SURVIVAL OF *HELICOVERPA ZEA* ON BT SWEET CORN

R. C. Venette, P. K. O'Rourke, W. D. Hutchison,
E. C. Burkness and D. A. Andow
USDA-APHIS
University of Minnesota
St. Paul, MN

Abstract

Helicoverpa zea (Boddie) causes significant damage to several economically important crops throughout the United States. To control this insect and other lepidopteran pests, many host species have been genetically modified to express δ-endotoxins from the soil bacterium *Bacillus thuringiensis* (Bt) Berliner. Populations of *H. zea* are potentially exposed to cry1Ac in transgenic cotton in the south and to cry1Ab in transgenic corn in the north. Widespread production of transgenic crops has raised concerns about targeted insects developing resistance to Bt and the potential for cross resistance. In Minnesota, an in-field screen using late-planted Bt sweet corn has been used since 1997 to detect insects with potential resistance to Bt and to monitor changes in the frequency of these individuals. Larvae ($\geq 3^{rd}$ instar) actively feeding on Bt expressing tissues are of particular concern. Paired plots of transgenic sweet corn (event BT-11) and its non-Bt isoline ('Bonus'), respectively, were planted at 5 locations throughout the southern half of the state. In 1997, no *H. zea* larvae ($\geq 3^{rd}$ instar) were found after sampling 4,200 ears of Bt corn; nearby non-Bt sweet corn averaged 0.79 larvae/ear. In 1998, 131 late-instar larvae were recovered after sampling 19,744 ears; the average density of larvae on non-Bt sweet corn was 0.95 larvae/ear. In 1999, 33 late instars were found after sampling 36,120 ears; non-Bt sweet corn averaged 0.41 larvae/ear. Frequencies of late-instar larvae surviving on Bt sweet corn increased from 3.01×10^{-4} in 1997 to 5.84×10^{-3} in 1998 and declined to 2.3×10^{-3} in 1999. As *H. zea* does not overwinter in Minnesota, different source populations (e.g., southern U.S.) with differing susceptibilities to Bt may account for variability in frequency estimates.

Introduction

Widespread production of transgenic crops modified to express δ-endotoxins from the soil bacterium *Bacillus thuringiensis* (Bt) Berliner has generated concerns over targeted insects developing resistance. Increased tolerance to the cry1Ac endotoxin has already been observed for *Helicoverpa zea* (Boddie) (Sumerford et al., 1999). Resistance to cry1Ac may also confer resistance to cry1Ab, the endotoxin produced by transgenic corn, as has been demonstrated with other leipodpteran pests (Bolin et al., 1999).

Resistance management strategies assume that Bt resistant mutants are scarce. An in-field screen using Bt sweet corn (Venette et al., 2000) provides an estimate of the frequency of insects with potential resistance to Bt. The basic premise of the in-field screen is to compare the density of insects on transgenic sweet corn to the density of insects on nearby non-Bt sweet corn. Larvae ($\geq 3^{rd}$ instar) that are actively feeding on Bt-expressing tissues are at greatest risk of being resistant. The ratio of larvae (on a per ear basis) from the Bt crop to larvae from the non-Bt crop provides a measure of the frequency of resistance.

The objective of the current study is to detect *H. zea* larvae with potential resistance to Bt in Minnesota and monitor annual changes in the frequency of resistant individuals.

Materials and Methods

In 1999, trials using transgenic (based on event BT-11) and non-transgenic (non-Bt isoline) sweet corn were planted in a randomized complete block design with 4 replications of each treatment. Each plot was approximately 0.05 ha (0.125 acre) and separated from other plots by a minimum of 1.5m (5 ft). Trials were conducted near Becker, Lamberton, Morris, Rosemount, and Waseca, MN. Corn was planted between 15 June and 15 July to maximize insect pressure at ear maturity. For a given trial, both Bt and non-Bt hybrids were planted on the same date.

Approximately 20 d after silking, 50 randomly selected ears were sampled from each non-Bt plot. For each ear, the number of *H. zea* larvae in each instar, their location, and the amount of feeding damage was recorded. By assuming that potentially resistant larvae occur at a frequency of 1 in 1000 and applying the average density of larvae observed on non-Bt corn, we estimated the number of samples necessary to locate at least 1 larva ($\geq 3^{rd}$ instar) on Bt sweet corn with 95% probability. Calculations were based on formulas described in Venette et al. (2000). The predicted number of Bt-ears was divided by 4 to provide the appropriate sample size per plot. Ears were inspected for larvae and damage as before. Damaged ears were brought to the laboratory to confirm expression of Bt using the GeneCheck™ strip test.

Larvae from Bt and non-Bt ears were placed on artificial diet and brought to the lab. Larvae were sent to Dr. Blair Siegfried (Univ. of Nebraska, Lincoln) for discriminatory-dose bioassays.

The frequency of potentially resistant *H. zea* larvae was calculated following:

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$$f=(S+1)/(ML),$$

where f is the frequency of potentially resistant larvae, S is the number of larvae ($\geq 3^{rd}$ instar) observed on Bt corn, M is the number of Bt-ears sampled, and L is the average density of *H. zea* larvae on non-Bt ears.

Field trials conducted in 1997 and 1998 followed similar protocols.

Results and Discussion

As in previous years, the in-field screen proved to be very efficient in locating larvae with potential resistance to Bt. Depending on the pest density in non-Bt sweet corn, sampling Bt ears by a crew of 5-8 people was completed in 1-2 days just before harvest. Labor for sampling constituted the primary cost for the screen. As laboratory bioassays are currently in progress, total costs for the screen have yet to be determined. However, when resistance is based on a dominant allele, the in-field approach is the most cost effective method available to screen for resistant larvae (Venette et al. 2000).

In 1999, the average density of *H. zea* on non-Bt sweet corn ranged from 0.09 to 0.96 larvae per ear (Table 1). Consequently, between 1,672 and 8,184 Bt ears had to be collected at an individual site to have a 95% probability of detecting an appropriate instar. The total number of late-instar larvae observed on Bt ears ranged from 1 to 12 (Table 1). The frequency of late-instar *H. zea* larve on Bt corn varied from 6.7×10^{-4} to 1.2×10^{-2}. After pooling all data to generate a statewide average, we found that the average density of *H. zea* was 0.41 larvae per non-Bt ear. More than 36,000 Bt ears were sampled and a total of 33 larvae were observed. In comparison, no *H. zea* larvae were observed after sampling 4,200 ears in 1997. In 1998, 131 late-instar *H. zea* larvae were recovered after sampling 19,744 ears of Bt sweet corn.

The statewide average frequency of late-instar larvae in 1999 was 2.3×10^{-3}. The frequency was greater than the frequency observed in 1997 (3.0×10^{-4}) but less than the frequency determined for 1998 (5.8×10^{-3}). Annual variability in the in-field screen may be due to the fact that *H. zea* does not overwinter in Minnesota. Populations annually disperse into the state from more southern climes. The location of source populations may vary from year to year, and these populations may have different susceptibilities to Bt (Stone and Sims, 1993).

References

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Table 1. Helicoverpa zea larvae observed on ears of non-Bt and Bt sweet corn in Minnesota, 1999.

	Non-Bt Ears		Bt-Ears		
Location	**n**	**larvae/ear**	**n**	**larvae**	**f**
Becker	200	0.49	6821	2	8.98E-04
Lamberton	200	0.28	3875	12	1.20E-02
Morris	200	0.37	8184	1	6.70E-04
Rosemount	205	0.09	4488	1	4.79E-03
Rosemount	200	0.08	6102	4	1.02E-02
Waseca	205	0.59	4978	6	2.38E-03
Waseca	200	0.96	1672	7	4.98E-03
Summary	1410	0.41	36120	33	2.31E-03

f = frequency of late-instar larvae

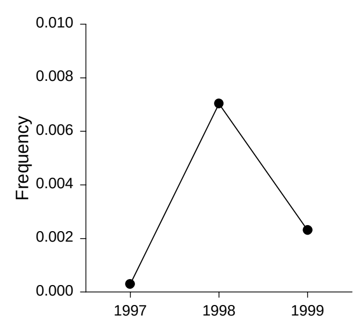

Figure 1. Annual variation in the frequency of late-instar *H. zea* observed on Bt sweet corn.