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Research Article

Using Seed Mixes in Conjunction with Structured Refuges

Caprio[*1], M. A., & J. C. Martinez[1]
[1]Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology
Mississippi State University

*corresponding author email: mcaprio@entomology.msstate.edu

Abstract: The U.S. Environmental Protection Agency recently approved, for areas of the Southern U.S., a 5% seed-mixture (Refuge-In-A-Bag or RIB) combined with a 20% external structured refuge for pyramided Bt corn. In this analysis we compared the durability of a pyramided PIP (plant incorporated protectant) deployed with a 20% refuge versus a 5% RIB and an additional 20% external refuge when the larval movement proportion was varied. The results showed that when the PIP expressed a very high dose (overall mortality of susceptibles = 99.99%) and larval movement was 50% and 90%, the 20% external refuge was 38% and 52% more durable, respectively, than the external + RIB refuge strategy despite the presence of additional refuge in the latter scenario. When the PIP was a high dose (overall mortality = 99.75%) and larval movement was 50% and 90%, then the 20% block refuge was 10% and 20% more durable, respectively. Conversely, for a low dose PIP, the combined refuge strategy with 50% and 90% larval movement was 10% and 5% more durable, respectively, than the 20% external refuge. *Helicoverpa zea* has been shown to be highly mobile, and one should therefore proceed with caution before deploying RIBs in major cotton growing regions until a careful analysis of each PIP has been conducted.

Keywords: Resistance management, *Bacillus thuringiensis*, larval movement

Introduction

Seed blends incorporating *Bacillus thuringiensis* (Bt) and non-Bt seeds, also known today as Refuge-in-a-Bag (RIB), have been a topic of discussion for over two decades. Various researchers have indicated that under some circumstances this insect resistance management (IRM) approach could lead to increased resistance evolution in some pests (Mallet and Porter 1992, Tabashnik 1994, Onstad and Gould 1998, Davis and Onstad 2000). For example, Lepidopteran larvae that are mobile and engage in inter-plant movement may have the opportunity to escape Bt exposure in RIB fields and finish development on non-Bt expressing plants. This scenario describes one possible pathway of heterozygote (*rs*) selection in RIBs that increases the rate of resistance and was the FIFRA (Federal Insecticide, Fungicide and Rodenticide Act) Science Advisory Panels' (SAPs) primary concern when they discouraged the use of seed blends to control Lepidoptera pests (EPA SAP 1998, EPA SAP 2000). Conversely, the 2009 SAP (EPA SAP 2009) concluded that a 20% seed blend strategy for Bt corn with less than high dose effects on corn rootworm was supported by the insect's limited larval movement. The 2010 SAP (EPA Scientific Advisory Panel 2011) reiterated the original concern with seed blends for mobile Lepidoptera if the Bt toxins were not high dose. *Helicoverpa zea*, a major pest of corn and cotton, is a general example of the SAPs' arguments: it has been shown to engage in significant larval movement, and none of the single toxins currently registered in Bt corn or Bt cotton is truly high-dose. It is even questionable whether the currently registered pyramided PIPs (plant incorporated protectants) express a high-dose against *H. zea* (EPA 2010a). Additionally, in a RIB, field pollination of corn will occur with a mixture of Bt and non-Bt pollen, ensuring a mosaic of Bt and non-Bt kernels in a corn ear. *Helicoverpa zea* larvae may, therefore, selectively feed on less toxic kernels in this configuration. An ear of corn becomes a "RIB" within a RIB. Such sub-lethal exposure would give heterozygous larvae a fitness advantage, and resistance would increase as a result.

A 20% external refuge has been the standard requirement by EPA for pyramided Bt corn in the major U.S. cotton growing regions (Gould and Tabashnik 1998). Recently though, EPA registered Bt corn pyramided PIPs with a 5% RIB, but with an additional external 20% refuge, to target Lepidopteran pests such as *H. zea* in the South (e.g., EPA 2010b). It is unclear at this point whether this external refuge will be sufficient to mediate the increased selection pressure in RIBs and *H. zea.* The seed blend strategy may increase the risk of

resistance relative to pure stand Bt with an external refuge. Our analysis aims to provide some first insights into the uncertainties with RIBs and *H. zea* in major cotton growing regions.

Materials and Methods

The EPA-ORD (Office of Research and Development) model (Caprio and Glaser 2010), which included either RIBs with larval movement or a structured refuge without larval movement, was modified to include both strategies simultaneously. This model, a two locus monogenic resistance model, assumes that all adults form a common random mating pool will produce a cohort of eggs in Hardy-Weinberg equilibrium. The population had an initial resistance allele frequency of 0.001. These eggs were divided into two pools, one representing the Bt fields and the second representing the structured (external) non-transgenic refuges. There was no larval movement between these two pools. The pool representing Bt fields was further divided into Bt plant and non-Bt plant portions in simulations of RIBs. Because these were components of a seed-mix, larval movement was allowed between these two pools of insects. Each insect population pool consisted of a complete 3x3 array representing the relative frequencies of all nine genotype combinations. Because all adults were assumed to mate at random, the total population at the start of each generation was renormalized so that the frequencies summed to 1.0. A proportion R of this population moved into the structured refuge pool. Of the proportion (1-R) that moved into the Bt field, a proportion M ended up in the non-Bt pool of the seed-mix. The program then looped through D segments of larval development. D was the number of segments into which larval development was divided, often, though not necessarily, in days. Natural mortality was assumed constant in all habitats and therefore not implicitly included in the model. If the larval survivorship in response to Bt-toxins over D segments was W_{ssss} ($W_{s1s1} * W_{s2s2}$), then at the start of each loop $\sqrt[D]{W_{ssss}}$ proportion of the population survived. This larval survivorship varied with genotype of the insect and the habitat (plant type) the insect was on for any given day. Larval movement followed survivorship. If a proportion G of the larval population was expected to remain on the same plant throughout development, then $1-\left(\sqrt[D]{G}\right)$ proportion of the population would move during each larval developmental segment. When a larva moved in the seed-mix, it had a probability M of moving to a non-Bt plant and a probability (1-M) of ending up on a Bt plant. The objective of the

model was that it should yield the same result when larval movement was set to zero (G = 1) as the original EPA-ORD model when used without larval movement. We believe this model design exceeds the suggestion by the EPA-SAP (2011) of four larval movement scenarios as it simultaneously includes potential movement of susceptibles that initially start on Bt plants onto non-Bt plants, as well as movement over time of susceptibles and heterozygotes off of non-Bt plants and onto Bt plants. Because the relative survival of heterozygotes compared to susceptible homozygotes varies with time of movement, movement at different age classes will have a different impact on the evolution of resistance. The current model incorporates this variation without forcing parameters to limit larval movement to one or two episodes of movement. (The model, for two larval segment development periods (D=2), would proceed as follows (substituting the appropriate fitness W for other genotypes):

Habitat   Proportion of original eggs

Population distribution after initial oviposition

Refuge   R

RIB   M(1-R)

Bt plants   (1-M)(1-R)

Population distribution after selection in segment 1

Refuge   R

RIB   M(1-R)

Bt plants $(1\text{-}M)(1\text{-}R)\ \sqrt[D]{W_{ssss}}$

Population distribution after movement in segment 1

Refuge   R

RIB $\left[\left(\sqrt[D]{G}\right)+[\sqrt[D]{W_{ssss}}\ (1-M)+(M)](1-\left(\sqrt[D]{G}\right))\right](M)(1-R)$

Bt plants $\left[\left(\sqrt[D]{G}\right)\sqrt[D]{W_{ssss}}+[\sqrt[D]{W_{ssss}}\ (1-M)+(M)](1-\left(\sqrt[D]{G}\right))\right](1-M)(1-R)$

Population distribution after selection in segment 2

Refuge   R

RIB $\left[\left(\sqrt[D]{G}\right)+[\sqrt[D]{W_{ssss}}\ (1-M)+(M)](1-\left(\sqrt[D]{G}\right))\right](M)(1-R)$

Bt plants $\left[[\left(\sqrt[D]{G}\right)\sqrt[D]{W_{ssss}}+[\sqrt[D]{W_{ssss}}\ (1-M)+(M)](1-\left(\sqrt[D]{G}\right))](1-M)(1-R)\right]\ \sqrt[D]{W_{ssss}}$

Movement in segment 2 is unimportant because it is followed by complete random mating and mixing of all populations.

　　　To understand the impact of combining a seed-mixture strategy with a structured refuge, the model was modified to include both a seed-mixture refuge (with larval movement) and a structured refuge (Figure 1).
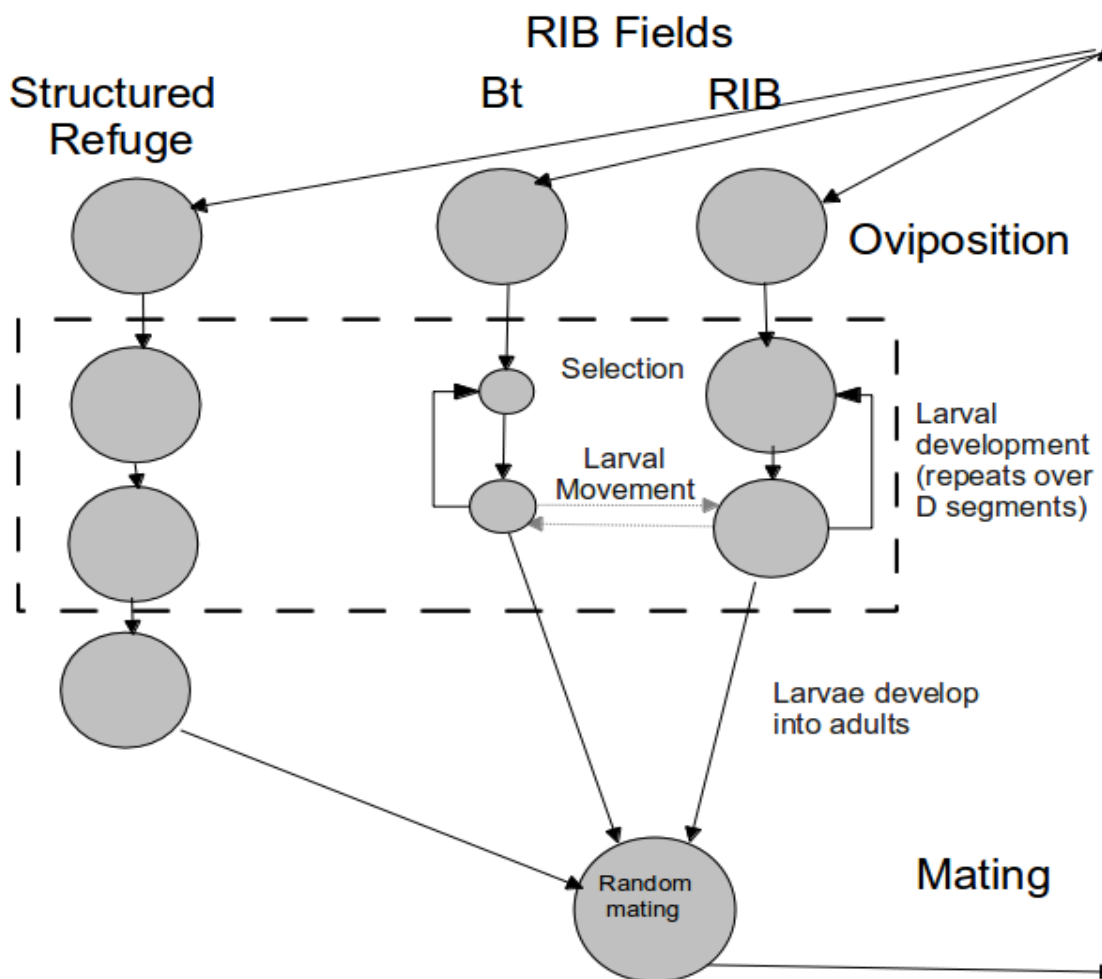

Figure 1. A schematic of the relationships between the different compartments of the model. Each compartment is a portion of the population that is treated uniformly except for differences in survivorship due to genotype.

Three scenarios were examined, a very high dose (0.01% survivorship in the presence of both toxins, each toxin caused 99% mortality to homozygous susceptible insects; a high dose (0.25% overall survivorship, each toxin caused 95% mortality to homozygous susceptible insects); and a low dose environment (5% overall survivorship, each toxin caused 77.7% mortality to homozygous susceptible insects). The fitness of resistant homozygotes was assumed to be 1.0. The high dose scenarios used a dominance of 0.1, while the dominance was raised to 0.3 for the low dose scenario as data suggest that dominance normally increases with decreasing dose (Caprio and Sumerford 2007).

Results and Discussion

Combining seed-mixtures with structured refuges has been suggested as a method to increase overall refuge size (Tabashnik 1994, Carriere et al. 2004). When a 5% seed-mixture was simulated in combination with a 20% refuge in high-dose environments, the results were dependent on the amount of larval movement (Figures 2-4).
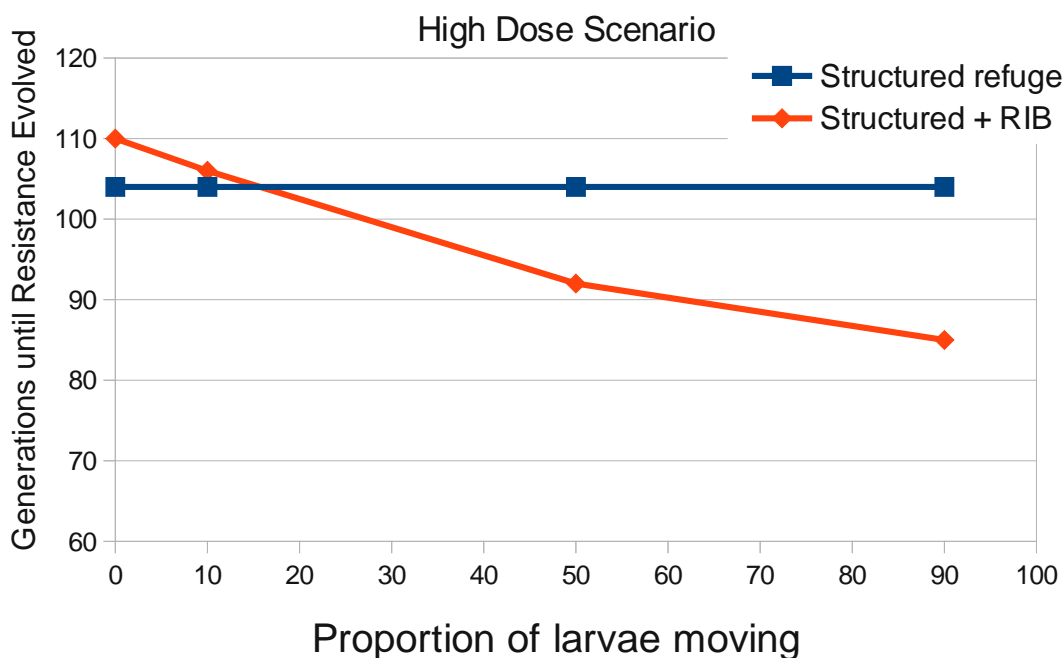

Figure 2. The effect of combining a RIB (Refuge-In-A-Bag) with an external refuge for different movement rates for a dual toxin high dose product (overall mortality of homozygous susceptible individuals due to both toxins = 99.75%). The dominance in these simulations was 0.1.
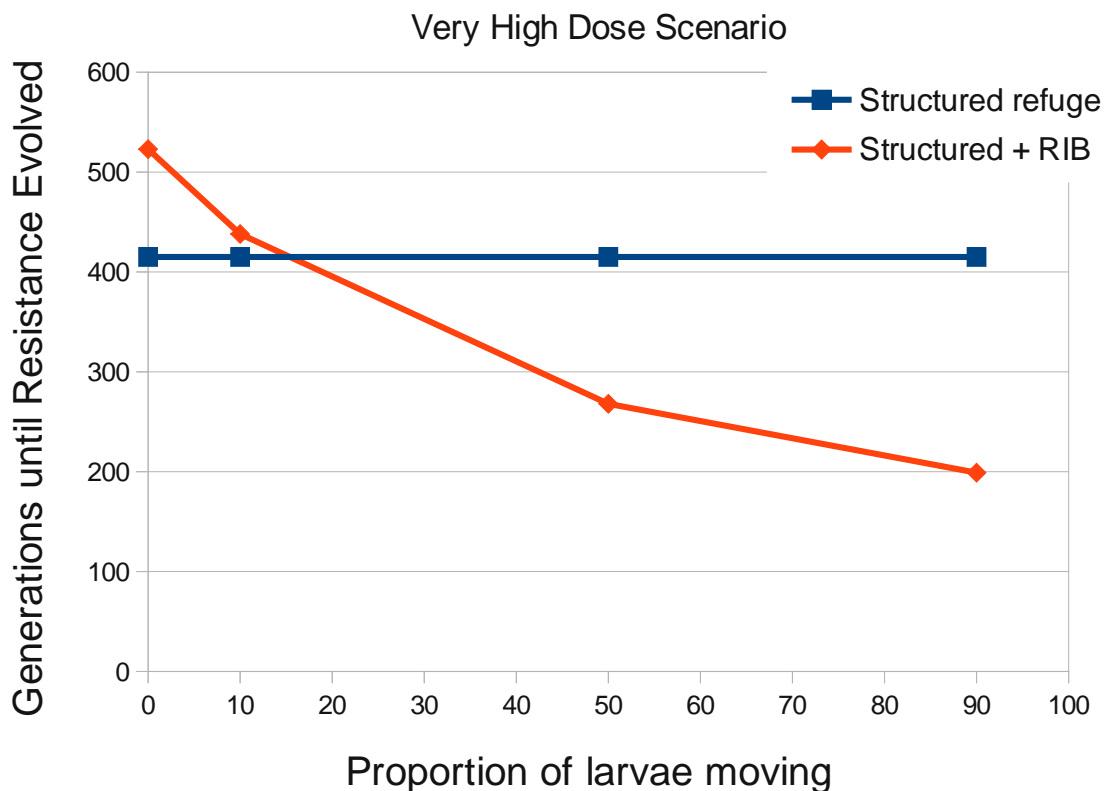
Very High Dose Scenario

Figure 3. The effect of combining a RIB (Refuge-In-A-Bag) with an external refuge for different movement rates for a dual toxin very high dose product (overall mortality of homozygous susceptible individual due to both toxins = 99.99%). The dominance in these simulations was 0.1.

When there was no larval movement, the combination of seed-mixture and structured refuge delayed resistance longer than the structured refuge alone because there were more non-Bt plants across all habitats (24% versus 20%). A 24% structured refuge with no seed-mixture also yielded the same amount of delay demonstrating that it is increased proportion of non-Bt plants that causes increased delay in resistance. As the amount of movement increased, the combination seed-mixture/structured refuge became less effective at managing resistance than the structured refuge strategy, even though the latter had less total refuge.

In a low-dose environment, where the overall survivorship to the combined toxins was 5% and the dominance was increased to 0.3, the seed-mixture added durability to the structured refuge, at least with movement rates as high as 90% (Figure 4).
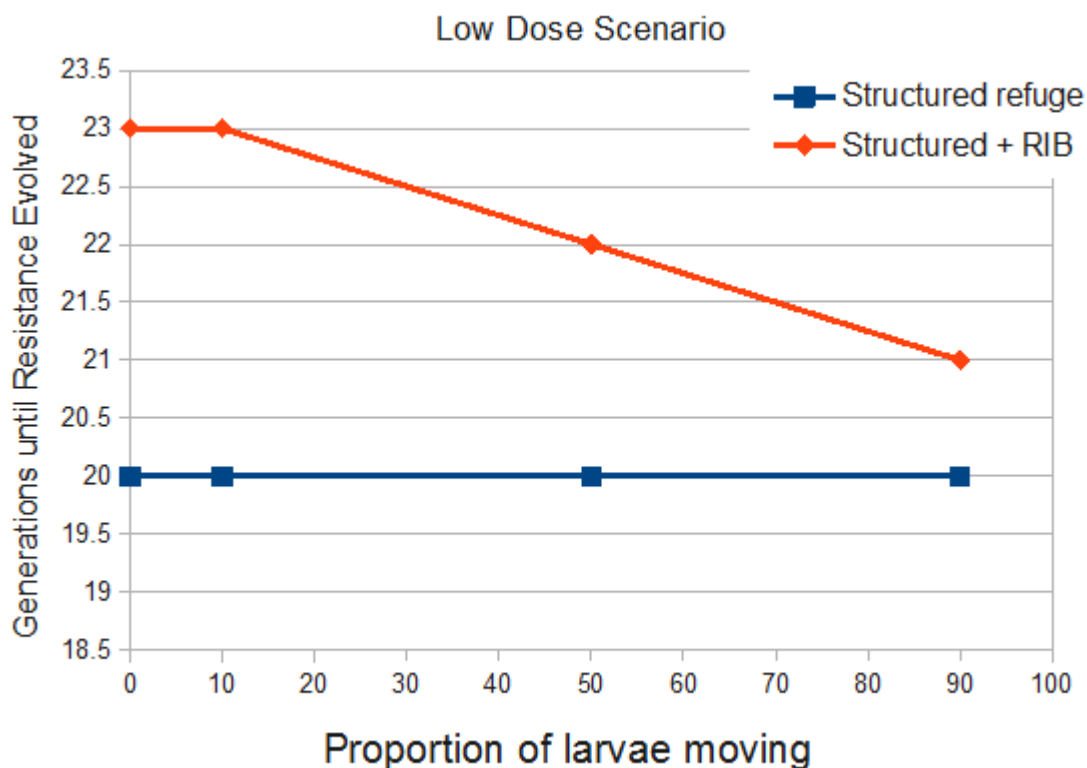

Figure 4. The effect of combining a RIB (Refuge-In-A-Bag) with an external refuge for different movement rates for a dual toxin low dose product (overall mortality of homozygous susceptible individual due to both toxins = 95%). The dominance in these simulations was 0.3.

The presence of the RIB had the most negative impact when the movement rate was high and the dose was very high (it decreased the time for resistance evolution by 52% at a movement rate of 90%). In the high dose scenario, the decrease in durability due to having the RIB present was 18% at 90% movement. Conversely, at the lowest dose, the presence of the RIB increased the durability and time for resistance evolution by 5% even at a movement rate of 90%. For *H. zea* and *H. armigera* (a close relative), studies have shown that typical movement rates on non-Bt cotton ranged from a high of 80-95% and movement on Bt cotton exceeded 95%

(Gore et al. 2002, Men et al. 2005). Based on these modeling results and previously published data on larval movement, care should be taken before a Bt-corn RIB with an external refuge is deployed in major cotton growing regions. Additionally, should *H. zea* exhibit lower rates of plant-to-plant movement on corn than previously documented in cotton, then there still exists the concern that a corn ear presents a RIB within the RIB field (because of pollination with Bt and non-Bt pollen). Corn ears that express a mosaic of lethal and sub-lethal doses of the toxin could allow selectively feeding, heterozygous larvae with a fitness advantage and increase the rate of resistance evolution. Further work is needed to theoretically evaluate the 'RIB within the RIB' (+ external refuge) for *H. zea* in major cotton growing regions.

Acknowledgements

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