

EVALUATING BT IRM REQUIREMENTS IN NORTH CAROLINA THROUGH COMPUTER MODELING

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Abstract

A simulation model of *Helicoverpa zea* resistance development to Bt crops in eastern North Carolina (Storer et al. 2003) was modified in order to evaluate the impact of EPA mandated non-Bt refuges and soybean acreage on the rate of resistance evolution. The model was further refined to include pyramided corn and cotton cultivars; however, *H. zea* survival on pyramided cultivars was based solely on theoretical data. To convincingly identify the most influential factors in resistance evolution, empirical estimates of *H. zea* survival on these cultivars were incorporated into the model. The model predicts that pyramided Bt cultivars and soybean as a host both greatly delay resistance development. With pyramided Bt cultivars, the model also predicts that the 20% sprayed non-Bt cotton refuge's contribution to delaying resistance evolution is greatly supplemented by other non-Bt sources of susceptible moths and has less of an effect on Bt resistance management than the non-Bt corn refuge.

Introduction

The percentage of North Carolina cotton acreage planted to Bollgard® varieties has risen from 3% in 1996 to approximately 90% in 2004 (J. Bacheler, pers. comm. Oct. 2005). While *Helicoverpa zea* lab colonies have developed resistance to Bt toxins, no populations of any insect pest have shown resistance to Bt plants in the field (Tabashnik et al. 2003). Furthermore, there has been no detectable increase in resistance allele frequency in North Carolina (Jackson et al. 2002). Since the introduction of Bt cotton, the EPA has mandated plantings of non-Bt crop refuges to provide a source of susceptible insects necessary to delay resistance evolution.

Recent empirical evidence indicates that the mandated 20% sprayed non-Bt cotton refuge may not be contributing substantially to delaying resistance evolution in *H. zea* to Bt crops. A stable carbon isotope analysis of *H. zea* wings (Gould et al. 2002) indicated that C4 plants (e.g. corn) are the major larval hosts in the early-to-mid season in LA and TX; 90 -100% of tested moths developed as larvae on a C4 host. Later in the season when cotton is an attractive host, <50% of moths were produced from C3 hosts (i.e. cotton, soybean, and other non-grass hosts). However, stable carbon isotope analysis cannot differentiate among C3 host crops. Data from unsprayed field plots in eastern North Carolina provide estimates of *H. zea* adult production on corn, cotton, peanut, and soybean late in the season. Based on equal plot size, 19.17% of the adults were produced from corn, 36.09% on cotton, 5.64% on peanut, and 39.10% on soybean (Jackson et al. 2003). Since these estimates were based on equal plot sizes, average planted acres for 2000-2002 for each crop (NCDA, 2003) were used to estimate the proportion of adults produced from each crop in eastern NC. This resulted in an estimated 13.78% of the late season adults produced on corn, 33.18% on cotton, 0.63% on peanut, and 52.40% on soybean. Since nearly 100% of the early season moths were produced from corn, non-Bt corn appears to be the most useful early season crop host refuge. Soybean serves as a major late season non-Bt host and may play a more pivotal role than a 20% sprayed or 5% un-sprayed non-Bt cotton refuge in delaying resistance evolution to Bt in eastern North Carolina. The above concerns only production on the major crop hosts and ignores production on the numerous *H. zea* minor crop hosts, wild hosts, and immigration of moths from other areas with different crop mixes.

Another factor that will influence Bt resistance evolution is the introduction of pyramided Bt cultivars containing two different toxins. Pyramiding Bt events exploits "redundant killing" where totally susceptible insects are killed by both toxins and insects resistant to one toxin are killed by the other toxin. This approach will only be effective in the absence of cross-resistance between the pyramided toxins and when the doses of each toxin are high enough to result in substantial redundant killing (Andow and Hutchinson 1998, Gould 1998). In 2003, Monsanto marketed Bollgard II® that expresses Cry1Ac and Cry2Ab and prior to 2003 was testing an experimental pyramided field corn hybrid (Mon84006/Mon810) that expresses Cry1Ab and Cry2Ab. Pyramided cultivars provide increased efficacy against *H. zea* and may decrease the rate at which resistance evolves (Jackson et al. 2004, Roush 1998).

Herein we use an existing simulation model originally described in Storer et al. (2003) and modified by Livingston (unpublished manuscript) to simulate the use of pyramided Bt corn and cotton cultivars. This work does not represent an in depth sensitivity analysis of parameter interactions but purely uses current empirical data to evaluate the expected role of EPA mandated non-Bt refuges, pyramided Bt cultivars, and soybean as a host in *H. zea* resistance development to Bt in eastern North Carolina.

Methods and Materials

As described by Storer et al. (2003a & b), "The model [was] developed ... to accommodate a spatial mix of two host crops (corn and cotton), and to reflect the agronomic practices, as well as the spatial and temporal population dynamics of *H. zea* in eastern North Carolina." The original model was further refined by Storer to include soybean as a host and by Mike Livingston to include pyramided corn and cotton cultivars; however, at that time there were no empirical data on which to base the fitness of *H. zea* genotypes susceptible to Cry2Ab and those susceptible to both Cry2Ab and Cry1A. To convincingly identify the most influential factors in resistance evolution, empirical estimates of *H. zea* survival on cultivars containing these Bt Cry proteins both singly and pyramided was needed for incorporation into the model. For these estimates, we utilized *H. zea* adult production data on pyramided and single toxin Bt corn and cotton from our research (Table 1 & 2).

We used fitnesses of susceptible ($rr\ r'r'$) larvae on non-Bt, Cry1A, Cry2Ab, and pyramided plants to calculate fitnesses of all genotypes (Table 3). On the pyramided plants, the genotype with resistance to Cry1A and Cry2Ab ($RR\ R'R'$) was assumed to have equal fitness to susceptible larvae on non-Bt plants, minus the general fitness cost (0.025) of carrying each resistance allele. Those larvae only resistant to Cry1A ($RR\ r'r'$) received the fitness value of a susceptible individual on a plant with only Cry2Ab since that was the only toxin affecting their survival. Similarly, those larvae that are only resistant to Cry2Ab ($rr\ R'R'$) have the fitness value of susceptible larvae on Cry1A plants. Larvae susceptible to both toxins ($rr\ r'r'$) simply were assigned a fitness value calculated directly from field collected susceptible larvae that developed on pyramided plants. Heterozygote fitness was calculated based on additive inheritance.

The model was run under a specific set of assumptions not limited to but including: 1) initial Cry1A resistance allele frequency = 0.00043 and Cry2A = 0.00039 (Burd et al. 2003), 2) a single gene associated with resistance to each toxin (R and r = alleles for resistance or susceptibility to Cry1Ac and Cry1Ab; R' and r' = alleles for resistance or susceptibility to Cry2Ab), 3) Cry1Ab and Cry1Ac share complete cross-resistance, 4) 0.025 additive fitness cost for carrying each resistance allele, 5) degree of dominance = 0.5, 6) development time delay for susceptible larvae = 6 days on corn and 8 days on cotton, 6) including soybean in the model, 47% of the acreage is planted to soybean, 24% to corn, and 29% to cotton; excluding soybean in the model, 0% of the acreage is planted to soybean, 44% corn, and 56% cotton. (NCDA, 2003) 8) Replacement rates with soybean: $R1 = 4$, $R2 = 35$, $R3 = 6$; Replacement rates without soybean: $R1 = 1.5$, $R2 = 75$, $R3 = 10$. (Storer 1999)

The model was run with five different Bt cultivar combinations: 1) Bollgard cotton & Yieldgard® corn (BG/YG), 2) Bollgard II cotton & Yieldgard corn (BGII/YG), 3) Bollgard II cotton & pyramided field corn (BGII/PYG), 4) Bollgard cotton & Pyramided field corn (BG/PYG), 5) Bollgard II cotton & pyramided field corn phase out Bollgard cotton & Yieldgard corn over a three year period (Phase out). Also, five non-Bt refuge plans were simulated: 1) the currently EPA mandated 50% non-Bt corn refuge and 20% sprayed cotton refuge, 2) a 0% non-Bt corn refuge and a 20% sprayed cotton refuge, 3) a 50% non-Bt corn refuge and a 0% non-Bt cotton refuge, 4) a 20% non-Bt corn refuge and a 20% sprayed cotton refuge, 5) and a 0% non-Bt corn refuge and a 0% non-Bt cotton refuge. When simulating the Bollgard cotton & Yieldgard corn phase out, we assumed a 33.33% adoption rate resulting in all

farms planting pyramided cultivars by the third year. Model runs without mandated EPA non-Bt refuges were compared to the currently mandated EPA plan for each cultivar combination to estimate the contribution of each non-Bt crop refuge in delaying Bt resistance evolution. To evaluate the role of soybean as an alternate host in delaying resistance evolution, the model was run with Bt cultivar combinations at the currently mandated refuge sizes both with and without soybean as a host. Resistance was considered problematic when resistance allele frequencies for both Cry1A and Cry2Ab are > 0.50 .

When the model was run assuming the current resistance allele frequency estimates for Cry1A (0.00043) and Cry2Aa (0.00039) (Burd et al. 2003), the combined effect of high mortality in pyramided corn, proportion of *H. zea* developing on corn in generations 1 and 2, and low winter survival resulted in population eradication when the non-Bt corn refuge was removed from the BGII/PYG and BG/PYG cultivar combinations. Thus, to make comparisons between the currently required refuge sizes and removal of the non-Bt corn refuge in these cultivar combinations, initial resistance allele frequencies 2.33 – 2.56 times higher than the empirical estimates and replacement rates approximately 2 times higher per generation than those assumed by Storer (1999) when modeling single gene crops with soybean as an alternate host had to be assumed when eliminating the non-Bt corn refuge. These assumptions hastened resistance development for all Bt cultivar combinations but did not drastically affect the relative rate of resistance development between refuge strategies. Results using these parameters are followed by an asterisk (*).

Results

Effects of soybean as a host and pyramided cultivars

Following current EPA refuge requirements and excluding soybean as an alternate host, the frequency of resistance alleles reached 0.50 after 6 years of growing BG/YG. However, the resistance allele frequency surpassed 0.50 after 10 years with soybean as a host, a 1.67 fold delay. Following current EPA refuge requirements and simulating a BGII/YG cultivar combination, the frequency of resistance alleles reached 0.50 after 14 years when soybean was excluded and 28 years when included, a 2 fold delay. Finally, following current EPA refuge requirements and simulating a BGII/PYG cultivar combination, the frequency of resistance alleles reached 0.50 after 17 years when soybean was excluded and 56 years when included, a 3.29 fold delay. From these results, the effect of pyramided gene cultivars versus single gene cultivars can also be determined. With soybean as a host, simulating a BGII/YG cultivar combination resulted in a 2.8 fold delay compared to the BG/YG cultivar combination, a BG/PYG cultivar combination resulted in a 4.70 fold delay compared to the BG/YG cultivar combination and a BGII/PYG cultivar combination resulted in a 5.60 fold delay versus the BG/YG cultivar combination (Figure 1).

Eliminating non-Bt cotton refuge (including soybean as a host)

When simulating a BG/YG cultivar combination with soybean as a host, eliminating the 20% sprayed non-Bt cotton refuge resulted in a > 0.50 resistance allele frequency after 7 years indicating a 1.14 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4). Eliminating the non-Bt cotton refuge from a BGII/YG cultivar combination resulted in a > 0.50 resistance allele frequency after 24 years indicating a 1.16 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4). Eliminating the non-Bt cotton refuge from a BG/PYG cultivar combination resulted in a > 0.50 resistance allele frequency after 23* years indicating a 1.86 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4). Finally, eliminating the non-Bt cotton refuge from a BGII/PYG cultivar combination resulted in a > 0.50 resistance allele frequency after 43 years indicating a 1.30 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4).

Reducing the non-Bt corn refuge (including soybean as a host)

When simulating a BG/YG cultivar combination, reducing the 50% unsprayed non-Bt corn refuge to a 20% unsprayed non-Bt corn refuge resulted in a > 0.50 resistance allele frequency after 7 years indicating a 1.43 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4). Reducing the non-Bt corn refuge with a BGII/YG cultivar combination resulted in an > 0.50 resistance allele frequency after 22 years indicating a 1.27 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4). Reducing the non-Bt corn refuge with a BG/PYG cultivar combination resulted in an > 0.50 resistance allele frequency after 25* years indicating a 1.68 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4). Finally, reducing the 50% unsprayed non-Bt corn refuge to a 20% unsprayed non-Bt corn refuge with a BGII/PYG cultivar combination resulted in a > 0.50 resistance

allele frequency after 41 years indicating a 1.37 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4).

Eliminating non-Bt corn refuge (including soybean as a host)

When simulating a BG/YG cultivar combination, eliminating the 50% unsprayed non-Bt corn refuge resulted in a > 0.50 resistance allele frequency after 5 years indicating a 2 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4). Eliminating the non-Bt corn refuge from a BGII/YG cultivar combination resulted in a > 0.50 resistance allele frequency after 13* years indicating a 1.62 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4). Eliminating the non-Bt corn refuge from a BG/PYG cultivar combination resulted in an > 0.50 resistance allele frequency after 12* years indicating a 3.5 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4). Finally, eliminating the non-Bt corn refuge from a BGII/PYG cultivar combination resulted in a > 0.50 resistance allele frequency after 14* years indicating a 2.29 fold delay realized by following the current refuge requirements with the same cultivar combination (Table 4).

Eliminating both non-Bt crop refuges (including soybean as a host)

For all cultivar combinations, eliminating both the non-Bt corn refuge & non-Bt cotton refuge resulted in a > 0.50 resistance allele frequency no more than one year sooner than only eliminating the non-Bt corn refuge (Table 4).

Phase out of Bollgard and Yieldgard

When simulating the phase out of Bollgard cotton & Yieldgard corn over a 3 year period, resistance allele frequency exceeded 0.50 in 50 years hastening resistance development by 6 years (a 1.12 fold increase) when compared to replacing single gene cultivars with pyramided cultivars in year one while following the current refuge requirements. When simulating the phase out with no non-Bt cotton refuge, resistance allele frequency exceeded 0.50 in 39 years hastening resistance development by 4 years (a 1.10 fold increase). When simulating the phase out with no non-Bt corn refuge, resistance allele frequency exceeded 0.50 in 8 years hastening resistance development by 6 years (a 1.75 fold increase). When simulating the phase out with a smaller 20% non-Bt corn refuge and a 20% sprayed cotton refuge resistance allele frequency exceeded 0.50 in 18 years hastening resistance development by 9 years (a 1.5 fold increase). When simulating the phase out with a smaller 20% non-Bt corn refuge and a 0% sprayed cotton refuge resistance allele frequency exceeded 0.50 in 16 years hastening resistance development by 8 years (a 1.5 fold increase) (Table 4).

Discussion

The mandated 50% non-Bt corn refuge and soybean as an alternate host are predicted to substantially slow the rate of resistance development in eastern North Carolina *H. zea* populations when pyramided crops are simulated. In comparison, the 20% sprayed cotton refuge appears to contribute very little in terms of slowing resistance development; this probably also describes the influence of the 5% un-sprayed or imbedded refuge options currently allowed in cotton. Since a 50% un-sprayed refuge in corn typically produces more susceptible moths than a 20 % sprayed refuge in cotton, and the *H. zea* population in eastern North Carolina primarily develops on C4 hosts (e.g. corn) during early season, it seems logical that the corn refuge plays a more vital role in resistance development than a 20% sprayed non-Bt cotton refuge. During the later part of the growing season, *H. zea* has both C3 and C4 hosts, and a large proportion of the larvae have been shown to develop on non-Bt C3 crops other than cotton (Jackson et al. 2003); consequently, a structured non-Bt cotton refuge may be less critical than a structured corn refuge. Previous transgenic crop evaluation using a version of this model (Storer 2003b) reports that the percentage of Bt cotton has a greater effect on resistance development than Bt corn when corn and cotton are modeled as the only two host crops. We do not refute that finding but simply report that the current 20% sprayed non-Bt cotton refuge's contribution to delaying resistance evolution is greatly supplemented by other non-Bt sources of susceptible moths during the cotton generations and has much less of an effect on Bt resistance management than the current 50% non-Bt corn refuge in eastern North Carolina.

This model also predicts Bollgard II and a pyramided field corn hybrid should greatly lengthen the effectiveness of the Bt technology and that completely replacing single gene cultivars with pyramided cultivars over a three year period does not result in a drastic reduction in the length of Bt crop effectiveness compared to an immediate switch. Though there is potential for large delays in resistance development with a Bollgard II and a pyramided field corn hybrid cultivar combination, both cultivar combinations with at least one pyramided crop out perform the single

gene cultivar combination. Furthermore, this model predicts that when soybean is included as a host, planting at least one pyramided cultivar and no structured non-Bt corn or cotton refuge results in superior resistance delay to a single gene cultivar combination following the current refuge requirements.

This model predicts that soybean as an alternate host can delay resistance development by a factor nearly as long as both mandated non-Bt crop refuges combined and removing soybean from the cropping system drastically reduces the time necessary for resistance development. When only corn and cotton are simulated as *H. zea* hosts, of course each structured non-Bt refuge is vital to delaying resistance development. The inclusion of non-Bt hosts other than the corn and cotton refuges is vital to determining the value of said crop refuges. Soybean acreage in North Carolina varies from year to year. So, it is unrealistic to assume that 50% of the crop acreage will be planted to soybean each year. The effects of intermediate soybean acreages are not directly modeled, but one could assume that as the soybean acreage declines resistance development would hasten and the cotton refuge would become more important in delaying resistance development. However, it is also unrealistic to assume that soybean is the only alternate host of *H. zea* and is completely erroneous to assume that *H. zea* larvae only develop on corn and cotton. This model is not a perfect representation of the relative abundance of *H. zea* hosts or a perfect representation of the relative productivity of the crop hosts modeled. Even with 50% of the crop acreage planted to soybean, the percentage of available non-Bt hosts and percentage of larvae developing on non-Bt hosts is still underrepresented. Consequently, assuming that 50% percent of *H. zea* host acreage is non-Bt each year is reasonable even though the actual soybean acreage will vary, and the observed effects of soybean acreage on resistance development are valid.

Under our set of assumptions, this model predicts the failure of single gene Bt cultivars within 10 years while following the currently required refuge sizes. Since single gene Bt cultivars (BG/YG) have been planted for 10 years now with no observable increase in resistance allele frequency in North Carolina, it seems improbable that the resistance allele frequency will exceed 0.50 by next year. There are a number of probable reasons why this model erroneously predicts the rate of increase in resistance allele frequency with single gene Bt cultivars. First, this model only includes soybean as an alternate host when in fact *H. zea* has many alternate hosts. Second, this model assumes complete cross resistance between Cry1Ab and Cry1Ac. Cross resistance to Cry1A Bt toxins has been observed in *Heliothis virescens* (Gould et al. 1992) and *Plutella xylostella* (Tabashnik et al. 1997) but may not occur in an *H. zea* field population. Third, the fitness cost of carrying a resistance allele may be greater than assumed. Fourth, this model tracks the rate of resistance allele frequency increase in a closed population and does not simulate the effect of long range migration by *H. zea* on resistance development. Finally, we assumed that 50% of the corn acreage is planted to Bt hybrids, but Bt hybrids have until recently accounted for less than 50% of the corn acreage (J. Van Duyn, pers. comm. Dec. 2005) Thus, the comparisons presented here are based on assumptions that make the development of Bt resistance more rapid than observed and are not representative of the exact time until resistance evolution but are an indicator of the relative importance of pyramided Bt cultivars, EPA mandated non-Bt refuges, and soybean as a host in *H. zea* resistance development to Bt in eastern North Carolina.

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Table 1. Mean (SE) number of bollworm adults produced per hectare from four cotton genotypes averaged across 2 locations in North Carolina (2005).

Genotype	Adults per Hectare ^a
Non-Bt (SG 521 RR)	36,903 (4,108)
Cry2Ab (15985X)	2,725 (1,450)
Bollgard (SG 215 BR)	9,645 (2,023)
Bollgard II (DP 424 BGII/RR)	1,467 (587)

Table 2. Mean number of bollworm adults produced per 100 ears from four corn genotypes in each of 2 years in North Carolina (2001 & 2002)

Genotype	Adults 2001	Adults 2002
Non-Bt	32.22	106
Yieldgard	4.69	2.33
Cry2Ab	3.67	12
Mon84006/Mon810	0.29	0

Table 3. Relative fitness values of all insect genotypes occurring on pyramided crops excluding fitness cost of carrying a resistance allele.

Insect Genotype	Relative fitness on Pyramided	Relative fitness on Pyramided
	Corn	Cotton
RR R'R'	1.000	1.000
RR R'r'	0.557	0.537
RR r'r'	0.114	0.075
Rr R'R'	0.542	0.631
Rr R'r'	0.312	0.368
Rr r'r'	0.059	0.039
rr R'R'	0.084	0.261
rr R'r'	0.044	0.133
rr r'r'	0.004	0.034

R = resistant to Cry1Ab r = susceptible to Cry1Ab R' = resistant to Cry2Ab r' = susceptible to Cry2Ab

Table 4. Years until resistance allele frequencies (Cry2Ab and/or Cry1A) are > 0.50 in a *H. zea* population including soybean as a host with different cultivar combinations and refuge sizes

Cultivar Combination	Non – Bt Refuge					
	50% corn / 20% cotton	20% corn / 20% cotton	0% corn / 20% cotton	50% corn / 0% cotton	20% corn / 0% cotton	0% corn / 0% cotton
BG/YG	10	7	5	10	7	5
BGII/YG	28	22	.	24	19	.
BGII/YG*	21	16	13	18	15	12
BG/PYG*	42	25	12	38	23	11
BGII/PYG	56	41	.	43	35	.
BGII/PYG*	32	27	14	26	24	13
Phase	50	33	.	39	28	.
Phase*	31	18	8	25	16	7

* initial resistance allele frequency and replacement rates higher than empirical estimates (see methods & materials)

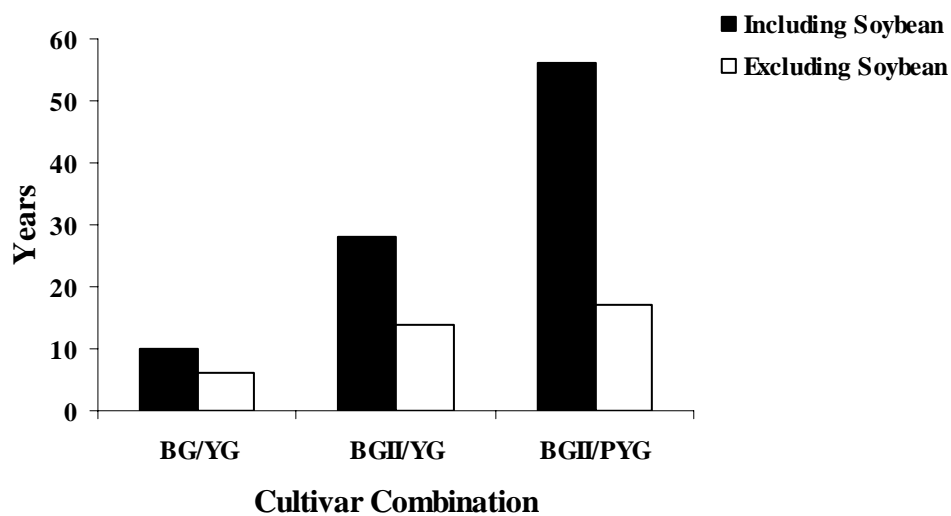


Figure 1. Year until resistance allele frequencies (Cry2Ab and/or Cry1A) are > 0.50 in a *H. zea* population including and excluding soybeans as a host following the currently mandated 50% non-Bt corn refuge and 20% sprayed non-Bt cotton refuge.