

HELICOVERPA ZEA FITNESS ON BT CORN AND COTTON IN EASTERN NORTH CAROLINA: POTENTIAL EFFECTS OF ALTERNATE HOST CROPS AND PYRAMIDED BT PLANTS

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Abstract

A deterministic population genetics model was developed to evaluate how corn and cotton with pyramided Bt toxins, Non-Bt cotton refuges, and alternate *H. zea* crop host refuges could affect Bt resistance development in a *H. zea* population in eastern North Carolina. Empirical estimates of resistance allele frequencies for *H. zea* in the literature, adult production on Bt and Non-Bt corn and cotton, and proportions of larvae developing on cotton, corn, soybeans, and peanuts during each of three generations were used as model parameters. The frequency of resistant individuals is calculated at the end of each year, and resistance is considered problematic in the year when 25% of the individuals are homozygous for all alleles conferring resistance to a specific Bt crop. Model runs predict that the planting of pyramided Bt cultivars, the Non-Bt corn refuge, and alternate host refuges will each have a greater impact on resistance development than a 5%, structured, Non-Bt cotton refuge.

Introduction

Farmers routinely plant transgenic Yieldgard® field corn, and Bollgard® cotton to minimize losses from insect pests including losses from the corn earworm, *Helicoverpa zea* (Boddie). Yieldgard® and Bollgard® express a single *Bacillus thuringiensis* (Bt) toxin (Cry1Ab and Cry1Ac, respectively) in the fruit, leaves, and stem over the entire growing season. Season-long Bt expression protects the plants from the caterpillar, but imposes a high selection pressure for resistance development to the Bt toxins. To mitigate resistance development in pest insects to single toxin Bt crops, the USDA and EPA have indicated that a high dose/refuge strategy will be most effective (US-EPA 1999). Unfortunately, Yieldgard® and Bollgard® crops impose only moderately high mortality on *H. zea*, and consequently, these cultivars are not considered to have a high dose for this pest (Gould 1998, Storer 2001, US-EPA 2001). With single toxin hybrids that do not produce a high dose for a primary pest, large Non-Bt refuges must be planted to delay resistance development for an acceptable period of time (Roush 1998). Currently, Bt corn acreage is restricted to 50% of field corn acreage in cotton-growing areas due to fear of cross-resistance between Cry1Ab in field corn and Cry1Ac in cotton. The EPA expects the 50% Non-Bt corn refuge to delay resistance evolution in the corn earworm to Cry1Ab at least until its registration runs out in October of 2008 (US-EPA 2001). There are currently three refuge options for cotton: 1) External 20% sprayed non-Bt cotton – within 1 mile of Bt cotton, with use of any insecticide except Bt sprays; 2) Embedded 5% - contiguous at least 150 ft wide within the Bt field and may be sprayed with any insecticide that is simultaneously used in Bt cotton; 3) External 5% unsprayed – may not be treated with any insecticide labeled for *H. zea*, at least 150 ft wide, and within 0.5 mile of Bt cotton. Jackson, et al. (unpublished data) are evaluating the role of hosts such as soybeans and peanuts as a refuge alternatives to Non-Bt cotton.

Another tactic for delaying resistance development is introducing a pyramided hybrid 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 (Andow and Hutchinson 1998, Gould 1998). In 2003, Monsanto introduced Bollgard II® expressing Cry1Ac and Cry2Ab and prior to 2003 was testing an experimental pyramided field corn hybrid (Mon84006/Mon810) expressing Cry1Ab and Cry2Ab. Pyramided cultivars are expected to have increased efficacy against *H. zea*, and are generally expected to decrease the rate at which resistance evolves.

Here we use a deterministic population genetics model to evaluate how pyramided crops, Non-Bt cotton refuges, and alternate *H. zea* crop host refuges could affect Bt resistance development in a *H. zea* population in Eastern North Carolina. The model developed here is very general. Therefore, the model results are not intended as a basis for setting specific refuge requirements or crop deployment strategies, but as an indicator of the relative importance of structured same crop refuges, alternate host crop refuges, and introduction of pyramided Bt crops.

Methods

A two locus deterministic population genetics model similar to that described in Gould (1986) was developed based on a set of assumptions: 1) Equal sex ratio and random mating, 2) all genotypes equally fecund, 3) obligate sexual reproduction and adult movement, 4) one time step equals one crop year with 3 *H. zea* generations, 5) 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). 6) Cry1Ab and Cry1Ac share complete cross-resistance, 7) Bt is the only mortality factor not held constant for all genotypes. 8) 0.025 fitness cost for carrying each resistance allele; and 9) heterozygote obtains 90% of its fitness from the susceptible parent and 10% from the resistant parent.

The model is initiated with genotypic frequencies of Cry1A and Cry2A resistance in a larval cohort based on empirical estimates of resistance allele frequencies for *H. zea* in the literature. Burd, et al. 2003, estimated the gene frequency of a resistance allele to Cry1Ab, R, as 0.00043 and the gene frequency of a resistance allele to Cry2Aa, R', at 0.00039. To err on the side of caution, the initial resistance allele frequencies were assumed at 0.001 for R and R', and the initial frequencies of the 9 *H. zea* genotypes were calculated assuming Hardy-Weinberg equilibrium and no linkage disequilibrium.

Within the model, larvae "feed" on either a Bt or Non-Bt plant with larval survival based on the relative fitness of each genotype and proportions of the *H. zea* population on Bt and non-bt crops for each generation determined by data from Jackson et al. (2002), Jackson et al. (unpublished data), and the NCDA (2003). On pyramided plants, we used fitnesses of rr r'r' larvae on Non-Bt, Cry1A, Cry2A, and pyramided plants to calculate fitnesses of all genotypes. On the pyramided plants the genotype with resistance to Cry1A and Cry2A (RR R'R') is assumed to have fitness equal to the fitness of 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 Cry1Ab (RR r'r') receive the fitness value of an rr r'r' individual on a plant with only Cry2Ab since that is 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 Cry1Ab plants. Larvae susceptible to both toxins (rr r'r') are simply given a fitness value calculated directly from field collected rr r'r' larvae that were placed on pyramided plants. Heterozygote fitnesses are calculated based on partially dominant inheritance as given in assumption #9 above.

Fitness values for rr r'r' larvae on all corn cultivars were calculated from field data on *H. zea* adult production per 100 ears from Yieldgard®, Cry2Ab, pyramided, and non-Bt corn (Kurtz, et. al. unpublished data)(Table 1). The data were obtained by collecting corn ears from field plots, and placed by plot into 68.1 L Rubbermaid® buckets holding 2 inches of potting soil and a wooden rack to elevate the ears above the soil. After storage in an open-sided shed for 7 -12 days, the potting soil from each bucket was sifted through a wire screen to collect pupae and pre-pupae. Pre-pupae were covered individually with potting soil in 240 ml plastic cups and left in the shed stacked inside a Rubbermaid bucket until pupation. Pupae were weighed, measured for length, sexed, put in 1oz. diet cups and stored in a rearing room until adult emergence. The 100 ears per plot originally collected were discarded after soil sifting for pupae and pre-pupae collection and replaced by 100 fresh ears per plot. This sampling process was performed three times in the Non-Bt and single toxin hybrids and twice in the dual toxin hybrid to accurately document the total moth production from each over time. In the model we assume similar survival of larvae in pre-tasseling and reproductive corn, but it is likely that survival is lower in pre-tasseling corn.

Adult production from cotton was taken from Jackson et al. (2002)(Table 2). No estimate was available for cotton expressing Cry2Ab alone; therefore, the difference between adult production in Bollgard® cotton and cotton expressing Cry2Ab alone was assumed to be proportionally equal to the difference in production between Yieldgard® and the corn hybrid containing Cry2Ab alone.

Estimates of moths originating from C4 plants in NC from Jackson et al. (unpublished data), and data from Jackson, et al. (2003) on larvae per acre in corn, cotton, soybeans and peanuts over the growing season were used in combination with the NCDA statistics on crop acreage in North Carolina for 2000, 2001, and 2002 to determine the proportion of *H. zea* larvae present on each crop type during each of the 3 *H. zea* generations. These data suggest that > 90% of moths from the first two generations developed on C4 plants such as corn (Fig 1). For this model, we assumed that all moths with a stable carbon isotope signature from a C4 plants (Gould et al. 2002) originated on corn. Based on these data, we set the proportion of larvae from the first 2 generations developing on corn at 90%. We assumed 5% on cotton, and 5% on weed hosts during these first two generations. Data from unsprayed field plots near Plymouth, NC give estimates of numbers of larvae developing on corn, cotton, peanuts, and soybeans during the third generation of 2002 in eastern North Carolina. Based on equal plot size, 17.39% of the larvae were on corn, 36.34% on cotton, 0.05% on peanuts, and 40.99 % on soybeans. Since these estimates are based on equal plot sizes, average planted acres for 2000-2002 as listed by the NCDA for each crop were multiplied by the estimated larvae per acre then divided by the total estimated larvae for all crops to estimate the proportion of larvae developing on each crop in eastern NC (Table 3).

In the model, all larvae surviving to the end of a generation are assumed to pupate and successfully emerge as adults. Emerging moths disperse randomly between the Bt and Non-Bt populations and randomly mate. Offspring produced are in Hardy-Weinberg proportions based on allelic frequencies of the parents and represent the beginning of generation 2.

The selection and development process is repeated for generation 2 and 3; all larvae surviving to the end of generation 3 overwinter as pupae, and all emerge as moths to begin generation 1 of the next year. The frequency of resistant individuals is

calculated at the end of each year. Resistance is determined as the year when 25% of the individuals are homozygous for alleles conferring resistance to the specific cultivar being examined.

The model was run under varied cultivar deployment assumptions for Yieldgard®, Bollgard®, Mon84006/Mon810 and Bollgard2®. Refuge plans examined were: 1) current refuge requirements of 50% Non-Bt corn refuge and a 5% unsprayed cotton refuge (with and without alternate hosts); 2) a 50% Non-Bt corn refuge and no cotton refuge (with and without alternate hosts); and 3) no corn refuge and no cotton refuge (with and without alternate hosts).

Results

Yieldgard® and Bollgard® vs. Mon84006/MON810 and Bollgard II®

Following current EPA refuge requirements, planting new pyramided gene corn and cotton is expected to delay resistance evolution 6.08 times longer than planting single gene crops when alternate hosts are not considered a source of Non-Bt refuge and of 9.40 times longer when alternate hosts are assumed to be present. (Tables 5 & 6)

Alternate Hosts vs. No Alternate Hosts

Following current EPA refuge requirements and including peanuts and soybeans as alternate hosts in the third generation, the frequency of resistant *H. zea* is predicted to reach 0.25 in 235 years when both pyramided corn and Bollgard II® are planted; however, without these crops considered as alternate hosts, time to resistance is shortened by 156 years or 66.38%. (Tables 5 & 6)

No Structured Cotton Refuge

When the model is run simulating a 50% corn refuge, no structured cotton refuge, and peanuts and soybeans as alternate hosts in the third generation, the frequency of resistant *H. zea* is predicted to reach 0.25 in 228 years if both pyramided corn and Bollgard II® are planted. Without the alternate hosts as a source of Non-Bt refuge, the frequency of resistant *H. zea* is predicted reach 0.25 in 64 years. Eliminating the Non-Bt cotton refuge hastened resistance development by 18.99 % when alternate hosts were not included, but only by 2.98% when they are. (Tables 5 & 6)

No Structured Corn Refuge

When assuming no corn and no cotton refuge, but peanuts and soybeans as alternate hosts in the third generation, resistant insects reach a frequency of 0.25% in 18 years, and only 3 years when alternate hosts are not included. This suggests that alternate hosts could slow resistance development by a factor of 6 if they were considered as the only source of Non-Bt refuge. Also, eliminating the Non-Bt corn refuge hastened resistance development by 96.20% when alternate hosts were not included and 92.34% when they are. (Tables 5 & 6)

Discussion

Based on results from this model, growing pyramided plants versus single gene plants is expected to greatly lengthen the effectiveness of Bt technology, as is the effect of alternate hosts as a source of Non-Bt refuge. It is evident that a structured cotton refuge provided little impediment when alternate hosts are considered as a source of Non-Bt refuge. Since corn is the major host in the first and second generations, conventional corn refuge size appears to have a greater effect on resistance development than any other source. The model developed here is very general. Therefore, the results presented here are not intended as a basis for setting refuge requirements or crop deployment strategies, but as an indicator of the relative importance of structured same crop refuges, alternate host crop refuges, and introduction of pyramided Bt crops.

References

- Andow, D. A., and W. D. Hutchinson. 1998. Bt-corn resistance management. In *Now or never: serious new plans to save a natural pest control* (ed. M. Mellon and J. Rissler), pp. 19-66. Cambridge, MA: Union of Concerned Scientists.
- Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annu. Rev. Entomol.* 43:701-726.
- Gould, F. 1986. Simulation models for predicting durability of insect-resistant germ plasm: a deterministic diploid, two locus model. *Environ. Entomol.* 15:1-10.
- Gould, F., N. Blair, M. Reid, T.L. Rennie, J. Lopez, and S. Micinski. 2002. *Bacillus thuringiensis*-toxin resistance management: Stable isotope assessment of alternate host use by *Helicoverpa zea*. *Proc Natl Acad Sci USA.* Vol 99(26): 16581-19586.
- Jackson, R. E. Unpublished data.

Jackson, R. E. 2002. The influence of transgenic cottons expressing one or two *bacillus thuringiensis* proteins against *Helicoverpa zea* (Lepidoptera: Noctuidae) and factors affecting *B.T.* resistance in *H. zea*. Ph.D. dissertation, North Carolina State University, Raleigh.

Jackson, R.E., J.R. Bradley, Jr., and J.W. Van Duyn. 2003. Quantification of *Helicoverpa zea* populations in eastern North Carolina crop environments: implications for *B.t.* resistance management. Proc. Beltwide Cotton Conf.

North Carolina Department of Agriculture & Consumer Services. 2003. Agriculture statistics division-field crops. http://www.ncagr.com/stats/crop_fld/fldannyr.htm

Roush, R. T. 1998. Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not? Phil. Trans. R. Soc. Lond. B 353:1777-1786.

Ruberson, J. R. Unpublished data.

Storer, N. P., J. W. Van Duyn, and G. G. Kennedy. 2001. Life history traits of *Helicoverpa zea* (Lepidoptera: Noctuidae) on Non-Bt and Bt transgenic corn hybrids in eastern North Carolina. J. Econ. Entomol. 94:1268 - 1279.

US-EPA. 1999. EPA and USDA position paper on insect resistance management in Bt crops. U.S. Environmental Protection Agency. http://www.essentialbiosafety.info/docroot/articles/epa_irm.pdf

US-EPA. 2001. Biopesticides Registration Action Document, Bacillus thuringiensis Plant Incorporated Protectants. U.S. Environmental Protection Agency. http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm

Table 1. *H. zea* adult survival and relative fitness values from corn trials near Plymouth, NC 2001 & 2002.

Corn Hybrid	Adults/100 ears	Relative fitness values
Non-Bt	69.11	1
Yieldgard@(Cry1Ab)	3.51	0.084
Mon84006 Cry2Ab	7.84	0.114
Mon84006/Mon810	0.143	0.004

Table 2. *H. zea* adult survival and relative fitness values from cotton plots averaged across three locations in North Carolina 2000-2001.

Cotton Variety	Adults/hectare (SE)	Relative fitness values
Non-Bt	19,562 (4,060) a	1
Bollgard® (Cry1Ab)	1,115 (341) b	0.057
Cry2Ab	---	0.072
Bollgard II®	247 (146) b	0.013

Means within the same column followed by the same letter are not significantly different, Fisher's Protected Least Significant Difference Test ($P \leq 0.05$).

Table 3. Percent of *H. zea* larvae developing on each host per generation.

Generation	Corn	Cotton	Soybeans	Peanuts	Weeds
1	90%	5%	0%	0%	5%
2	90%	5%	0%	0%	5%
3	12.32%	32.93%	54.16%	0.58%	0%

Table 4. 2002 *H. zea* larval production per acre from small plot trial (Untreated) near Plymouth, NC.

Crop	Aug-8	Aug-14	Aug-21	Aug-30	Sept-9
Corn	7566	6190	5502	0	0
Cotton	14100	19258	534	344	0
Soybeans	3095	28888	12724	688	0
Peanuts	3095	1720	1032	0	0

Table 5. Years until resistant insect frequency = 25% without alternate hosts considered as a source of Non-Bt Refuge.

Non-Bt Refuge	Yieldgard®/Bollgard®	(Mon84006/Mon810)/Bollgard II®
50% corn; 95% cotton	13	79
50% corn; No cotton	12	64
No corn; No cotton	3	3

Table 6. Years until resistant insect frequency = 25% with alternate hosts considered as a source of Non-Bt Refuge.

Non-Bt Refuge	Yieldgard®/Bollgard®	(Mon84006/Mon810)/Bollgard II®
50% corn; 95% cotton	25	235
50% corn; No cotton	23	228
No corn; no cotton	5	18

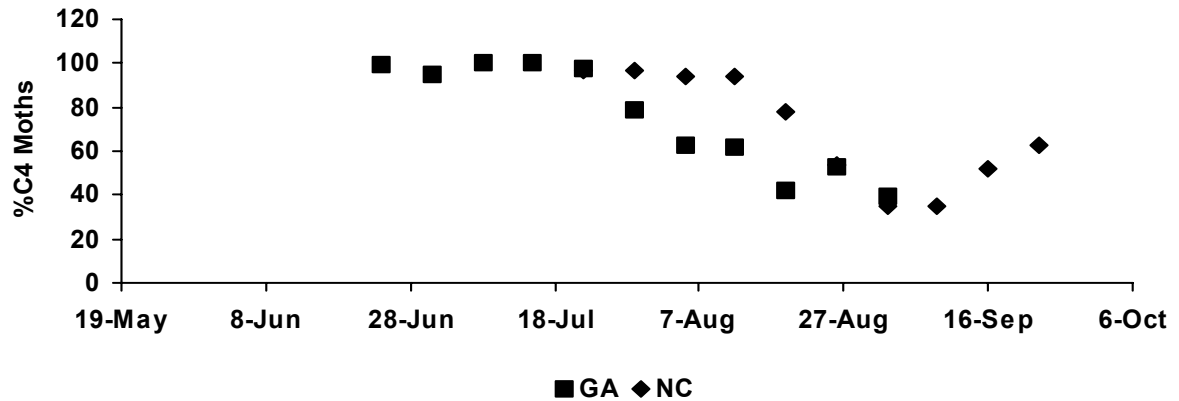


Figure 1. Moths originating from C4 (non-cotton) host.