

Oviposition of European Corn Borer (*Lepidoptera: Pyralidae*) and Impact of Natural Enemy Populations in Transgenic Versus Isogenic Corn

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ABSTRACT In a 1994 field experiment, oviposition, predation, and parasitism of the European corn borer, *Ostrinia nubilalis* (Hübner), were recorded in transgenic and isogenic corn, *Zea mays* L. Plots of plants expressing the Cry1A(b) protein of *Bacillus thuringiensis* Kurstaki and plots of isogenic plants both had 2nd-generation *O. nubilalis* egg mass densities of ≈ 1.1 per plant, indicating a lack of antixenosis by transgenic plants. Distribution and size of egg masses on plants also was unaffected by corn type. Size of plants was the same in both treatments. Levels of egg mass predation were 24.75 and 19.35%, respectively, but not significantly different between the transgenic and isogenic plots. Parasitism of egg masses was not significantly different between transgenic and isogenic plots, and was low at 6.31 and 4.41%, respectively. Percentage of eggs within masses which hatched was 10.2% lower in transgenic than in isogenic plots. However, neither predation, parasitism, or sloughing of eggs from plants were significantly different between the 2 treatments. Densities of *O. nubilalis* predators were not different between the 2 treatments throughout the *O. nubilalis* oviposition period. Parasitism of *O. nubilalis* larvae by *Eriborus terebrans* (Gravenhorst) and *Macrocentrus grandii* Goidanich was not significantly different between plots and ranged from 2.4 to 7.0%. Although most differences between transgenic and isogenic plants were nonsignificant, all observed differences in natural enemy population parameters under our conditions were in the direction opposite to that expected if transgenic plants had an adverse impact.

KEY WORDS *Ostrinia nubilalis*, *Bacillus thuringiensis*, *Zea mays*, transgenic, biological control

IN THE PAST decade, genetic transformation has been accomplished on >50 plant species for crop improvement in such areas as herbicide tolerance, insect and pathogen resistance, and seed storage (Kung and Wu 1993). The transformation of crop plants to express protectants such as *Bacillus thuringiensis* Kurstaki insecticidal proteins presents both tremendous opportunities and challenges in approaches to integrated pest management. For example, expression of the Cry1A(b) protein in hybrid field corn plants resulted in extremely high levels of resistance to European corn borer, *Ostrinia nubilalis* (Hübner), and improved yield 35% in comparison with *O. nubilalis*-susceptible genotypes (Koziel et al. 1993). However, the potential for adaptation of several insect and pathogen pests to transgenic crop plants has already been documented (Kareiva et al. 1993), and broad-spectrum resistance by *Heliothis virescens* (F.) to *B. thuringiensis* toxins has been reported (Gould et al. 1992).

A growing concern is that pest adaptation to transgenic resistance mechanisms may limit the

life span of this technology in pest management, and a number of authors have suggested approaches for extending this life span (e.g. Kennedy et al. 1987, Strong 1990, Kareiva et al. 1993, Tabashnik 1994). Long-term management of transgenic *B. thuringiensis* resistance in corn requires addressing questions of resistance management. An unknown area is the impact on natural enemies of *O. nubilalis* in commercial-scale fields of transgenic corn.

Resistance mechanisms developed through conventional plant breeding programs can have profound influences on natural enemy populations that may be either positive or negative (Boethel and Eikenbary 1986). Natural enemies may be affected directly, for example, by increased mortality and decreased searching efficiency as a result of high levels of chemical or mechanical antibiosis mechanisms. Indirect effects may include increased availability of susceptible pest life stages caused by extended development times induced by antibiosis, resulting in synergism. Theoretical and empirical evidence presented by Gould et al. (1991) and Johnson and Gould (1992) suggest that in some situations, pest adaptation to transgenic plants may be accelerated by the synergistic action of natural enemies.

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This experiment was conducted to estimate the effects of natural enemies on *O. nubilalis* in transgenic corn. In addition, the low levels of *O. nubilalis* larvae expected in transgenic blocks provided an opportunity to collect data on the potential impacts on density-dependent mortality of *O. nubilalis* from predators and parasitoids. Information on recruitment of *O. nubilalis* into transgenic versus nontransgenic corn also was collected.

Methods and Materials

The experiment was set up using a completely randomized design with 3 replications. The 2 treatments were transformed hybrid corn plants that expressed a version of the Cry1A(b) protein derived from *Bacillus thuringiensis* Berliner (developed by Ciba Seeds, Research Triangle Park, NC) (Koziel 1993); and nontransformed plants. Both the transgenic and isogenic plants were derived from inbred line 00526 and had a maturity date of 107 d.

Plots were managed using conventional agronomic practices; i.e., chisel plow, $\approx 53,340$ seeds per hectare in rows on 0.762-m centers, and recommended application of herbicide and fertilizer. The exceptions were that corn had been planted in the test field the previous season, and planting was delayed until all other corn fields in the surrounding area had been planted (May 19). These exceptions were made to ensure a local population of *O. nubilalis* and attractiveness of plots to 2nd-generation moths.

Individual plot size was 0.405 ha (64.05 by 62.83 m) to reduce edge effects and simulate commercial field conditions. Plots were arranged in a 2 \times 3 pattern, with the long axis of the pattern oriented north-south. Each plot was surrounded by a 4.58-m border of annual rye grass, *Lolium multiflorum* Lam., to act as an action site for *O. nubilalis* moths (Derrick and Showers 1990), and the entire experiment was surrounded by a 4.58-m isolation strip of isogenic corn plants. The experiment was located in a center-pivot irrigated soybean field at least 201.3 m from the nearest commercial corn field on the Hensell farm near Constantine, MI.

Oviposition by *O. nubilalis*, fate of eggs, and abundance of potential egg predators were recorded by scouting sample sites consisting of 5 consecutive plants. Within each plot (84 rows wide), 2 sample sites spaced 21 m apart were marked in rows 21, 32, 43, 54, and 65. All plant surfaces were examined, and the number of eggs in masses were counted using field magnifiers (20 \times).

Plant height and leaf surface area were determined with a tape measure and an area meter (Model#LI3100, Li-Cor, Lincoln, NE). Two plants were randomly selected from 2 plots, and a 3rd from the last plot so that in total 5 plants were measured for each plant type.

Scouting for eggs and predators was conducted simultaneously on 3 dates chosen to fall near the

beginning, peak, and end of *O. nubilalis* oviposition. These dates were selected based on pupation of 1st-generation *O. nubilalis* within the study field and 2 other sites in St. Joseph County, and catches of moths in a light trap at Three Rivers, MI. The condition of eggs (fresh, hatched, preyed upon, parasitized, sloughed off), number of eggs within masses, and the location of masses on plants were recorded. Previous experience monitoring the fate of freshly oviposited egg masses allowed us to categorize egg mass remains accurately.

Two scouts, 1 on each side of a sampled row, counted potential predator numbers. Predators recorded were *Ortus insidiosus* (Say); coccinellids, almost all of which were *Coleomegilla maculata* (DeGeer); and lacewing larvae (species not determined). All of these are generally recognized in the literature as predators of *O. nubilalis* eggs (e.g., Hudon and LeRoux 1986b, Andow 1990).

Parasitism of *O. nubilalis* larvae was determined by artificially infesting microplots with blackhead-ing laboratory-reared egg masses (DEKALB Genetics, Olivia, MN) on August 19 during the 2nd-generation moth flight. Because *O. nubilalis* larvae will not survive on transgenic corn, seeds within microplots in transgenic plots were replaced with isogenic seeds. Each microplot included 5 adjacent rows with 4 plants per row; 3 microplots were laid out 15.25 m apart around the center of each plot, approximating a triangle. All larvae were removed from 1 plant within every row of each microplot on 2 sampling dates (August 26, September 15), and placed individually into plastic cups (30 ml) containing meridic diet (Guthrie et al. 1972). Cups were held at $\approx 24^{\circ}\text{C}$, $\approx 50\%$ RH, ≈ 14 -h photophase until either moths or parasitoids emerged.

Means for each of the 6 plots were calculated for oviposition and egg fate, predator counts, and larval parasitism. Analysis of variance (ANOVA) was carried out on the plot means to compare the plant types. Distribution of *O. nubilalis* oviposition was analyzed using an analysis of covariance (ANCOVA) (Zar 1984). Plant size data were analyzed with 2-tailed Student *t*-tests (Zar 1984).

Results

The density of *O. nubilalis* egg masses oviposited by 2nd-generation moths was not significantly different between isogenic and transgenic plots ($F = 0.069$; $df = 1, 4$; $P = 0.81$) (Table 1). Distribution of egg masses on plants also was not significantly different ($F = 0.002$; $df = 1, 26$; $P = 0.97$) (Fig. 1). Peak oviposition occurred on the leaf just below the primary ear leaf, with 87.1% of egg masses laid within 3 leaves above and below this point (Fig. 1). Number of eggs per mass in isogenic plots, 15.34 ± 1.17 (mean \pm SD), was not significantly different from the number in transgenic plots, 15.24 ± 0.71 ($F = 0.016$; $df = 1, 4$; $P = 0.907$) (Table 1).

Table 1. Fate of 2nd-generation *O. nubilalis* egg masses in transgenic and isogenic hybrid field corn plots, Constantine, MI, 1994

Treatment	No. egg masses/plant	% of egg masses, mean \pm SD ^a							
		Hatched	Preyed upon	Parasitized ^b	Sloughed ^c	Hatched	Preyed upon	Parasitized ^a	Sloughed ^c
Isogenic	1.1 \pm 0.5a	87.3 \pm 4.0a	19.4 \pm 9.3a	4.4 \pm 2.2a	1.5 \pm 1.33a	86.4 \pm 4.2a	10.4 \pm 3.8a	2.1 \pm 1.9a	1.1 \pm 1.0a
Transgenic	1.2 \pm 0.2a	82.9 \pm 3.2a	24.8 \pm 3.1a	6.3 \pm 1.0a	2.4 \pm 1.12a	77.6 \pm 2.5b	15.8 \pm 1.1a	4.6 \pm 2.0a	2.0 \pm 0.6a

Within a column, values followed by different letters are significantly different (ANOVA; $P \leq 0.05$). Means were calculated using plot averages, and all analyses were carried out on plot averages.

^a Values within a row may not add up to 100% because >1 fate may befall different eggs within a mass.

^b Parasitoids had already emerged; therefore, specimens for identification could not be recovered.

^c Sloughed-off corn plant, presumably by leaf curling and flexing.

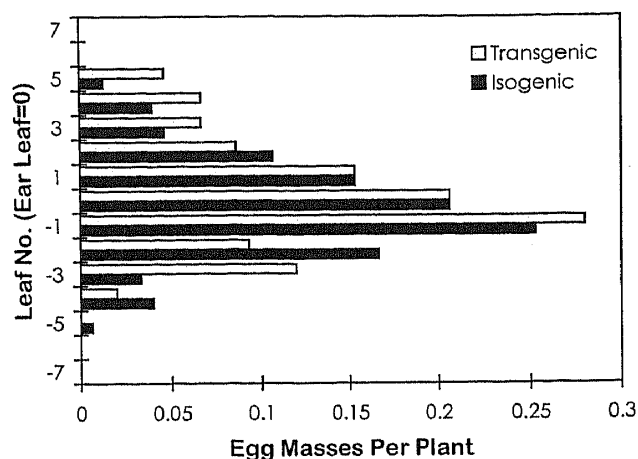


Fig. 1. Distribution of *O. nubilalis* oviposition on corn plants in isogenic and transgenic hybrid field corn plots, Constantine, MI, 1994.

Plant heights in transgenic plots, 2.73 ± 0.49 m, and isogenic plots, 2.84 ± 0.07 m, were not significantly different ($F = 0.27$; $df = 1, 8$; $P = 0.62$). Similarly, plant leaf area in transgenic, 5.55 ± 0.54 , and isogenic plots, 5.59 ± 0.29 , was not significantly different ($F = 0.024$; $df = 1, 8$; $P = 0.88$).

The percentage of *O. nubilalis* egg masses from which at least some of the eggs hatched was not significantly different between plots ($F = 2.12$; $df = 1, 4$; $P = 0.219$) (Table 1). Likewise, the percentages of egg masses which had been at least in part preyed upon, parasitized, or sloughed off of plants were not significantly different between isogenic or transgenic plots ($F = 0.92$; $df = 1, 4$; $P = 0.39$; $F = 1.83$; $df = 1, 4$; $P = 0.25$; $F = 0.80$; $df = 1, 4$; $P = 0.42$, respectively). Although the percentage of individual eggs within masses that hatched was significantly lower in transgenic plots ($F = 9.67$; $df = 1, 4$; $P = 0.036$), the levels of predation, parasitism, and sloughing were not significantly higher ($F = 0.84$; $df = 1, 4$; $P = 0.41$; $F = 4.0$; $df = 1, 4$; $P = 0.12$; $F = 0.70$; $df = 1, 4$; $P = 0.45$, respectively). With the sample sizes used, power is between 15 and 30% (with a 1-sided test) to detect an increase (or decrease) of 25% in the mean response of egg fate on transgenic plants. The exception is percentage hatch, for which power is about 99% to detect such an increase.

Numbers of predators were not significantly different (ANOVA; $df = 1, 4$; $P \geq 0.05$) between isogenic and transgenic plots except for number of lacewing larvae on August 2 ($F = 40.5$; $df = 1, 4$; $P = 0.003$), and number of coccinellid adults on August 17 ($F = 12.89$; $df = 1, 4$; $P = 0.023$) (Table 2). The most abundant predator on all 3 sample dates was *O. insidiosus* (Table 2).

Parasitism of *O. nubilalis* larvae was not significantly different between plots on either of the collection dates ($F = 0.037$; $df = 1, 4$; $P = 0.86$; $F = 0.079$; $df = 1, 4$; $P = 0.79$) (Table 3). Most of the parasitism resulted from *Eriborus terebrans* (Gravenhorst) (Hymenoptera: Ichneumonidae), but *Ma-*

Table 2. Number (mean \pm SD) of *O. nubilalis* egg predators per corn plant in transgenic and isogenic hybrid field corn plots over the 2nd-generation *O. nubilalis* oviposition period, Constantine, MI, 1994

Predator	2 Aug.		17 Aug.		12 Sept.	
	Isogenic	Transgenic	Isogenic	Transgenic	Isogenic	Transgenic
<i>O. insidiosus</i> nymphs	0.127 \pm 0.070a	0.573 \pm 0.959a	3.313 \pm 1.997a	2.553 \pm 1.529a	0.047 \pm 0.064a	0.033 \pm 0.031a
<i>O. insidiosus</i> adults	1.107 \pm 0.272a	1.107 \pm 0.512a	1.453 \pm 0.329a	1.420 \pm 0.410a	0.680 \pm 0.197a	0.487 \pm 0.257a
Coccinellid larvae	0.993 \pm 0.826a	1.387 \pm 0.493a	0.313 \pm 0.175a	0.293 \pm 0.081a	0.007 \pm 0.012a	0.007 \pm 0.012a
Coccinellid adults	0.060 \pm 0.040a	0.087 \pm 0.099a	0.053 \pm 0.031b	0.180 \pm 0.053a	0.007 \pm 0.012a	0.047 \pm 0.031a
Lacewing larvae	0.013 \pm 0.012b	0.073 \pm 0.012a	0a	0.053 \pm 0.042a	0.007 \pm 0.012a	0.007 \pm 0.012a
Lacewing adults	0a	0a	0a	0.020 \pm 0.020a	0.007 \pm 0.012a	0a

Within a row, values for a given date followed by different letters are significantly different (ANOVA; $P \leq 0.05$). Means \pm SD were calculated using plot averages, and all analyses were carried out on plot averages.

crocentrus grandii Goidanich (Hymenoptera: Braconidae) also was present (Table 3).

Discussion

This study failed to find significant differences in egg populations of *O. nubilalis*, or its predators and parasitoids, between transgenic and isogenic plots. Sample sizes (3 replications per genotype) were admittedly small, but all observed differences were in a direction opposite from that expected if transgenic plants had a negative impact on predator populations.

The density, distribution, and size of *O. nubilalis* egg masses were unaffected by transgenic corn in our study. Egg mass size and pattern of distribution on plants were similar to values reported by Hudson and LeRoux (1986a) on nontransgenic field corn plants. The setting of each plot in our study was very consistent. Size of corn plants was the same between treatments, plots were large (0.41 ha), habitat surrounding each plot was the same (i.e., annual rye grass), the experimental plot area was surrounded by a 4.58-m border of isogenic corn, and the entire experimental area was in the center of a soybean field that had been planted to corn the previous season. The lack of difference in *O. nubilalis* oviposition patterns under these conditions indicates a lack of antixenosis by transgenic plants.

The fate of egg masses and eggs within masses under the above conditions should reflect the level of mortality factors, especially natural enemy populations, in transgenic and isogenic corn. The lack of differences noted in our study indicates that

mortality factors of *O. nubilalis* eggs were consistent in all plots, and that corn type had no impact on these factors.

In fact, we did not find that population density of predators was different for the transgenic corn in our study. Predators such as coccinellids, lacewings, and anthocorids are probably attracted to corn by the presence of resources such as pollen, aphids, and thrips, which are the major components of their diets (Gordon 1985, Hudson and LeRoux 1986a, Andow 1996), and they feed on *O. nubilalis* eggs and young larvae opportunistically. The density of predators does not appear to be affected by *O. nubilalis* density.

Because survival of *O. nubilalis* larvae on transgenic corn is extremely low (Koziel et al. 1993), density-dependent mortality factors such as parasitism would be expected to be different between transgenic and isogenic plots. There were no differences in larval parasitism levels between plots in our study, suggesting density-independence of parasitism. For 1 of the parasitoids we recovered, *M. grandii*, density independence has been previously reported (Onstad et al. 1991).

In conclusion, under the conditions we studied, *O. nubilalis* oviposition patterns, fate of *O. nubilalis* egg masses and eggs, and egg and larval predation and parasitism were largely not affected by transgenic field corn. Plot size in our experiment was large (0.41 ha), which we feel to be adequate to test the parameters measured in this study. However, it could be argued they were not large enough to see landscape-scale population effects. In either event, we feel this information could be

Table 3. Fate of 2nd-generation *O. nubilalis* larvae collected from isogenic microplots within transgenic and isogenic hybrid field corn plots, Constantine, MI, 1994

Treatment	% <i>O. nubilalis</i> larvae from collection 1, 26 Aug., mean \pm SD				% of <i>O. nubilalis</i> larvae from collection 2, 15 Sept			
	n	Pupated	Parasitized ^a	Dead	n	Pupated	Parasitized ^b	Dead
Isogenic	118	80.36 \pm 5.19a	2.40 \pm 2.21a	16.27 \pm 1.73a	131	54.69 \pm 9.90a	6.19 \pm 0.45a	38.02 \pm 7.56a
Transgenic	99	78.23 \pm 9.23a	3.03 \pm 5.25a	18.74 \pm 7.92a	87	61.46 \pm 13.59a	7.00 \pm 5.03a	31.54 \pm 12.07a

Within a column, values followed by different letters are significantly different (ANOVA; $P \leq 0.05$). Means were calculated using plot averages and all analyses were carried out on plot averages.

^a Isogenic: 75% *E. terebrans*, 25% *M. grandii*; transgenic: 100% *E. terebrans*.

^b Isogenic: 88.9% *E. terebrans*, 11.1% *M. grandii*; transgenic: 100% *E. terebrans*.

used to assist in development of strategies for managing *B. thuringiensis* resistance and conserving natural enemies.

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