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BT RESISTANCE MANAGEMENT FOR AN EASTERN NORTH CAROLINA HELICOVERPA ZEA POPULATION: POTENTIAL ROLE OF SOYBEANS, MANDATED REFUGES AND PYRAMIDED BT PLANTS

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Abstract

The simulation model originally described in Storer et al. 2003 is used to evaluate the role of pyramided Bt cotton and corn cultivars, EPA mandated non-Bt refuges, and soybean as a host in *H. zea* resistance development to Bt in eastern North Carolina. 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 in eastern North Carolina.

Introduction

The percentage of North Carolina cotton acreage planted to Bollgard® varieties has risen from 3% in 1996 to approximately 80% in 2004 (J. Bacheler, pers. comm. Dec. 2004). 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. There is evidence that the mandated 20% sprayed non-Bt cotton refuge may not be necessary to adequately delay resistance evolution in *H. zea* to Bt crops in North Carolina.

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; 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, 2002) 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 non-Bt cotton in delaying resistance evolution to Bt in eastern North Carolina. The above concerns only production on the major crop hosts and ignores production on *H. zea* wild hosts.

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 are generally expected to decrease the rate at which resistance evolves.

Herein we use an existing simulation model originally described in Storer et al. (2003) and modified by Livingston to simulate the use of pyramided Bt corn and cotton cultivars. This model allows us to evaluate the role 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.

Methods and materials

As described by Storer et al. (2003), "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 model was further refined by Storer to include soybean as a host and by Mike Livingston 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 was needed for incorporation into the model. For these estimates, we utilized *H. zea* survival data on pyramided and single toxin Bt corn and cotton from our research and from that of Ryan Jackson, respectively. A detailed description of data collection and results can be found in Kurtz et al. (2004) and Jackson (2002). The model was run under a specific set of assumptions not limited to but including: 1) initial resistance allele frequency = 0.001, 2) fitness on Bt cultivars based on data from Jackson (2002) & Kurtz et al. (2004), 3) 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), 4) Cry1Ab and Cry1Ac share complete cross-resistance, 5) 0.025 fitness cost for carrying each resistance allele, 6) heterozygote obtains 50% of its fitness from the susceptible parent and 50% from the resistant parent, 7) including soybean in the model, 47% of the acreage is planted to soybean, 23% to corn, and 30% to cotton; excluding soybean in the model, 0% of the acreage is planted to soybean, 44% corn, 56% cotton.

We used fitnesses of susceptible (rr r'r') larvae on non-Bt, Cry1A, Cry2Ab, and pyramided plants to calculate fitnesses of all genotypes. On the pyramided plants, the genotype with resistance to Cry1A and Cry2Ab (RR R'R') was assumed to have equal fitness to rr r'r' 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 an rr r'r' 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 rr r'r' larvae on Cry1A plants. Larvae susceptible to both toxins (rr r'r') simply used a fitness value calculated directly from field collected rr r'r' larvae that developed on pyramided plants. Heterozygote fitnesses were calculated based on additive inheritance. 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 pyramided cultivars were too toxic to support a *H. zea* population. Thus, to make comparisons between all cultivar combinations, initial resistance allele frequencies 2.33 – 2.56 times higher than the empirical estimates had to be assumed.

The model was run with three 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). Three 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. 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 each crop type and refuge combination both with and without soybean as a host. Resistance was considered problematic when resistance allele frequencies for both Cry 1A and Cry2Ab are > 0.50.

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 4 years of growing BG/YG. However, the resistance allele frequency surpassed 0.50 after 7 years with soybean as a host, a 1.75 fold delay. Following current EPA refuge requirements and simulating a

BGII/YG cultivar combination, the frequency of resistance alleles reached 0.50 after 11 years when soybean was excluded and 20 years when included, a 1.82 fold delay. Finally, following current EPA refuge requirements and simulating a BGII/PYG cultivar combination, the frequency of resistance alleles reached 0.50 after 12 years when soybean was excluded and 42 years when included, a 3.5 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 2.86 fold delay compared to the BG/YG cultivar combination, while simulating a BGII/PYG cultivar combination resulted in a 6.00 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, eliminating the 20% sprayed non-Bt cotton refuge resulted in a > 0.50 resistance allele frequency after 6 years indicating a 1.17 fold delay realized by following the current refuge requirements with the same cultivar combination. Eliminating the non-Bt cotton refuge from a BGII/YG cultivar combination resulted in a > 0.50 resistance allele frequency after 18 years indicating a 1.11 fold delay realized by following the current refuge requirements with the same cultivar combination. Finally, eliminating the non-Bt cotton refuge from a BGII/PYG cultivar combination resulted in a > 0.50 resistance allele frequency after 35 years indicating a 1.20 fold delay realized by following the current refuge requirements with the same cultivar combination (Figure 2).

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 1.4 fold delay realized by following the current refuge requirements with the same cultivar combination. Eliminating the non-Bt corn refuge from a BGII/YG cultivar combination resulted in an > 0.50 resistance allele frequency after 12 years indicating a 1.67 fold delay realized by following the current refuge requirements with the same cultivar combination. Finally, eliminating the non-Bt corn refuge from a BGII/PYG cultivar combination resulted in a > 0.50 resistance allele frequency after 18 years indicating a 2.33 fold delay realized by following the current refuge requirements with the same cultivar combination (Figure 2).

Discussion

Based on the results from this model, Bollgard II and a pyramided field corn hybrid should greatly lengthen the effectiveness of the Bt technology. When pyramided plants are simulated, the mandated non-Bt corn refuge and soybean as a host substantially slow the rate of resistance development in eastern North Carolina *H. zea* populations. In comparison, the 20% sprayed cotton refuge appears to contribute very little in terms of slowing resistance development. During early season, the *H. zea* population in eastern North Carolina primarily develops on C4 hosts (e.g. corn); therefore, it seems reasonable that the corn refuge plays a major role in resistance evolution. 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 feed on non-Bt C3 crops other than cotton. It appears 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 in eastern North Carolina. Though there are increased delays in resistance development with a BGII/PYG cultivar combination, all refuge strategies simulated meet EPA expectations for resistance delay when simulating a BGII/YG cultivar combination.

Under our set of assumptions, this model predicts the failure of single gene Bt cultivars within seven years while following the current refuge strategy and that neither non-Bt crop refuge contributes appreciably to delaying resistance evolution with single gene Bt cultivars since resistance evolves so quickly. However; single gene Bt cultivars (BG/YG) have been planted for nine years now with no observable increase in resistance allele frequency in North Carolina. There are a number of probable reasons why this model erroneously predicted the rate of increase in resistance allele frequency with single gene Bt cultivars. First, the initial Cry1A resistance allele frequency was set 2.33 times higher than the current field estimate by Burd et al. (2003). Second, this model assumes complete cross resistance between Cry1Ab and Cry1Ac. Cross resistance to Cry1A Bt toxin has been observed in *Heliothis virescens* (Gould et al. 1992) and *Plutella xylostella* (Tabashnik et al. 1997) but may not occur in a *H. zea* field population. Third, the fitness cost of carrying a resistance allele may be greater than assumed. Finally, we assumed that 50% of the corn acreage is planted to Bt hybrids, but Bt hybrids typically only account for approximately 30% of the corn acreage (J. Van Duyn, pers. comm. Dec. 2004). The comparisons presented here were based on assumptions that make rapid evolution of Bt resistance more likely than expected with current field estimates. Therefore, results presented here 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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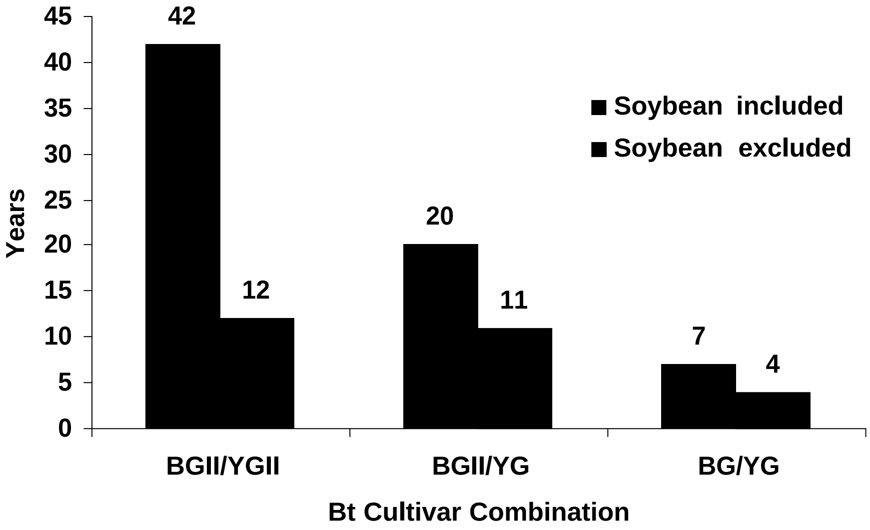

Figure 1. Years until Cry1A and Cry2Ab resistance allele frequencies are > 0.50 in a *H. zea* population including and excluding soybeans as a host following the currently mandated 50% non-Bt corn refuge and 20% sprayed non-Bt cotton refuge.

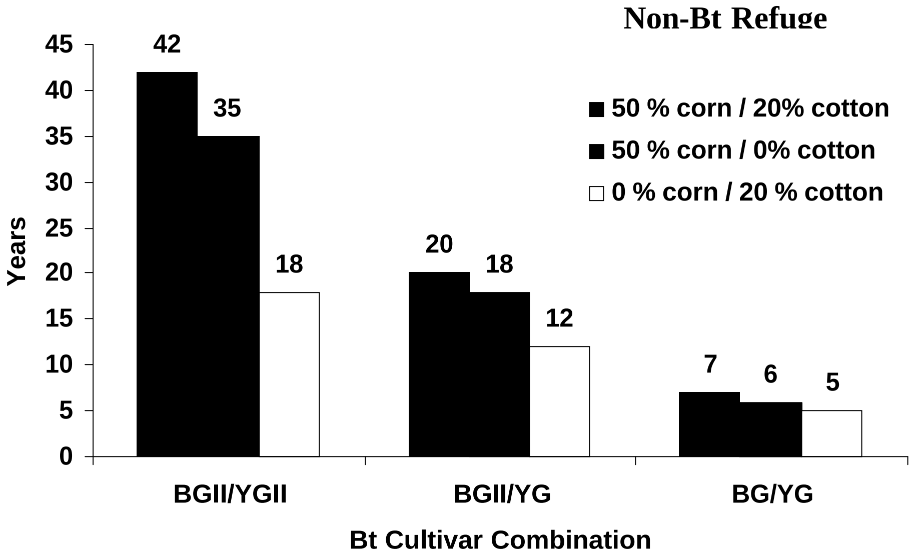

Figure 2. Years until Cry1A and Cry2Ab resistance allele frequencies are > 0.50 in a *H. zea* population with different Bt cultivar combinations and refuge requirements