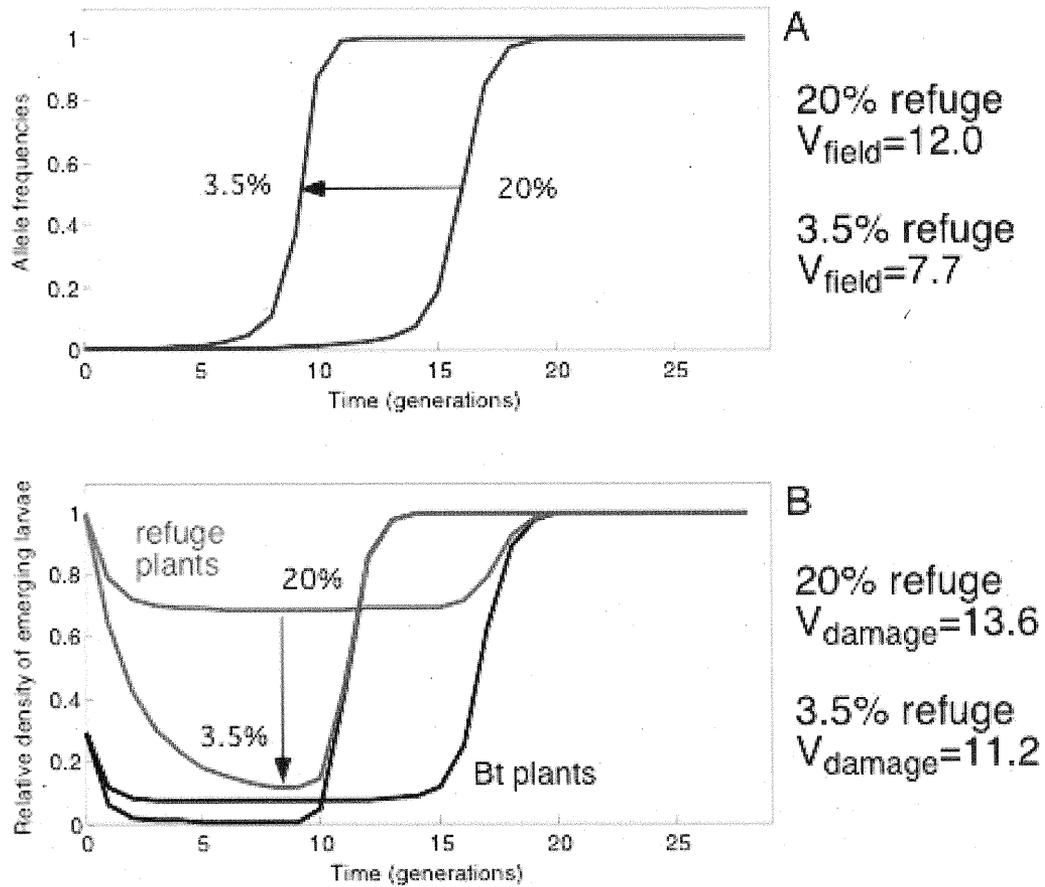


Figure A8: Consequences of reducing a 20% spatial refuge to a 3.5% spatial refuge for the case of a resistance trait but no tolerance trait. (A) Frequencies of the resistance allele. (B) The density of adults emerging per Bt plant (black line) and per non-Bt plant in the spatial refuge (green line). Definitions of  $V_{\text{field}}$  and  $V_{\text{damage}}$  are given in the appendix text.

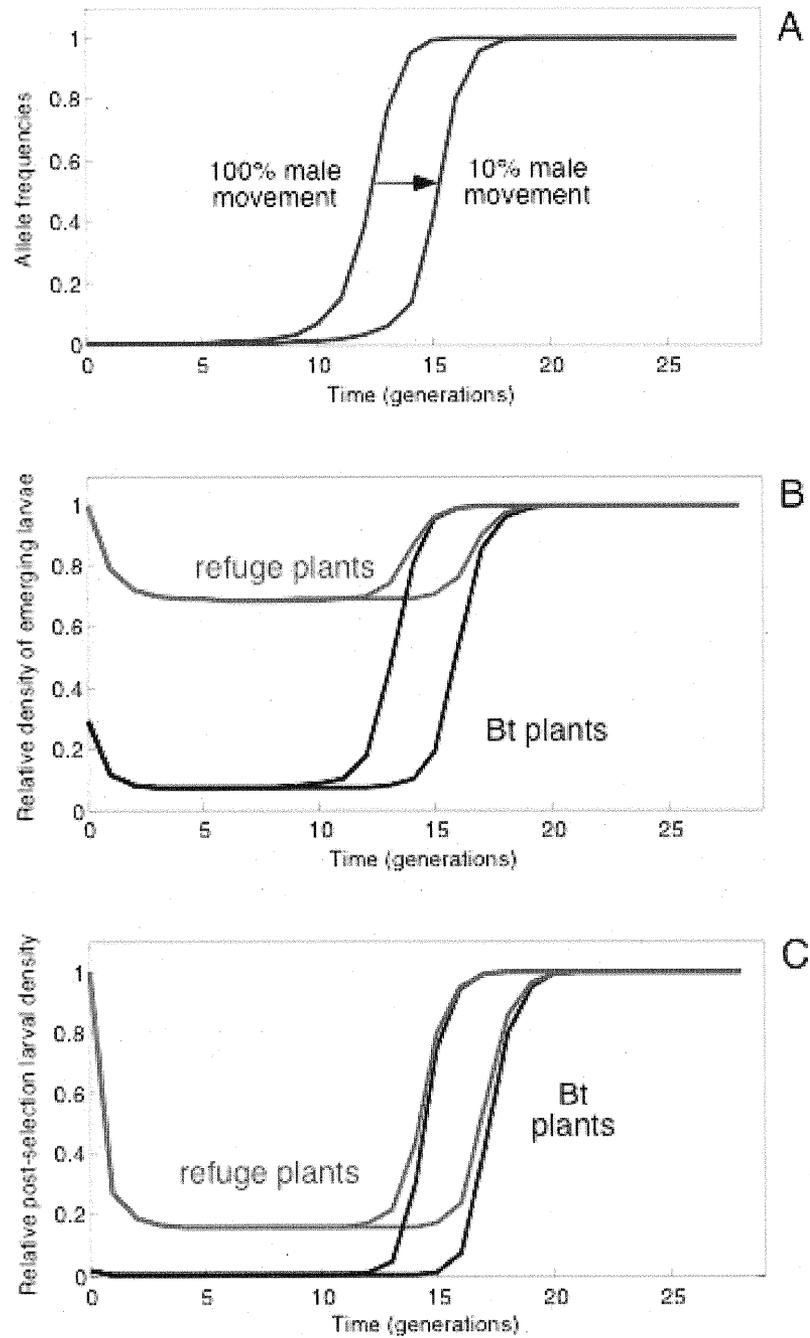


Figure A9: Resistance dynamics when male movement from natal fields is reduced from 100% to 10% for the case with 20% spatial refuge. The tolerance allele frequency is assumed to be zero. (A) The resistance allele frequencies. (B) The density of larvae emerging from Bt plants (black line) and non-Bt plants in the spatial refuge (green). (C) The density of larvae after selection but before density-dependent mortality in Bt plants.

## SAP Model Code

```
% Matlab program for resistance evolution of CRW based upon The
Pioneer model (Study ID PHI-2007-144).

% 4 March, 2009

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% parameter assignment
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% dispersal of males (rm) and from Bt (1) and refuge (2) fields
r1m=1;
r2m=r1m;

% dispersal of females (rf) and from Bt (1) and refuge (2) fields
r1f=1;
r2f=r1f;

% larval movement from Bt and non-Bt plants
L1=.625;
L2=.5;
SM=.5;

% proportion of males mating between emergence groups
pm=1;

% number of times larve can move among plants in Bt fields (given
a maximum
% value of 1)
Tmove=1;

% scaled cost of larvae "sampling" Bt plants
sm=1;

% winter egg survival
wintersurv=.5;

% initial gene frequencies for the "tolerance" gene p1 and the
"resistance"
% gene p2
initp1=.1;
initp2=.0005;

% survivals of genotypes to p1
h1=.5;
s1L=.0125;
s1H=.2;
s1=[s1L s1L*(1-h1)+s1H*h1 s1H];

% survivals of genotypes to p2
h2=.05;
s2L=.01;
```

```

s2H=1;
s2=[s2L s2L*(1-h2)+s2H*h2 s2H];

% obtaining fitnesses for genotypes at both loci
Z=1-(1-s1')*(1-s2);
Z=Z(:);

sAABB=Z(1);
sAaBB=Z(2);
saaBB=Z(3);
sAABb=Z(4);
sAaBb=Z(5);
saaBb=Z(6);
sAAAb=Z(7);
sAabb=Z(8);
saabb=Z(9);

s=Z;

% fecundities
FL=114;
FH=356;

F1=[FL FL FL FL*(1-h2)+FH*h2 h2*(FH*h2+FL*(1-h2))+(1-h2)*FL (1-
h2)*FL+FH*h2 FH FH FH]';
F1=diag(F1);

F2=FH*ones(9,1);
F2=diag(F2);

% calculate V for mating of genotypes
VV(:,:,1)=[1 .5 0;.5 .25 0;0 0 0];
VV(:,:,2)=[0 .5 1;.5 .5 .5;1 .5 0];
VV(:,:,3)=[0 0 0; 0 .25 .5;0 .5 1];
for i2=1:3
    for i1=1:3
        V(:,:,3*(i2-1)+i1)=kron(VV(:,:,i2),VV(:,:,i1));
    end
end

% O1 is the selection on larvae on Bt plants; O2 is selection on
non-Bt
% plants
O1=diag(s);
O2=eye(9);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% simulation
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

for caseflag=1:2

    %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

```

```

% set up initial conditions
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

if caseflag==1
    % seed blend

    % proportion of field type 2 (refuge)
    Q=10^-10;

    % proportion non-Bt plant in field types 1 and 2
    q1=.035;
    q2=1-10^-10;
else
    % spatial refuge

    % proportion of field type 2 (refuge)
    Q=.035;

    % proportion non-Bt plant in field types 1 and 2
    q1=10^-10;
    q2=1-10^-10;
end

% insect density without Bt
FF=FH;
a=.5*FF*wintersurv*(sv*L2+(1-L2))^Tmove;
x0=((a-2.59)/1.29)^(1/.88)/a;

% initial density of males and females
% X(1:9) = genotype numbers in type 1 (Bt) fields
% X(10:18) = genotype numbers in type 2 (refuge) fields

% female genotypes raised on Bt plants
X_1=zeros(18,1);

% female genotypes raised on non-Bt plants
X_2=zeros(18,1);
X_2(1)=.5*(1-Q)*x0;
X_2(10)=.5*Q*x0;

% male genotypes raised on Bt plants
Y_1=zeros(18,1);

% male genotypes raised on non-Bt plants
Y_2=zeros(18,1);
Y_2(1)=.5*(1-Q)*x0;
Y_2(10)=.5*Q*x0;

X=zeros(18,1);

Wm=zeros(18,1);
Wf=zeros(18,1);

```

```

tStart=1;
tEnd1=0;
tEnd2=0;

Pflag1=0;
Pflag2=0;
Xflag=0;

output=[];
Tstop=0;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% iterate model
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

for t=1:10^4
    % pre-mating movement of males
    d11=(1-r1m)+(1-Q)*r1m;
    d12=(1-Q)*r2m;
    d22=(1-r2m)+Q*r2m;
    d21=Q*r1m;
    Dm=[d11 d12;d21 d22];
    Dm=kron(Dm,eye(9));

    Y_1=Dm*Y_1;
    Y_2=Dm*Y_2;

    % no pre-mating female movement
    X_1=X_1;
    X_2=X_2;

    % mating assuming random mating between males and females
of
    % type 1, assuming mixing of a proportion of pm males
    Wm(1:9)=(Y_1(1:9)+pm*Y_2(1:9))/sum(Y_1(1:9)+pm*Y_2(1:9));

    Wm(10:18)=(Y_1(10:18)+pm*Y_2(10:18))/sum(Y_1(10:18)+pm*Y_2(10:18)
);

    Wf(1:9)=X_1(1:9)/sum(X_1(1:9));
    Wf(10:18)=X_1(10:18)/sum(X_1(10:18));

    W=[];
    for i=1:2
        Wmi=Wm(9*(i-1)+1:9*i);
        Wfi=Wf(9*(i-1)+1:9*i);
        for j=1:9
            W=[W;sum(sum((Wfi*Wmi') .*V(:, :, j)))]];
        end
    end
    X_1(1:9)=sum(X_1(1:9))*W(1:9);
    X_1(10:18)=sum(X_1(10:18))*W(10:18);
    X_1(isnan(X_1))=0;

```

```

of
    % mating assuming random mating between males and females
    % type 2, assuming mixing of a proportion of pm males
    Wm(1:9)=(pm*Y_1(1:9)+Y_2(1:9))/sum(pm*Y_1(1:9)+Y_2(1:9));
    Wm(10:18)=(pm*Y_1(10:18)+Y_2(10:18))/sum(pm*Y_1(10:18)+Y_2(10:18)
);

    Wf(1:9)=X_2(1:9)/sum(X_2(1:9));
    Wf(10:18)=X_2(10:18)/sum(X_2(10:18));

    W=[];
    for i=1:2
        Wmi=Wm(9*(i-1)+1:9*i);
        Wfi=Wf(9*(i-1)+1:9*i);
        for j=1:9
            W=[W;sum(sum((Wfi*Wmi') .*V(:, :, j)))]];
        end
    end
    X_2(1:9)=sum(X_2(1:9))*W(1:9);
    X_2(10:18)=sum(X_2(10:18))*W(10:18);
    X_2(isnan(X_2))=0;

    % post-mating movement of females
    d11=(1-r1f)+(1-Q)*r1f;
    d12=(1-Q)*r2f;
    d22=(1-r2f)+Q*r2f;
    d21=Q*r1f;
    Df=[d11 d12;d21 d22];
    Df=kron(Df,eye(9));

    X_1=Df*X_1;
    X_2=Df*X_2;

    % reproduction
    X(1:9)=F1*X_1(1:9)+F2*X_2(1:9);
    X(10:18)=F1*X_1(10:18)+F2*X_2(10:18);

    % overwintering survival
    X=wintersurv*X;

    %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% larval movement and selection in field
types 1 and 2 %%%%%%%%%%
    Xp=[];
    Xe=[];
    for fieldtype=1:2
        if fieldtype==1
            q=q1;
            U1=(1-q)*X(1:9);
            U2=q*X(1:9);
            QQ=1-Q;
        else

```

```

        q=q2;
        U1=(1-q)*X(10:18);
        U2=q*X(10:18);
        QQ=Q;
    end

    U1t=U1;
    U2t=U2;

    % movement among plants
    m11=(1-q)*sv*L1+(1-L1);
    m21=q*sv*L1;
    m22=q*sv*L2+(1-L2);
    m12=(1-q)*sv*L2;

    M=[m11 m12;m21 m22];
    M=kron(M,eye(9));

    % assume resistant larvae move from Bt plants with
    prob L2
    pickgenotypes=find(s==1);
    for i=1:length(pickgenotypes)
        M(pickgenotypes(i),pickgenotypes(i))=(1-
q)*sv*L2+(1-L2);
        M(pickgenotypes(i)+9,pickgenotypes(i))=q*sv*L2;
    end

    for tt=1:Tmove
        % sampling mortality on Bt plants
        U1=(eye(9)-(1-sm)*(eye(9)-O1))*U1;
        U2=(eye(9)-(1-sm)*(eye(9)-O2))*U2;
        U=[U1;U2];
        U=M*U;
        U1=U(1:9);
        U2=U(10:18);
    end

    % selected mortality
    U1=O1*U1;
    U2=O2*U2;

    % pre-density-dependent larval densities
    Xp=[Xp sum(U1)/((1-q)*QQ*(sv*L2+(1-
L2))^Tmove*.5*FF*wintersurv*x0) ...
        sum(U2)/(q*QQ*(sv*L2+(1-
L2))^Tmove*.5*FF*wintersurv*x0)];

    % density dependence assuming competition occurs on
    individual plants
    U1=U1/(2.59+1.29*(sum(U1)/((1-q)*QQ))^0.88);
    U2=U2/(2.59+1.29*(sum(U2)/(q*QQ))^0.88);

    % save density of emerging adults

```

```

Xe=[Xe sum(U1)/((1-q)*QQ*x0) sum(U2)/(q*QQ*x0)];

if fieldtype==1
    X_1(1:9)=.5*U1;
    X_2(1:9)=.5*U2;
    Y_1(1:9)=.5*U1;
    Y_2(1:9)=.5*U2;
else
    X_1(10:18)=.5*U1;
    X_2(10:18)=.5*U2;
    Y_1(10:18)=.5*U1;
    Y_2(10:18)=.5*U2;
end
end

% introduce resistance alleles
if t==tStart
    % initial gene frequencies
    p1=initp1;
    p2=initp2;

    % initially assume genotypes at H-W and unlinked
    Wi=[(1-p1)^2 2*p1*(1-p1) p1^2];
    Wj=[(1-p2)^2 2*p2*(1-p2) p2^2];

    W=Wi'*Wj;
    W=W(:);

    X_1(1:9)=sum(X_1(1:9))*W;
    X_1(10:18)=sum(X_1(10:18))*W;
    Y_1(1:9)=sum(Y_1(1:9))*W;
    Y_1(10:18)=sum(Y_1(10:18))*W;

    X_2(1:9)=sum(X_2(1:9))*W;
    X_2(10:18)=sum(X_2(10:18))*W;
    Y_2(1:9)=sum(Y_2(1:9))*W;
    Y_2(10:18)=sum(Y_2(10:18))*W;
end

% compute frequencies
P1=(sum(X_1([3 6 9]))+.5*sum(X_1([2 5 8])) + ...
    sum(X_1([12 15 18]))+.5*sum(X_1([11 14 17])) + ...
    sum(X_2([3 6 9]))+.5*sum(X_2([2 5 8])) + ...
    sum(X_2([12 15 18]))+.5*sum(X_2([11 14
17]))) / sum(X_1+X_2);

P2=(sum(X_1([7 8 9]))+.5*sum(X_1([4 5 6])) + ...
    sum(X_1([16 17 18]))+.5*sum(X_1([13 14 15])) + ...
    sum(X_2([7 8 9]))+.5*sum(X_2([4 5 6])) + ...
    sum(X_2([16 17 18]))+.5*sum(X_2([13 14
15]))) / sum(X_1+X_2);

if P1>.5 && Pflag1==0

```

```

        tEnd1=t;
        Pflag1=1;
    end

    if P2>.5 && Pflag2==0
        tEnd2=t;
        Pflag2=1;
    end

    if sum(X_1(1:9)+X_2(1:9)) > 0.9*0.5*x0*(1-Q) && t>tStart
&& Xflag==0
        tEndX=t;
        Xflag=1;
    end

    if Xflag
        Tstop=Tstop+1;
    end

    if Tstop==20
        break
    end

    output=[output;t-tStart P1 P2 Xe Xp];

end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% model output
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

GensToFailure2=tEnd2-tStart-1

cumValue=sum(1-((1-Q)*(1-q1)*output(:,4)+(1-
Q)*q1*output(:,5)+...
Q*(1-q2)*output(:,6)+Q*q2*output(:,7)))

figure(caseflag)
if caseflag==1
    set(caseflag,'Position',[272 75 403 724])
else
    set(caseflag,'Position',[677 74 403 724])
end
subplot(3,1,1)
plot(output(:,1),output(:,2),'b',output(:,1),output(:,3),'r')
xlabel('Time (generations)')
ylabel('Allele frequencies')
axis([output(1,1) output(end,1)+1 0 1.1])
title('red=resistance, blue=tolerance')

hold on

subplot(3,1,2)

```

```

if caseflag==1
    plot(output(:,1),output(:,4),'k',...
          output(:,1),output(:,5),'k--')
else
    plot(output(:,1),output(:,4),'k',...
          output(:,1),output(:,7),'g')
end
xlabel('Time (generations)')
ylabel('Relative density of emerging larvae')
axis([output(1,1) output(end,1)+1 0 1.1])
title('black=Bt fields, green=refuge')

hold on

subplot(3,1,3)
if caseflag==1
    plot(output(:,1),output(:,8),'k',...
          output(:,1),output(:,9),'k--')
else
    plot(output(:,1),output(:,8),'k',...
          output(:,1),output(:,11),'g')
end
xlabel('Time (generations)')
ylabel('Relative post-selection larval density')
axis([output(1,1) output(end,1)+1 0 1.1])
title('black=Bt fields, green=refuge')

hold on

end

```

## Appendix B: Analysis of Average Date of Emergence

Table B1. Arithmetic average dates of emergence (DOE, day of year) and differences in DOE (d) by gender and blend proportions of western corn rootworm adults produced in field plots of maize containing various proportions of HX1 and HXX corn genotypes replicated at four geographic locations: YK (York, NE), JH (Johnston, IA), MK (Mankato, MN), JV (Janesville, WI).

		Gender				
		Males		Females		
Location	Blend <sup>1</sup>	Ave(SE,N)	$\Delta$ Blend <sup>2</sup>	Ave(SE,N)	$\Delta$ Blend	$\Delta$ Gender <sup>3</sup>
YK	100:0	ND <sup>4</sup>	ND	212.1(1.7,2)	10.3	
	95:5	196.8(1.6,2)	1.0	203.1(1.2,5)	0.9	6.3
	90:10	194.0(2.3,1)	-1.8	209.1(2.0,3)	7.3	15.1
	80:20	197.9(1.3,4)	2.1	202.2(1.0,5)	0.4	4.3
	0:100	195.8(0.5,5)	0.0	201.8(0.5,5)	0.0	6.0
JH	100:0	ND		ND		
	95:5	196.2(1.2,4)	4.5	204.6(1.7,4)	3.6	8.4
	90:10	194.9(0.9,5)	3.2	201.8(1.2,4)	0.8	6.9
	80:20	194.8(0.7,5)	3.1	205.7(1.6,5)	4.7	10.9
	0:100	191.7(0.1,5)	0.0	201.0(0.2,5)	0.0	9.3
MK	100:0	196.2(0.4,4)	4.5	199.3(1.2,5)	3.1	3.1
	95:5	194.5(0.4,4)	2.8	199.1(0.5,5)	2.9	4.6
	90:10	194.9(0.4,5)	3.2	199.3(0.4,5)	3.1	4.4
	80:20	194.8(0.4,5)	3.1	197.5(0.3,5)	1.3	2.7
	0:100	191.7(0.1,5)	0.0	196.2(0.1,5)	0.0	4.5
JV	100:0	202.8(0.5,5)	7.7	209.1(0.6,5)	9.2	6.3
	95:5	202.6(0.4,5)	7.5	207.2(0.4,5)	7.3	3.6
	90:10	200.2(0.3,5)	5.1	205.5(0.3,5)	5.6	5.3
	80:20	200.3(0.2,5)	5.2	205.1(0.2,5)	5.2	4.8
	0:100	195.1(0.1,5)	0.0	199.9(0.1,5)	0.0	4.8
Pooled			Ave(SE,N); $p^5$		Ave(SE,N); $p$	Ave(SE,N); $p$
	0:100		6.1(1.6,2);>.05		7.5(2.2,3);>.05	4.7(1.6,2);>.05
	5:95		4.0(1.4,4);>.05		3.7(1.3,4);>.05	5.7(1.1,4);<.01
	10:90		3.8(1.5,4);>.05		4.2(1.4,4);>.05	7.9(2.5,4);=.05
	20:80		3.8(0.7,4);<.05		2.9(1.2,4);>.05	5.7(1.8,4);<.05
	100:0		0.0		0.0	6.2(1.1,4);<.01

<sup>1</sup>Percentages HXX:HX1

<sup>2</sup>{Ave[DOE 0:100]-Ave[DOE X:Y]}

<sup>3</sup>{Ave[DOE Females]-Ave[DOE Males]}

<sup>4</sup>No Data (n<2)

<sup>5</sup>( $H_0: \Delta=0$ )

Table B2. Numbers of western corn rootworm adults emerging from field plots containing various blend proportions of HX1 and HXX corn genotypes and replicated at four geographic locations.

Blend <sup>1</sup>	York, NE	
	Male	Female
0:100	0.2 (0.2) <sup>2</sup>	3.4 (0.9)
5:95	3.0 (1.3)	6.6 (1.3)
10:90	1.2 (0.6)	4.0 (1.2)
20:80	7.4 (2.0)	13.8 (4.3)
100:0	40.0 (4.3)	50.4 (6.2)
	Johnston, IA	
	Male	Female
0:100	0.20 (0.20)	0.40 (0.4)
5:95	3.6 (0.51)	4.6 (1.2)
10:90	10.0 (3.2)	4.8 (2.2)
20:80	18.0 (4.2)	12.6 (3.9)
100:0	198.6 (26.1)	150.2 (27.8)
	Mankato, MN	
	Male	Female
0:100	9.8 (2.9)	19.0 (4.8)
5:95	29.6 (12.8)	69.8 (26.6)
10:90	52.8 (24.4)	78.2 (33.9)
20:80	30.6 (5.9)	91.4 (24.7)
100:0	437.0 (94.7)	614 (107.9)
	Janesville, WI	
	Male	Female
0:100	30.8 (6.4)	49.2 (9.6)
5:95	59.4 (8.1)	86.8 (15.4)
10:90	102.0 (18.4)	156.2 (29.1)
20:80	200.6 (22.5)	296.6 (22.5)
100:0	1,421.6 (149.9)	1,496.4 (197.0)

<sup>1</sup>Percentages HX1:HXX

<sup>2</sup>Mean (SEM)

Figure B1. Emergence of western corn rootworm (WCR) males from field plots at Janesville, WI (JV) with various proportions of HXX and HX1 corn genotypes: (a) 100% HXX, (b) 5, 10, and 20% HX1, and (c) 100% HX1.

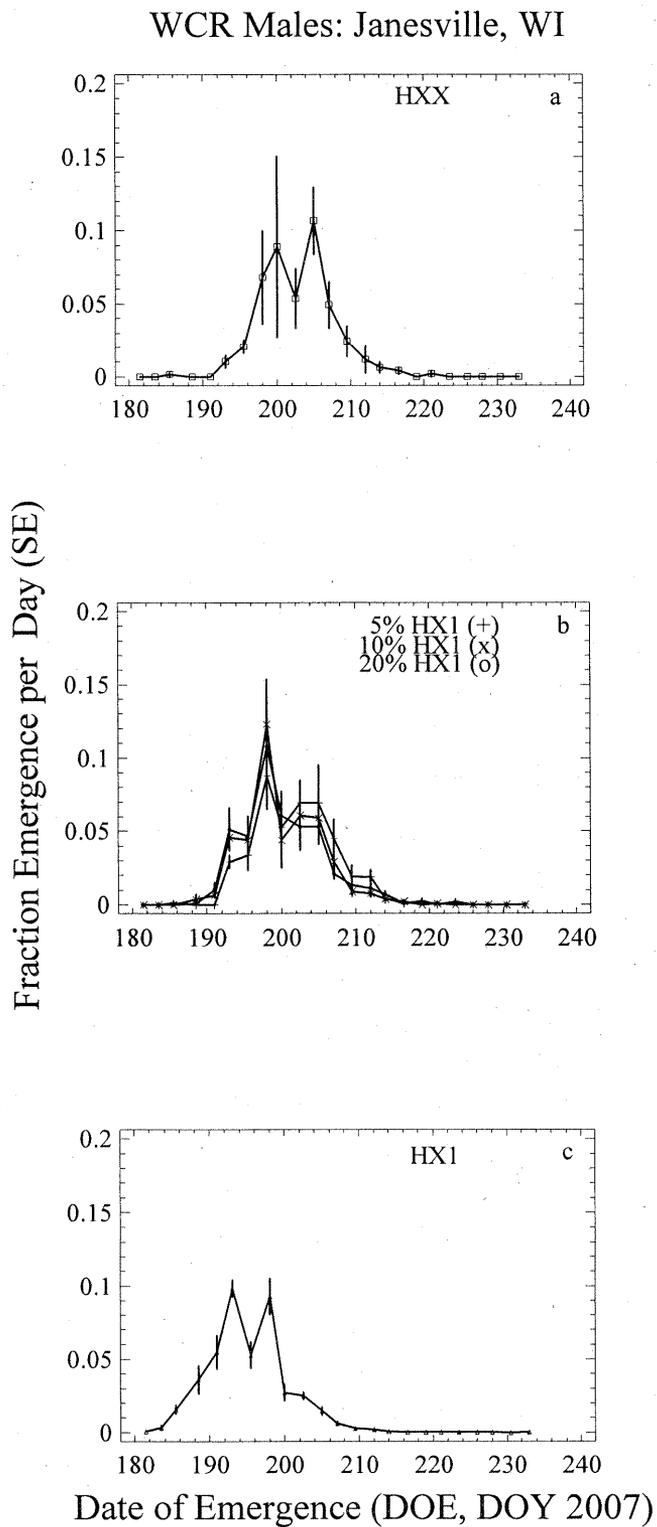


Figure B2. Emergence of western corn rootworm (WCR) females from field plots at Janesville, WI (JV) with various proportions of HXX and HX1 corn genotypes: (a) 100% HXX, (b) 5, 10, and 20% HX1, and (c) 100% HX1.

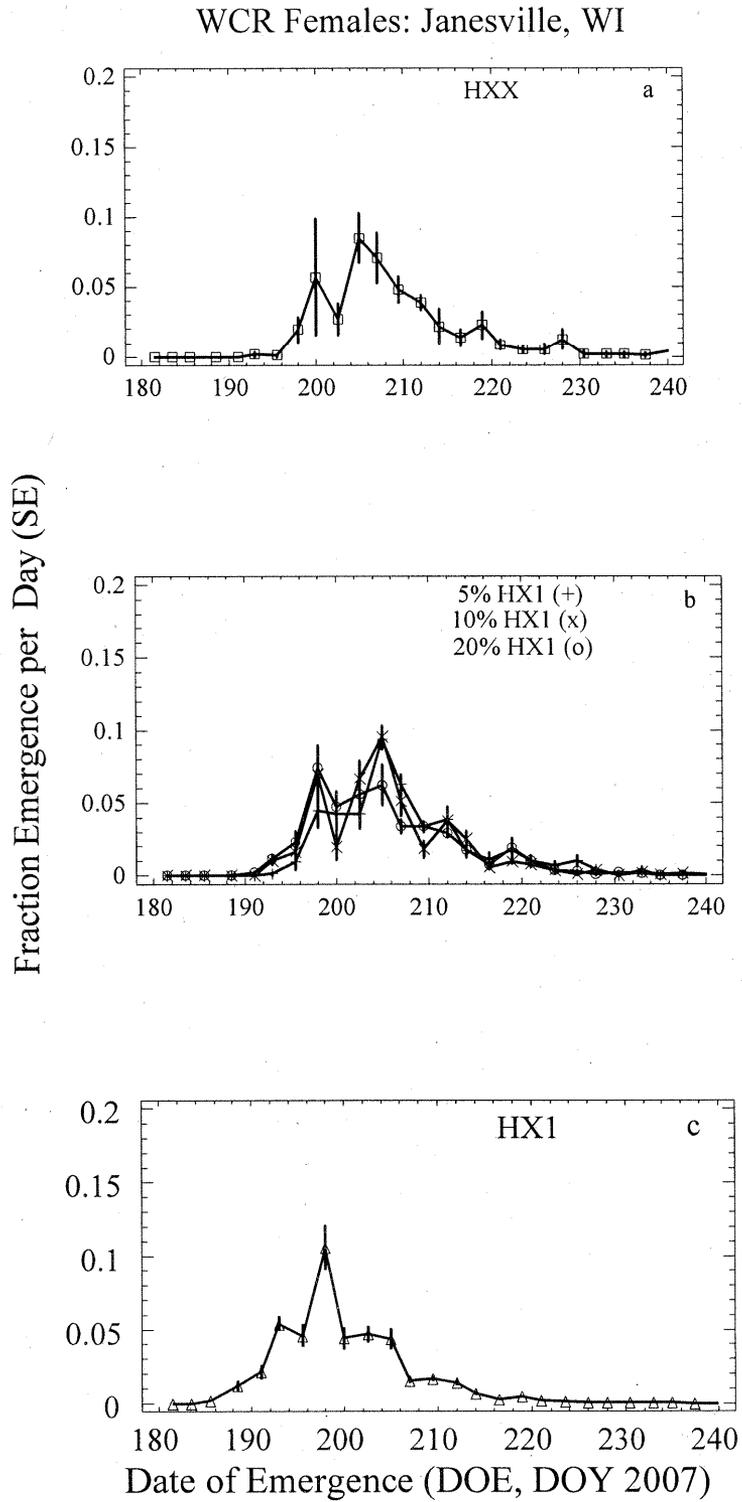


Figure B3. Emergence of western corn rootworm (WCR) males from field plots at Mankato, MN (MK) with various proportions of HXX and HX1 corn genotypes: (a) 100% HXX, (b) 5, 10, and 20% HX1, and (c) 100% HX1.

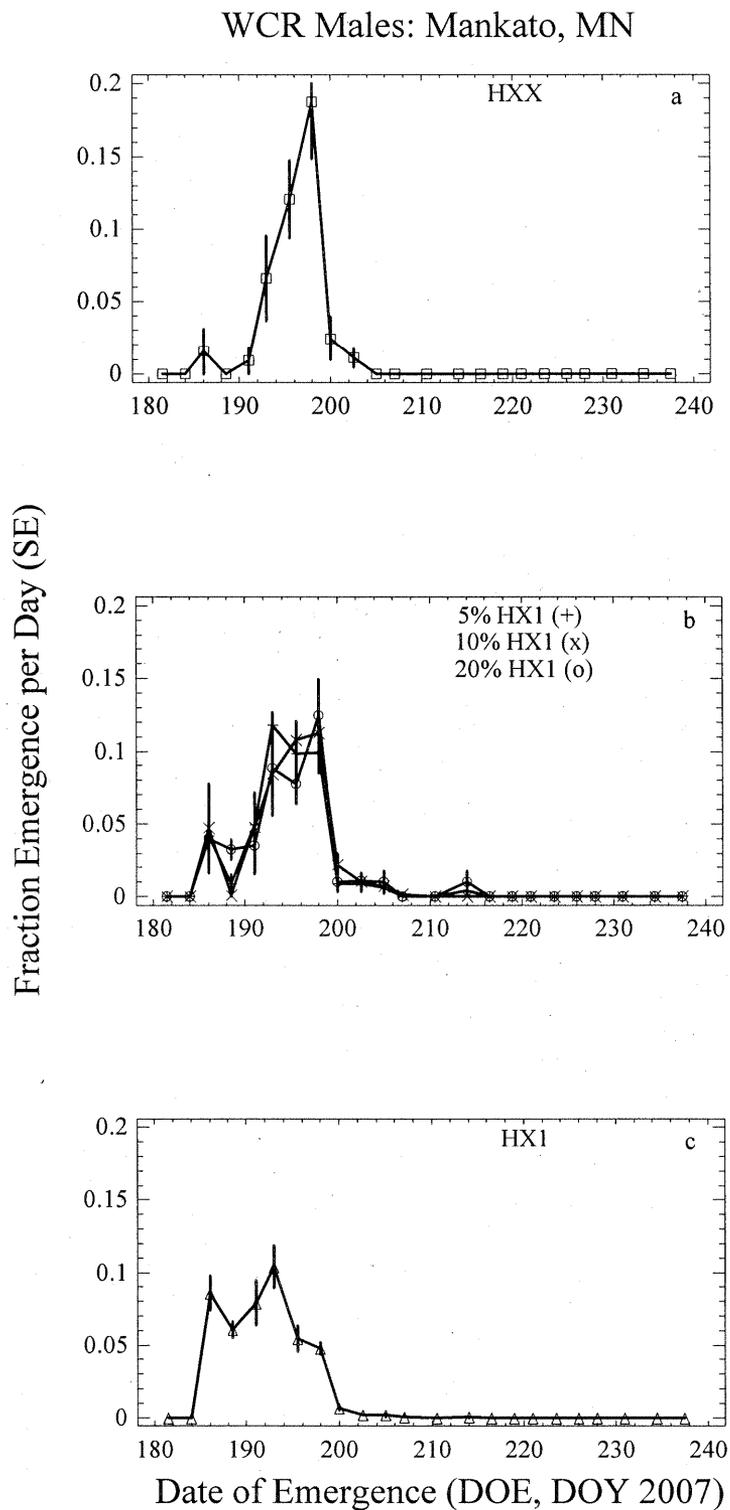


Figure B4. Emergence of western corn rootworm (WCR) females from field plots at Mankato, MN (MK) with various proportions of HXX and HX1 corn genotypes: (a) 100% HXX, (b) 5, 10, and 20% HX1, and (c) 100% HX1.

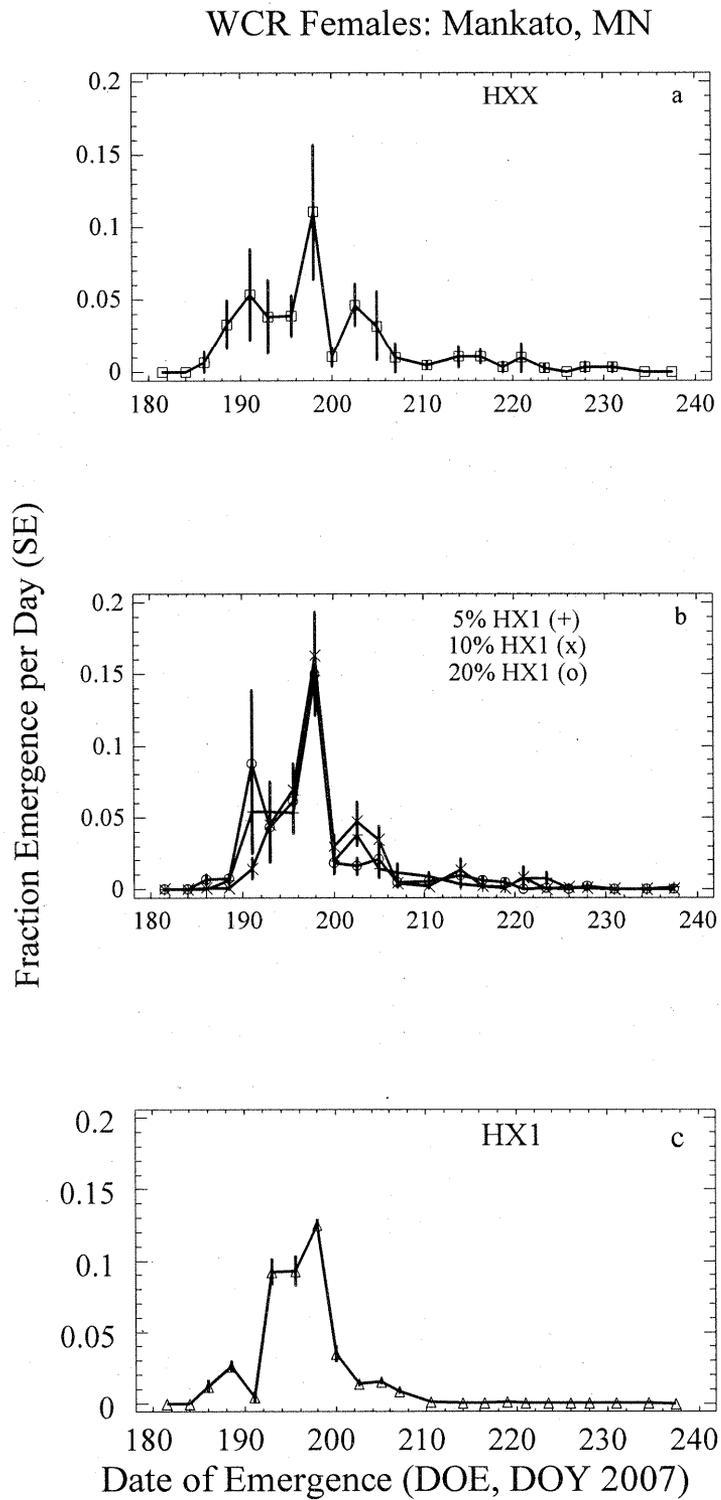


Figure B5. Emergence of western corn rootworm (WCR) males from field plots at Johnston, IA (JH) with various proportions of HXX and HX1 corn genotypes: (a) 100% HXX, (b) 5, 10, and 20% HX1, and (c) 100% HX1.

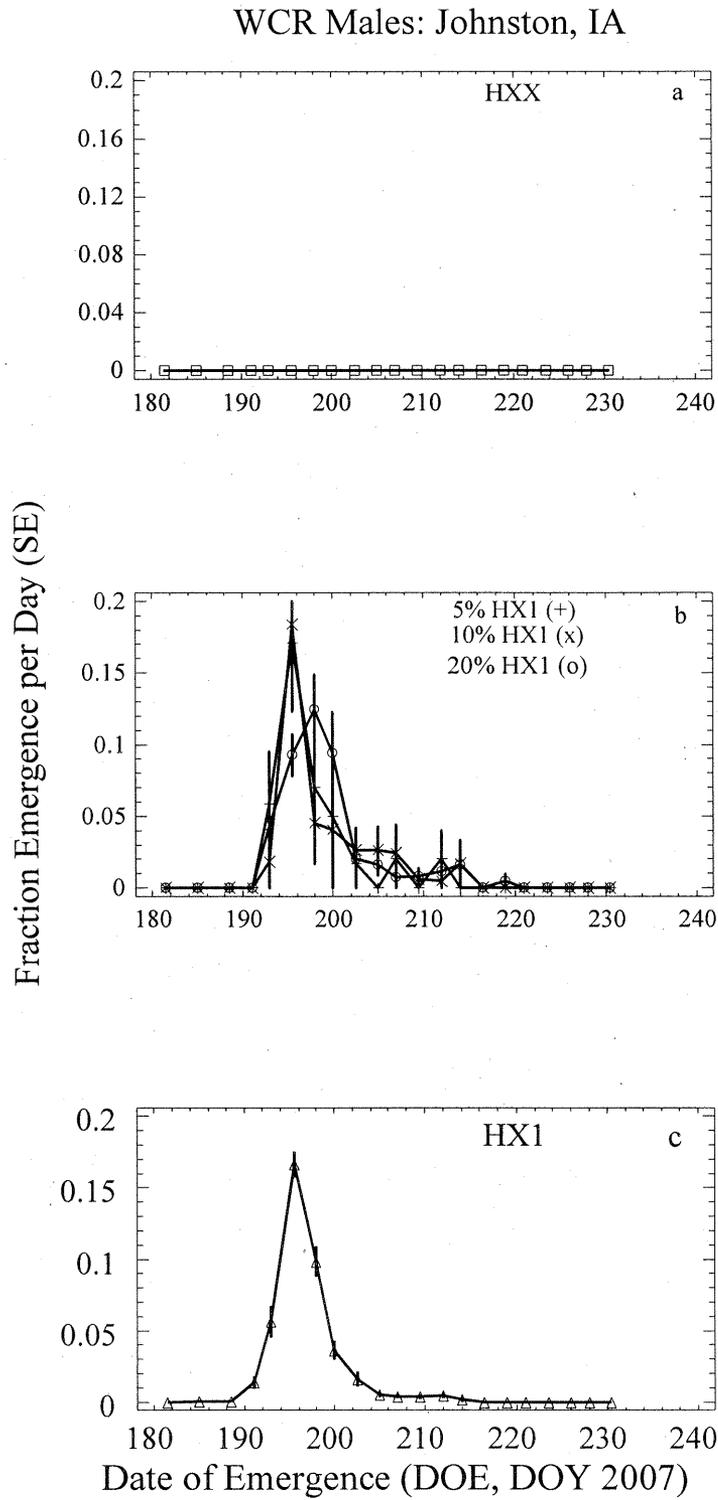


Figure B6. Emergence of western corn rootworm (WCR) females from field plots at Johnston, IA (JH) with various proportions of HXX and HX1 corn genotypes: (a) 100% HXX, (b) 5, 10, and 20% HX1, and (c) 100% HX1.

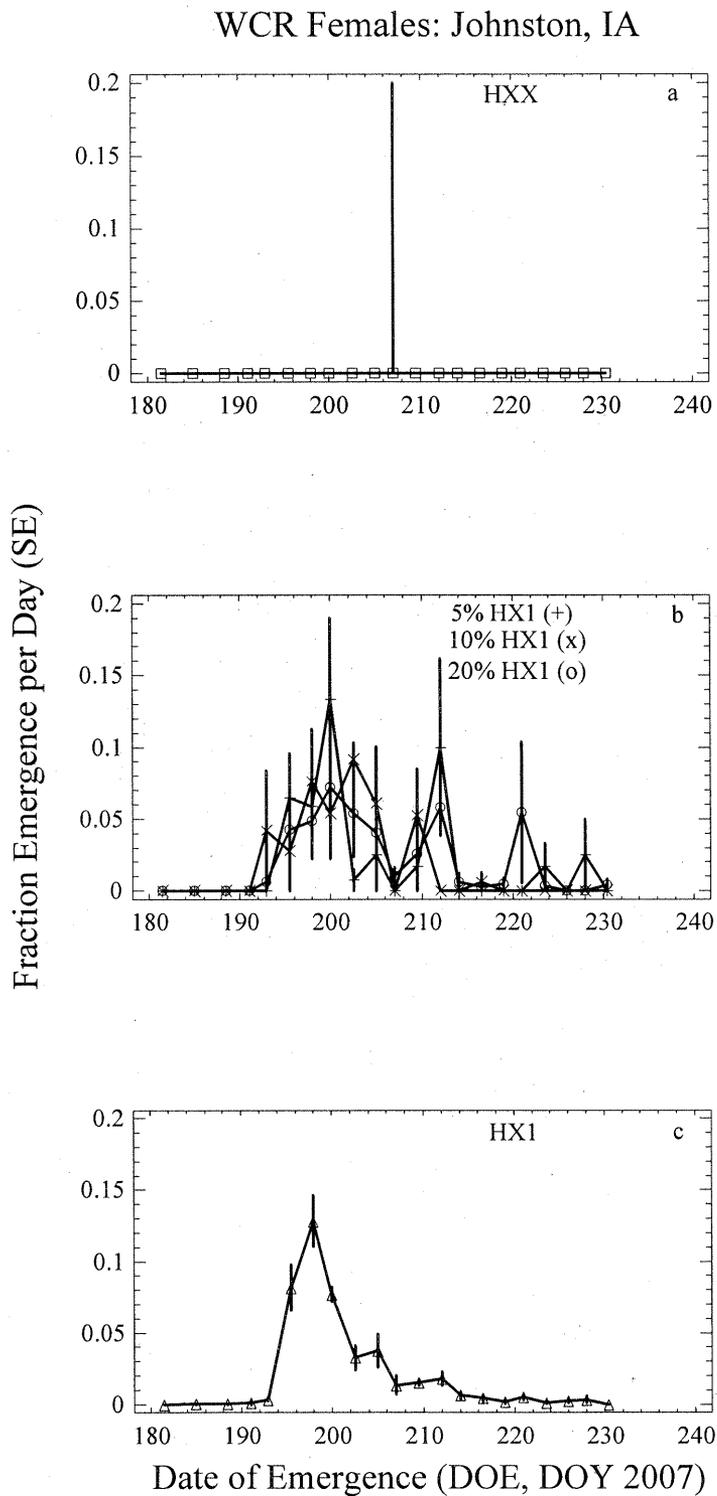


Figure B7. Emergence of western corn rootworm (WCR) males from field plots at York, NE (YK) with various proportions of HXX and HX1 corn genotypes: (a) 100% HXX, (b) 5, 10, and 20% HX1, and (c) 100% HX1.

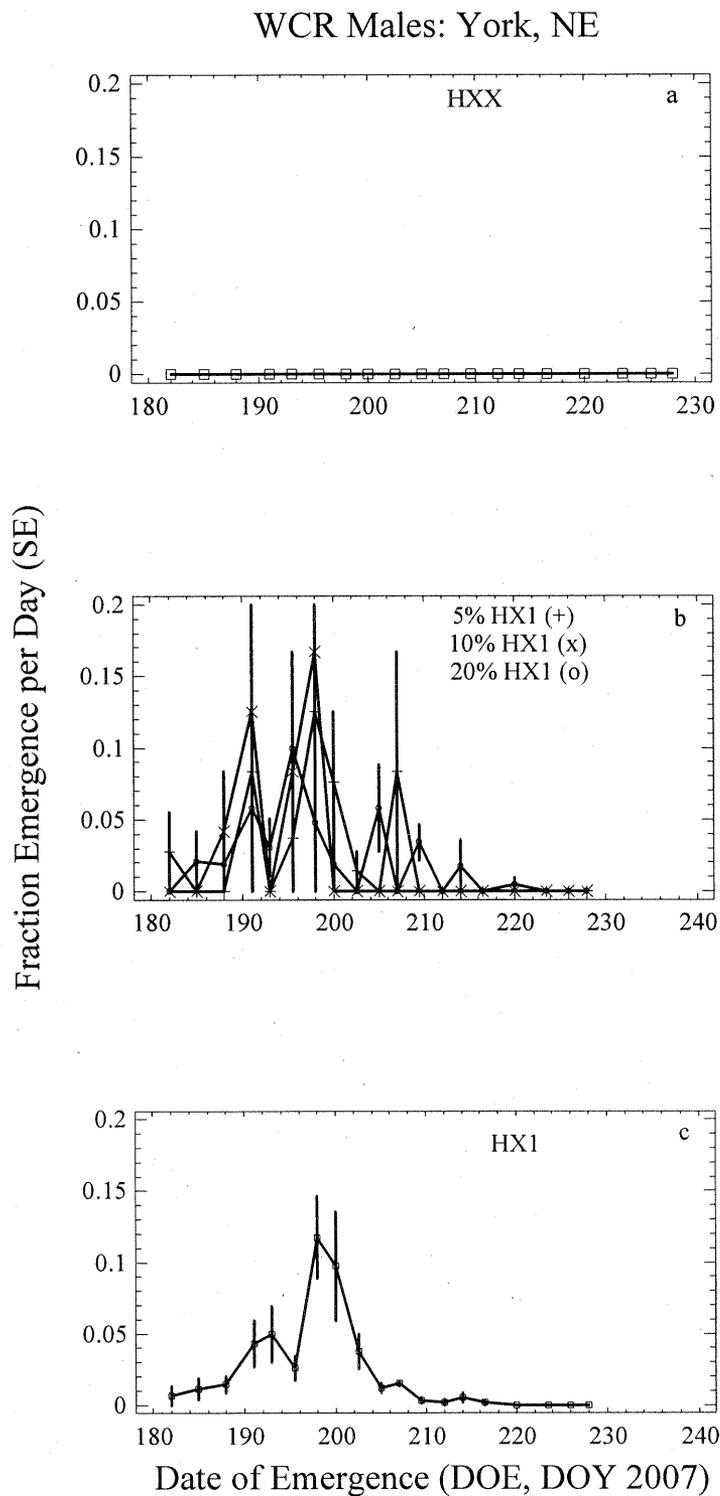


Figure B8. Emergence of western corn rootworm (WCR) females from field plots at York, NE (YK) with various proportions of HXX and HX1 corn genotypes: (a) 100% HXX, (b) 5, 10, and 20% HX1, and (c) 100% HX1.

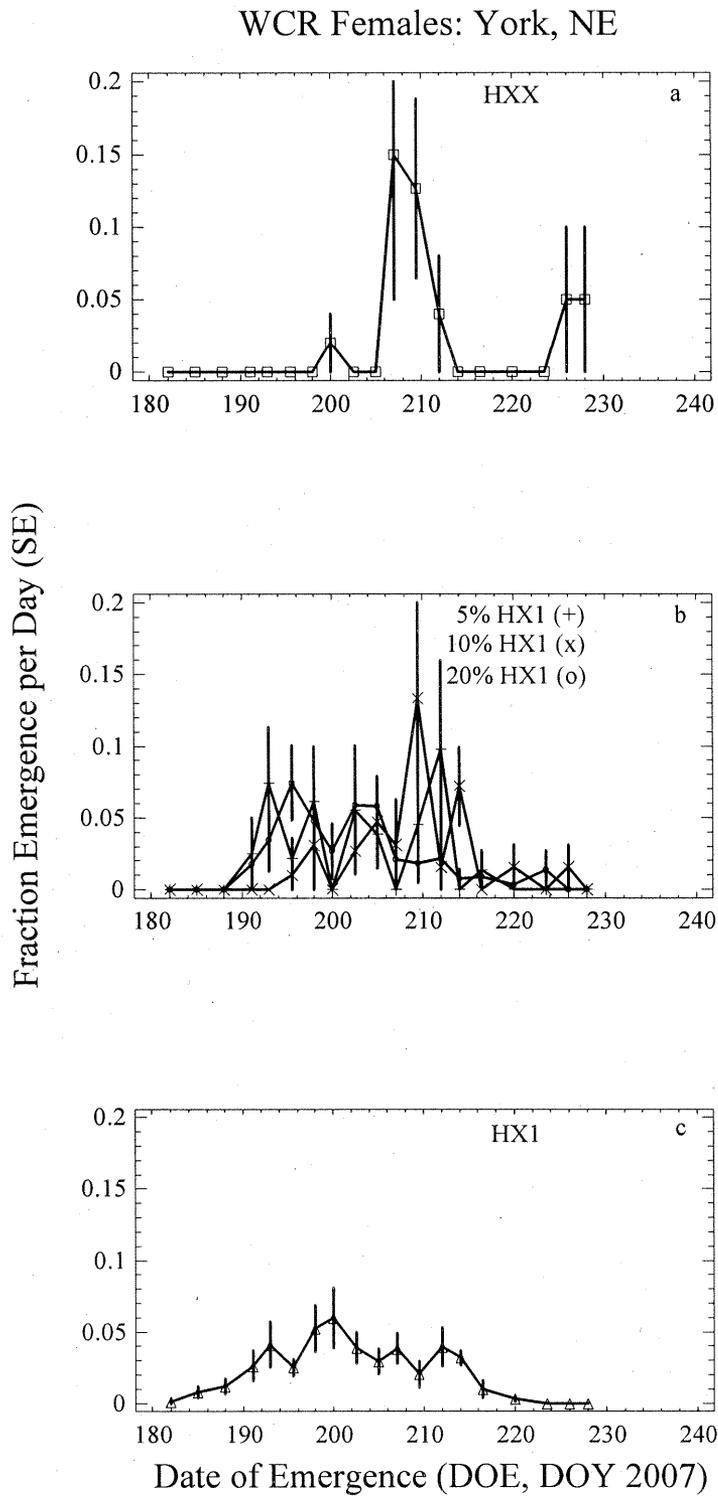


Figure B9. Net effect of the delay in date of emergence (DOE) of WCR males from seed blend corn fields relative to 100% HX1 fields and of the normal advance in DOE of males over females on the DOE of susceptible males from seed blend field/refuges relative to the median DOE of resistant females from the same fields; and a similar comparison of the DOE of susceptible males from 100% HX1 field/refuges relative to the median DOE of resistant females from seed blend fields.

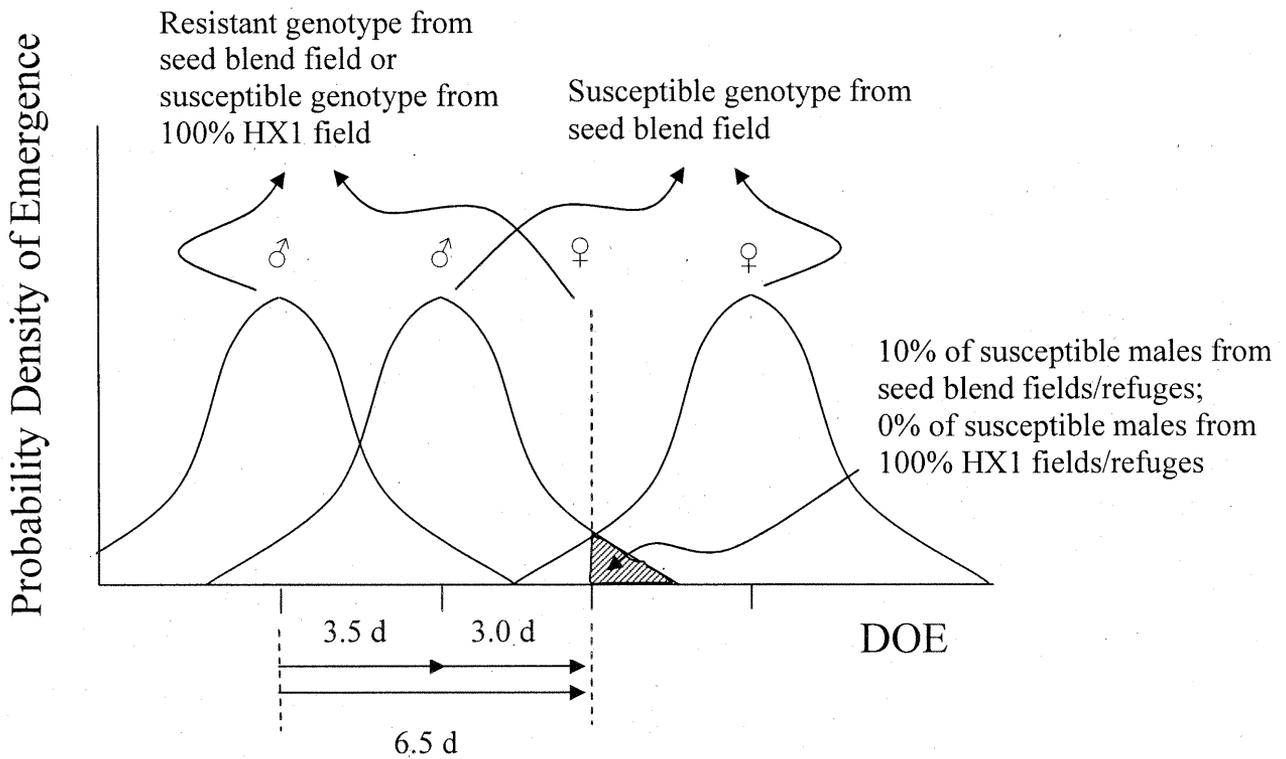


Figure B10. Fraction of adult WCR that are male emerging from field plots of corn containing various proportions of HX1 and HXX maize genotypes replicated at four geographic locations: YK (York, NE), JH (Johnston, IA), MK (Mankato, MN), JV (Janesville, WI).

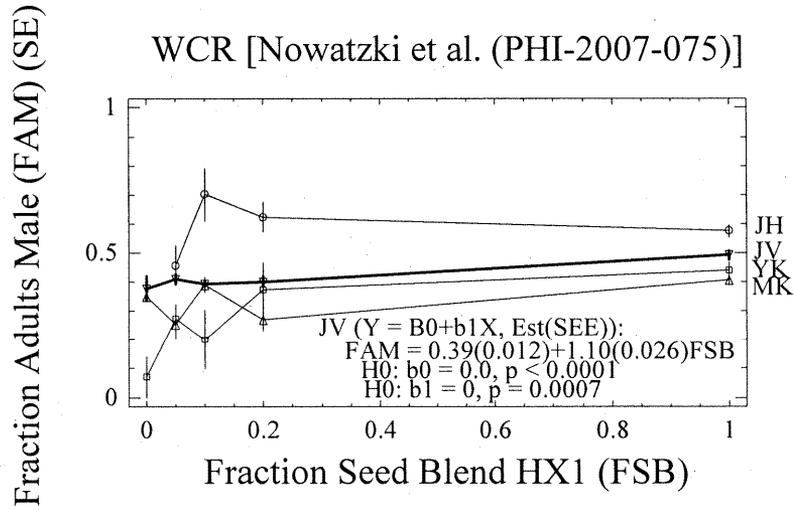


Figure B11. Probability of western corn rootworm larvae leaving the corn plant with which they are initially associated as a function of the fraction of the corn plants that are susceptible HX1 genotype under a model of larval movement based on the probabilities of association of resistant and susceptible corn genotypes;  $\text{Pr}[\text{Disp}] = 1 - p^2$ .

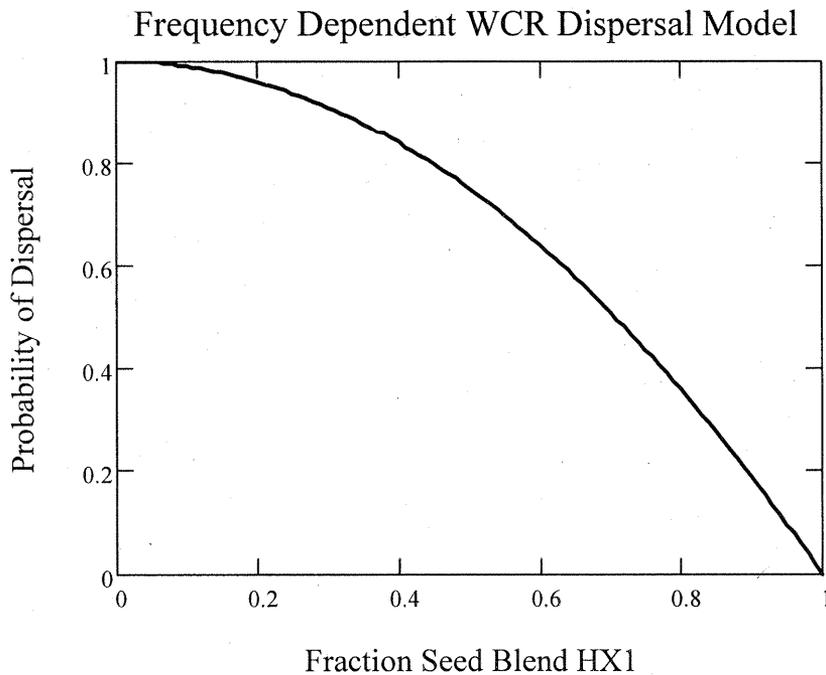


Figure B12. Expected standardized emergence of western corn rootworm adults from plots of corn as a function of the fraction of corn plants that are susceptible to WCR larvae (HX1 genotype) under either a model assuming no dispersal or a model assuming dispersal with probability equal to  $1-p^2$  and the indicated probabilities of dispersal-associated mortality.

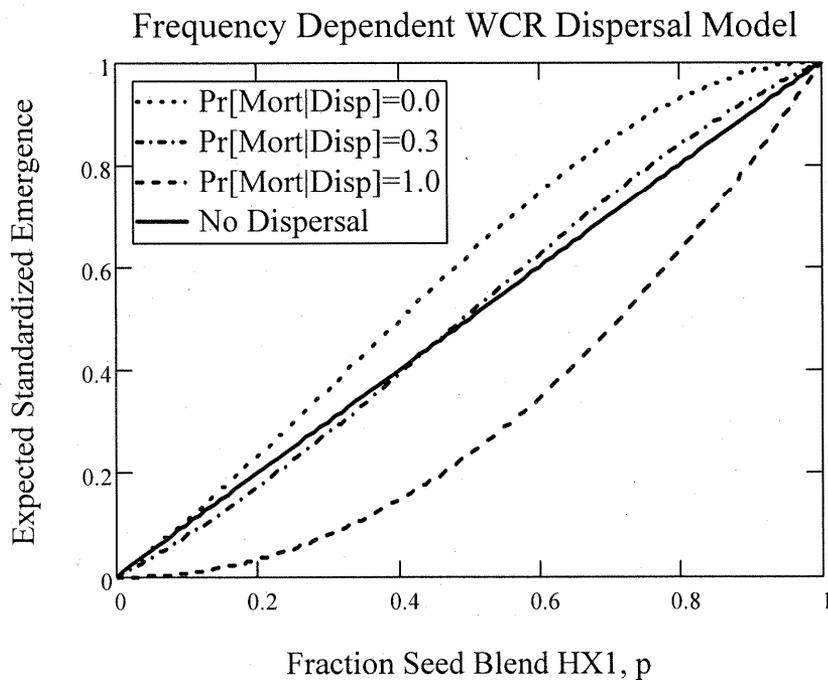


Figure B13. Expected standardized emergence of western corn rootworm adults from plots of corn as a function of the fraction of corn plants that are susceptible to WCR larvae (HX1 genotype) under either a model assuming no dispersal or a model assuming dispersal with probability equal to  $1-p^2$  and the indicated probabilities of dispersal-associated mortality and observed standardized emergence values from two of the four study sites in Nowatzki et al. (2009): Johnston, IA, and Janesville, WI.

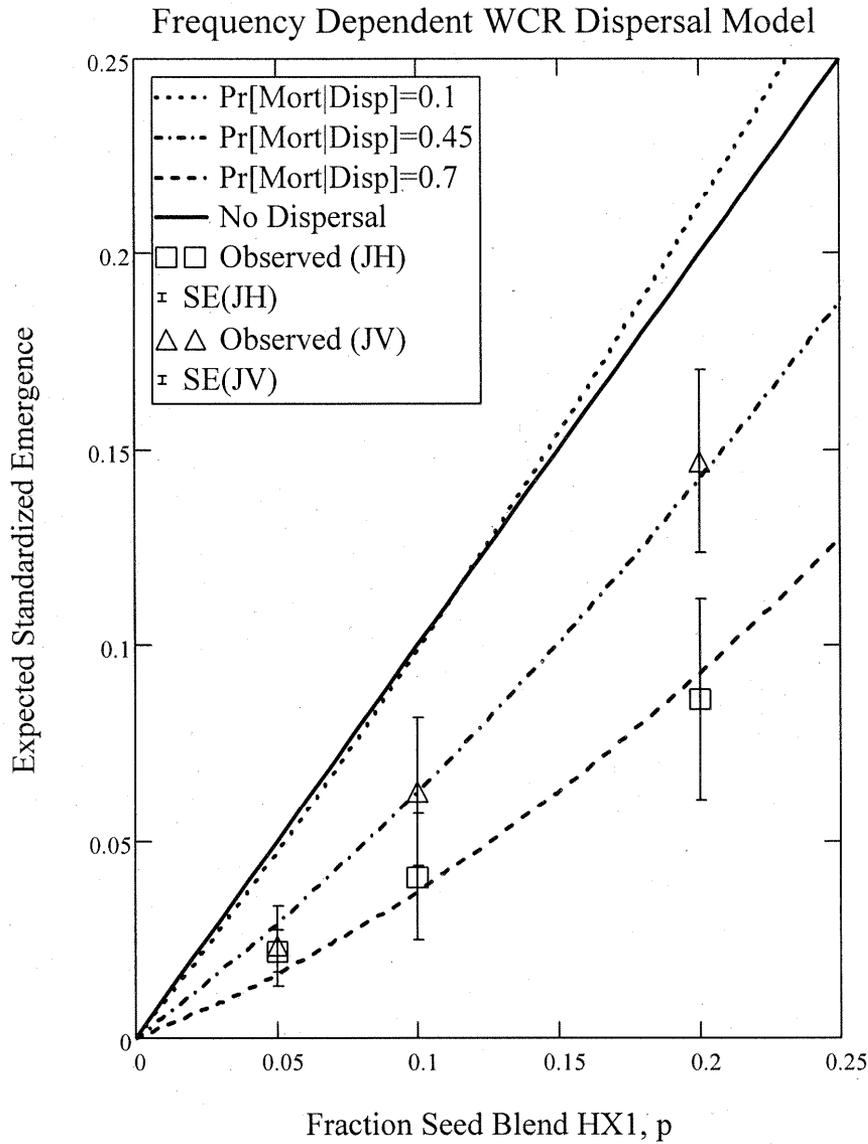
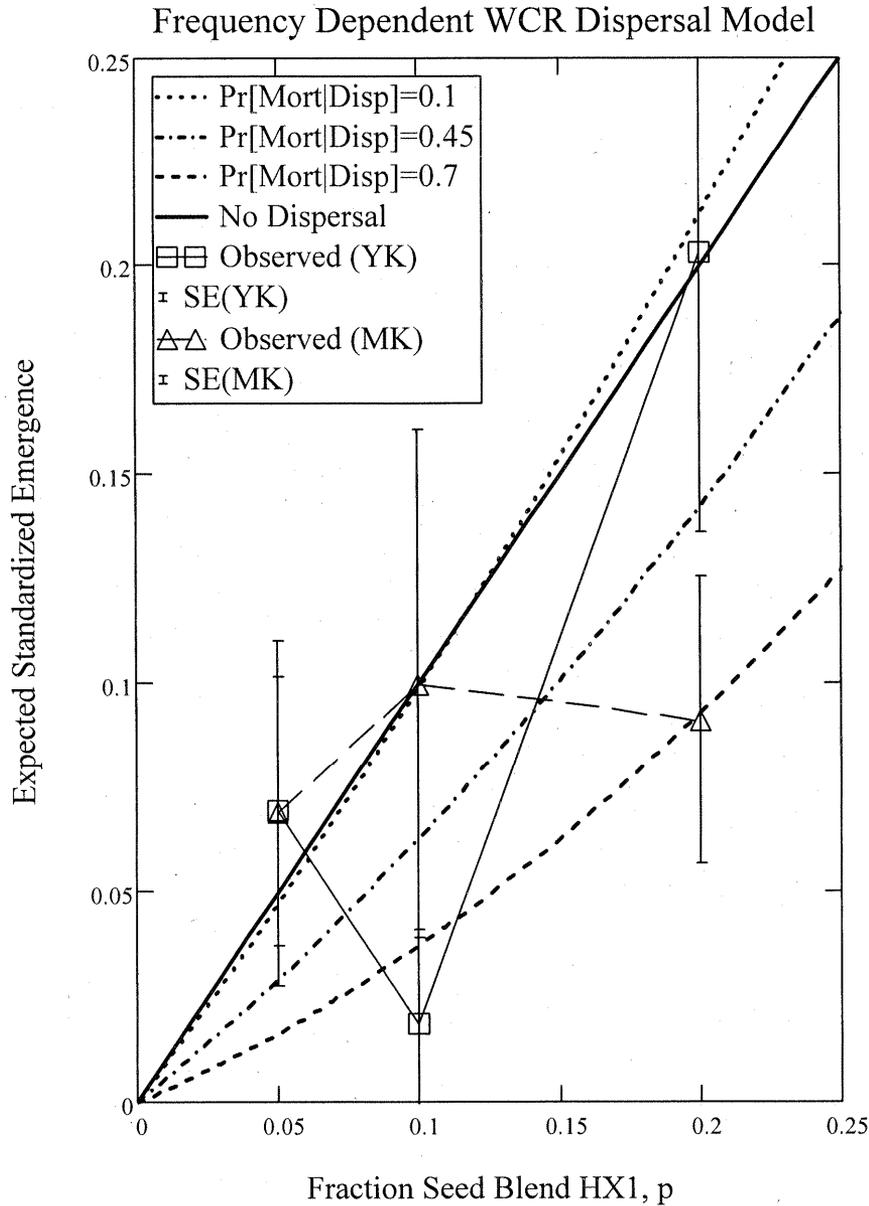


Figure B14. Expected standardized emergence of western corn rootworm adults from plots of corn as a function of the fraction of corn plants that are susceptible to WCR larvae (HX1 genotype) under either a model assuming no dispersal or a model assuming dispersal with probability equal to  $1-p^2$  and the indicated probabilities of dispersal-associated mortality and observed standardized emergence values from two of the four study sites in Nowatzki et al. (2009): York, NE, and Mankato, MN.



## Appendix C: Modeling Larval Movement

The following is a model predicting standardized emergence of the western corn rootworm adults as a function of HX1:HXX corn genotype proportions ( $p:q$ ). Probability of larval dispersal is determined implicitly by the structure of the model and probability of dispersal-associated mortality is incorporated as an adjustable parameter. Under the null hypothesis,  $H_0$ , of no larval movement and no dispersal-related mortality, the expected emergence from a given blend,  $\text{Exp}[{}_{H_0}E_{(p,q)}]$ , is given by the following:

$$\text{Exp}[{}_{H_0}E_{(p,q)}] = p \cdot E_{(100:0)} + q \cdot E_{(0:100)} = (E_{(100:0)} - E_{(0:100)})p + E_{(0:100)}, \quad (\text{Eq. 1})$$

for  $E_{(100:0)}$ , emergence from 100% HX1 plots and  $E_{(0:100)}$ , emergence from 100% HXX plots. From Eq. 1 it would appear useful to define a "standardized" measure of emergence,  ${}_{H_0}EST_{(p,q)}$ , for which the expected value under  $H_0$  can be derived and compared to its observed value:

$${}_{H_0}STE_{(p,q)} \equiv [(E_{(p,q)} - E_{(0:100)}) / (E_{(100:0)} - E_{(0:100)})] \quad (\text{Eq. 2})$$

$$\text{Exp}[{}_{H_0}STE_{(p,q)}] = [\text{Exp}[{}_{H_0}E_{(p,q)}] - E_{(0:100)}) / (E_{(100:0)} - E_{(0:100)})] = p \quad (\text{Eq. 3})$$

$$\text{Obs}[{}_{H_0}STE_{(p,q)}] \stackrel{?}{=} \text{Exp}[{}_{H_0}STE_{(p,q)}] \Rightarrow [(E_{(p,q)} - E_{(0:100)}) / (E_{(100:0)} - E_{(0:100)})] \stackrel{?}{=} p \quad (\text{Eq. 4})$$

Under the proposed alternative model,  $H_A$ , one has the following corresponding relationships:

$$\text{Exp}[{}_{H_A}E_{(p,q)}] = p[p^2 \cdot E_{(100:0)} + 2pq(\frac{1}{2} \cdot E_{(100:0)} + \frac{1}{2}(1-d)(p \cdot E_{(100:0)} + q \cdot E_{(0:100)})) + q^2(1-d)(p \cdot E_{(100:0)} + q \cdot E_{(0:100)})] + q[q^2(1-d)(p \cdot E_{(100:0)} + q \cdot E_{(0:100)})] \quad (\text{Eq. 5})$$

$${}_{H_A}STE_{(p,q)} \equiv [(E_{(p,q)} - E_{(0:100)}) / (E_{(100:0)} - E_{(0:100)})] \quad (\text{Eq. 6})$$

$$\begin{aligned} \text{Exp}[{}_{H_A}STE_{(p,q)}] &= [\text{Exp}[{}_{H_0}E_{(p,q)}] - E_{(0:100)}) / (E_{(100:0)} - E_{(0:100)})] \\ &= \{p^2 + (1-d)(1-p^2)p + (1-d)[(1-p^2)q - 1] (E_{(0:100)} / E_{(100:0)})\} \\ &\quad / [1 - (1-d) (E_{(0:100)} / E_{(100:0)})] \quad (\text{Eq. 7}) \end{aligned}$$

$$\begin{aligned} \text{Obs}[{}_{H_A}STE_{(p,q)}] &\stackrel{?}{=} \text{Exp}[{}_{H_A}STE_{(p,q)}] \Rightarrow \\ &[(E_{(p,q)} - E_{(0:100)}) / (E_{(100:0)} - E_{(0:100)})] \stackrel{?}{=} \{p^2 + (1-d)(1-p^2)p + (1-d)[(1-p^2)q - 1] (E_{(0:100)} / E_{(100:0)})\} \\ &\quad / [1 - (1-d) (E_{(0:100)} / E_{(100:0)})] \quad (\text{Eq. 8}) \end{aligned}$$

The following is an implementation of the above described model in MathCad. (Version 13, Mathsoft Engineering & Education, Inc., Cambridge, MA).

$p$   $\equiv$  fraction HX1 in seed blend  
 $d$   $\equiv$  probability of dispersal-associated mortality  
 $ER_{HX1toHXX}$   $\equiv$  ratio emergence from 100% HX1 to 100% HXX  
 $ExpEHA$   $\equiv$  expected standardized emergence (ESE)  
 $M$   $\equiv$  matrix of observed standardized emergence OSE and SE[OSE]  
 by blend proportion and location  
 $PrDisp$   $\equiv$  probability of dispersal by WCR larva from initial plant

$$i := 0..100$$

$$j := 0, 1..100$$

$$p_i := \frac{i}{100}$$

$$q_i := (1 - p_i)$$

$$d_j := \frac{j}{100}$$

$$ER_{HX1toHXX} := \frac{1}{30}$$

$$ExpEHA_{i,j} := \frac{\left[ (p_i)^2 + (1 - d_j) \cdot [1 - (p_i)^2] \cdot p_i \right] + \left[ [1 - (p_i)^2] \cdot q_i - 1 \right] \cdot ER_{HX1toHXX}}{1 - ER_{HX1toHXX}}$$

$$M := \begin{pmatrix} 0.05 & 0.0693 & 0.0371 & 0.1015 & 0.0219 & 0.0166 & 0.0272 & 0.0691 & 0.0277 & 0.1105 & 0.0234 & 0.0133 & 0.0335 \\ 0.10 & 0.0185 & -0.0041 & 0.0411 & 0.0409 & 0.0249 & 0.0569 & 0.1000 & 0.0393 & 0.1607 & 0.0628 & 0.0437 & 0.0819 \\ 0.20 & 0.2032 & 0.1364 & 0.2700 & 0.0863 & 0.0605 & 0.1121 & 0.0912 & 0.0569 & 0.1255 & 0.1470 & 0.1235 & 0.1705 \end{pmatrix}$$

$$x := M^{(0)}$$

$$y_{YK} := M^{(1)} \quad y_{JH} := M^{(4)} \quad y_{MK} := M^{(7)} \quad y_{JV} := M^{(10)}$$

$$SELy_{YK} := M^{(2)} \quad SELy_{JH} := M^{(5)} \quad SELy_{MK} := M^{(8)} \quad SELy_{JV} := M^{(11)}$$

$$SEUy_{YK} := M^{(3)} \quad SEUy_{JH} := M^{(6)} \quad SEUy_{MK} := M^{(9)} \quad SEUy_{JV} := M^{(12)}$$

$$PrDisp_i := 1 - (p_i)^2$$