FIELD EFFECTS OF BT CORN ON THE IMPACT OF PARASITOIDS AND PATHOGENS ON EUROPEAN CORN BORER IN ILLINOIS

M.E. Venditti and K.L. Steffey
Department of Crop Sciences, University of Illinois, Urbana-Champaign, Illinois, U.S.A.

INTRODUCTION

The European corn borer, *Ostrinia nubilalis* (Hübner), is one of the most economically important insect pests of corn in North America. Ostlie *et al.* (1997) reported that losses resulting from European corn borer damage and control costs exceed $1 billion in North America each year. Briggs and Guse (1986) estimated that European corn borers cause $50 million in yield losses annually in Illinois.

The European corn borer completes two to three generations per year in Illinois. Fourth and fifth instars tunnel in stalks, causing disruption in the flow of water and nutrients in the plants. The cavities created by the borers may weaken stalks and ear shanks, resulting in lodging or dropped ears. Before the advent of Bt corn, producers attempted to control European corn borers, if the need arose, by applying insecticides before the larvae tunneled into the corn stalks. However, scouting for European corn borers, especially second generation borers, is arduous, and timing an insecticide application for maximum efficacy is difficult. Consequently, the acreage of corn in Illinois treated with insecticides for control of European corn borers has often been significantly lower than the acreage with economic infestations. In other words, many producers did nothing to manage European corn borers, similar to what has been reported throughout much of the Midwest (Rice and Ostlie, 1997). However, the promise of season-long control of European corn borers with transgenic corn expressing ´-endotoxin proteins from *Bacillus thuringiensis* Berliner genes (Bt corn) (e.g., Koziel *et al.*, 1993) has encouraged many producers to manage this perennial pest with transgenic Bt hybrids.

As with any new technology, the use of Bt corn has raised several questions regarding potential risks. Concerns about the development of populations of European corn borer resistant to Bt have been well documented. However, the potential effects of Bt corn on predators, parasitoids, and pathogens that affect populations of European corn borer have not been studied thoroughly, especially in the field. Pilcher *et al.* (1997) fed Bt corn pollen to three predatory species in the laboratory. They reported that direct consumption of Bt corn pollen by *Coleomegilla maculata* (DeGeer), *Orius insidiosus* (Say), and *Chrysoperla carnea* Stephens had no detrimental effects. Results from laboratory studies in Switzerland (Hilbeck *et al.*, 1998; 1999) revealed increased mortality of *C. carnea* larvae that preyed on European corn borers or other Lepidoptera that had fed on Bt corn. However, Zwahlen *et al.* (2000) reported no effects on the predator *Orius majusculus* (Reuter) that were fed the thrips *Anaphothrips obscurus* (Müller) that had fed on Bt corn.

Only a couple of published field studies have provided insight about the potential effects of Bt corn on natural enemies of the European corn borer. After placing laboratory-reared European corn borer eggs in microplots in Michigan, Orr and Landis (1997) determined that Bt corn had no effect on numbers of adults and larvae of *C. maculata* or *O. insidiosus*, nor any effect on parasitism by *Macrocentrus grandii* Goidanich or *Eriborus terebrans* (Gravenhorst). In field-plot studies in Iowa, Pilcher (1999) observed a 30 to 60% reduction in numbers of adult *M. grandii* captured on traps in Bt corn, compared with trap captures in non-Bt corn.

Small-scale field trials and laboratory studies cannot adequately address the concern regarding the ecological impact of Bt corn on predators, parasitoids, and pathogens. Therefore, we assessed the impact of natural mortality factors on populations of first- and second-generation European corn borers in commercial-scale Bt cornfields and compared these findings with the assessments of natural enemies found in non-Bt cornfields.

MATERIALS AND METHODS

Our study was conducted during 1997 and 1998 in two no-till commercial-scale fields of Bt corn and in two no-till commercial-scale fields of non-Bt corn. The fields were located near Auburn in Sangamon County, Illinois, United States. Fields ranged in size from 16 to 23 ha. Each field was divided into six zones to increase the possibilities of encountering detectable population densities of European corn borers. A buffer area of approximately 15 rows around all edges was designated for each field, and each zone was separated from adjacent zones by three buffer rows. Buffer areas were not sampled. The design used was a completely randomized design with two replications and six subsamples per week in each field.

Egg masses, larvae, and pupae of first generation European corn borers were collected from 100 consecutive plants in each zone. The starting point for sampling was determined by walking a randomly assigned number of paces along a systematically assigned row. No row was sampled twice for the duration of the study. Portions of leaves to which egg masses were attached were cut off and placed in paper bags. Infested corn stalks were dissected in the field, and *O. nubilalis* larvae and pupae were removed and placed in paper bags. All life stages of European corn borers recovered were taken to the laboratory in Urbana, Illinois, for further examination, and each egg mass, larva, and pupa was placed individually on meridic diet (Guthrie, 1989) (provided by personnel at the USDA-ARS Corn Insect and Crop Genetics Research Unit, Ames, Iowa). Diet cups were placed in a growth chamber at 25 °C, 60% RH, and a photoperiod of 16:8 h (L:D).

Egg masses, larvae, and pupae of second generation European corn borers were collected weekly as previously described. No row sampled during assessment of the first generation was sampled during assessment of the second generation. To terminate diapause of fifth instars at the end of the growing season, the larvae were placed in vials at 27 °C under a photoperiod of 24:0 h (L:D) and 75% RH.

Parasitism was verified by the emergence of parasitoids from egg masses, larvae, and pupae. The numbers of eggs per egg mass were counted by using a dissecting microscope (20–30x). Eggs that did not hatch and had not been parasitized were examined for infection by *Nosema pyrausta* (Paillot). Larvae that hatched from the eggs were sacrificed and examined for infection by *N. pyrausta.* Surviving larvae were allowed to develop to adults, and half of the emerging adults were sacrificed. Adults and larvae that died in the growth chambers were examined for infection with *N. pyrausta* by making abdominal cross-sections of the larvae and by examining the malpighian tubules by using a dissecting microscope (20–30x). Portions of the malpighian tubules were mounted onto slides and examined for the presence of spores by using a phase-contrast microscope (400x).

European corn borers (larvae and eggs) also were examined for infection by *Beauveria bassiana* (Balsamo) Vuillemin and parasitism by *Trichogramma* species. However, these data are not presented in this paper.

Data were analyzed by using analysis of variance (ANOVA), and means were separated using Fisher=s least significant difference test (Litell *et al.*, 1991). Differences among means were compared at the 5% level of significance. Due to a large number of zeros in some of the variables analyzed, a square root transformation ($\sqrt{x + 0.5}$) of the raw data was used.

RESULTS

The natural enemies found in Bt and non-Bt corn were the parasitoids *M. grandii* and *Trichogramma* sp., and the pathogens *N. pyrausta* and *B. bassiana*. However, as indicated previously, data regarding parasitism by *Trichogramma* and infection by *B. bassiana* are not presented.

First generation European corn borer larvae were found in Bt cornfields, but only in corn plants that were not producing Bt toxins, based on Gene-Check ELISA assays. Most of the early instars of second generation European corn borers present in Bt corn were also in plants that did not express the toxin. However, late in the season, plants in Bt cornfields in which late instar European corn borers survived were difficult to evaluate for the Bt toxin with the ELISA test because this assay did not react with mature corn plant tissue.

Climatic conditions were different between the years. Total precipitation during the first generation oviposition period (June) was 5.4 cm in 1997 and 25 cm in 1998. Numbers of first and second generation European corn borers were significantly lower in 1998 ($F = 35.2$, df = 1, $P = 0.001$) than in 1997.

There were no significant differences in densities of egg masses (number of egg masses per 100 plants) of first ($F = 0.72$, df = 1, $P = 0.401$) and second generation ($F = 3.28$, df = 1, $P = 0.077$) European corn borers in Bt and non-Bt corn in 1997 and 1998 (Tables 1 and 2). However, significant differences in numbers of first-generation ($F = 353.82$, df = 1, $P = 0.0001$) and second-generation ($F = 73.93$, df = 1, $P = 0.0001$) larvae per 100 plants in Bt and non-Bt corn occurred during both years (Tables 1 and 2). Significantly fewer first and second generation larvae occurred in Bt cornfields than in non-Bt cornfields in both years.

Survivorship of European corn borers, expressed as the ratio of the number of larvae divided by the number of eggs, also was significantly different between Bt and non-Bt corn for first ($F = 19.7$, df = 1, $P = 0.0001$) and second generation European corn borers ($F = 9.9$, df = 1, $P = 0.003$) in both years.

The percentages of first generation larvae parasitized by *M. grandii* were not significantly different between corn types in both years (Table 1). However, because no *M. grandii* were collected from second generation European corn borers in Bt corn in 1998, there was a significant difference in the percentage of parasitism between the two corn types ($F = 15.4$, df = 1, $P = 0.0003$) (Table 2).

The percentages of eggs and larvae infected by *N. pyrausta* in Bt cornfields were not significantly different from percentages infected in non-Bt cornfields for first or second generation *O. nubilalis* (Tables 1 and 2).

Based upon the numbers of European corn borers obtained from our sampling regime and using the plant populations of the fields sampled, we estimated the potential numbers of European corn borer larvae per ha likely to be parasitized by *M. grandii* or infected with *N. pyrausta*. For first generation European corn borers in 1997 and 1998, we estimated that there were averages of 1.26 and 0.15 parasitized larvae per ha, respectively, in Bt corn compared with 19.27 and 2.72 parasitized larvae per ha in non-Bt corn. For second generation European corn borers in 1997 and 1998, we estimated there were averages of 1.61 and 0 parasitized larvae per ha in Bt corn compared with 31.6 and 2 parasitized larvae per ha in non-Bt corn in 1997 and 1998, respectively. For first generation European corn borers in 1997 and 1998, we estimated that there were averages of 0.89 and 0.12 larva per ha infected with *N. pyrausta* in Bt corn, compared with 16.8 and 3.85 larvae per ha infected with *N. pyrausta* in non-Bt corn. For second generation European corn borers in 1997 and 1998, we estimated that there were averages of 3.71 and 0.54 larvae per ha infected with *N. pyrausta* in Bt corn, compared with 53.13 and 5.31 larvae per ha infected with *N. pyrausta* in non-Bt corn.

Table 1. Densities, percentage parasitism, and percentage infection of first generation European corn borers in Bt and Non-Bt cornfields, Illinois, 1997 and 1998

Type of corn	Mean no. egg masses per 100 plants[ns]	Mean no. larvae per 100 plants[a]	Percentage parasitism of larvae by *M. grandii*[ns]	Percentage infection of eggs by *N. pyrausta*[ns]	Percentage infection of larvae by *N. pyrausta*[ns]
Bt corn, 1997	3.9	2.9	25	20.2	10.2
Non-Bt corn, 1997	6.4	46.1	19.7	22.8	17.2
Bt corn, 1998	2.3	0.6	8.3	19.6	8.3
Non-Bt corn, 1998	2	8.4	14.2	22.8	19.7

[ns] Means within years not significantly different from each other.

[a] Mean number of larvae per 100 plants significantly fewer in Bt corn than in non-Bt corn in both 1997 and 1998 (F = 353.82, P = 0.0001).

Table 2. Densities, percentage parasitism, and percentage infection of second generation European corn borers in Bt and Non-Bt cornfields, Illinois, 1997 and 1998

Type of corn	Mean no. egg masses per 100 plants[ns]	Mean no. larvae per 100 plants[a]	Percentage parasitism of larvae by *M. grandii*	Percentage infection of eggs by *N. pyrausta*[a]	Percentage infection of larvae by *N. pyrausta*[a]
Bt corn, 1997	54.2	9.6	7.8	13.7	26.7
Non-Bt corn, 1997	74.6	115	12.6	12.8	22.7
Bt corn, 1998	1.2	0.8	0b	29.2	15.8
Non-Bt corn, 1998	1.3	10.5	8.6b	27.7	24.1

[ns] Means within years not significantly different from each other.

[a] Mean number of larvae per 100 plants significantly fewer in Bt corn than in non-Bt corn in both 1997 and 1998 (F = 73.93, P = 0.0001).

[b] Mean percentage parasitism of European corn borer larvae was significantly lower in Bt corn than in non-Bt corn in 1998 (F = 15.4, P = 0.0003).

DISCUSSION

In 1998, numbers of European corn borers were very low; the numbers of second generation egg masses present in both Bt and non-Bt corn were considerably lower than in 1997 (Table 2). Extensive rainfall (25 cm) occurred when females were laying first generation eggs (June) in 1998. It is possible that precipitation greatly affected populations of *O. nubilalis*.

The specialist larval parasitoid *M. grandii* is the only introduced parasitoid that seems to be an important biological mortality factor of European corn borers in Illinois (Siegel *et al.*, 1987). This parasitoid parasitized first and second generation *O. nubilalis* larvae in both Bt and non-Bt corn in 1997 and first generation larvae in both types of corn in 1998 (Tables 1 and 2).

Survival of European corn borer larvae was very low in Bt cornfields (Tables 1 and 2). However, despite the low numbers of larvae in Bt corn, there were no significant differences in the percentages of parasitism by *M. grandii* of first and second generation European corn borers in 1997 and first generation European corn borers in Bt and non-Bt corn in 1998 (Tables 1 and 2), suggesting that *M. grandii* was able to find hosts in Bt cornfields. Orr and Landis (1997) also detected no significant differences in the percentage of larvae parasitized by *M. grandii* in transgenic and isogenic corn. The high percentage of parasitism of first generation European corn borers by *M. grandii* in Bt cornfields in 1997 (Table 1) may suggest that parasitism is density independent, which was previously noted for *M. grandii* (Onstad *et al.*, 1991).

The naturally occurring pathogen *N. pyrausta* can suppress *O. nubilalis* populations in Illinois (Siegel *et al.*, 1987). We observed no significant differences in infection by *N. pyrausta* in egg masses or larvae of either generation in Bt and non-Bt cornfields (Tables 1 and 2), suggesting that corn type had no effect on this pathogen.

Ingestion of spores present in contaminated material (e.g., frass or larval cadavers) is the major route of spreading *N. pyrausta* through tunnels and to new feeding sites (Maddox, 1973). In 1998, horizontal transmission probably did not occur in Bt corn because densities of larvae were very low, reducing the probability of larvae ingesting spores in frass or European corn borer cadavers. Moreover, the level of infection by *N. pyrausta* in eggs was very similar to the level of infection in larvae (Tables 1 and 2). Of the 70 larvae collected from Bt cornfields in 1997, it is possible that horizontal transmission occurred in some of these larvae. The increase in the amount of infection in the larvae in relation to infection of eggs allows for such inference.

Our results suggest that corn type, in general, has no impact on the percentage of larvae parasitized by *M. grandii* and the percentages of eggs and larvae infected by *N. pyrausta* in populations of the European corn borer. The Bt cornfields in our study were surrounded by non-Bt cornfields, a possible explanation of why the percentages of European corn borers parasitized and infected by pathogens were not different in Bt corn and non-Bt corn. Nevertheless, although densities of European corn borers are greatly reduced in Bt cornfields, natural enemies had an impact on European corn borer mortality.

Numbers of natural enemies could be seriously reduced when densities of *O. nubilalis* are low. Our estimates revealed that approximately 97 to 98% fewer first generation European corn borer larvae per ha were parasitized by *M. grandii* in Bt corn than in non-Bt corn in 1997 and 1998. Approximately 95% fewer second generation European corn borer larvae per ha were parasitized in Bt corn than in non-Bt corn in 1997. An estimated 98 to 99% fewer first generation European corn borers per ha were infected with *N. pyrausta* in Bt corn than in non-Bt corn in 1997 and 1998. An estimated 90 to 93% fewer second generation European corn borers per ha were infected with *N. pyrausta* in Bt corn than in non-Bt corn in 1997 and 1998. If a large percentage of area is devoted to the production of Bt corn, the overall impact on specific natural enemies could be significant. Widespread use of Bt corn could have the same effect as using insecticides that affect populations of natural enemies, although the effect of Bt corn is indirect. The loss of specific natural enemies could have serious consequences if European corn borers become resistant to Bt corn. Therefore, refuges of non-Bt corn within Bt cornfields seem necessary not only for resistance management but also for conservation of natural enemies.

REFERENCES

Briggs, S. P. and C. A. Guse. 1986. Forty years of European corn borer data: What have we learned? pp. 169-173. *In* Thirty-eighth Illinois Custom Spray Operators Training School Manual. Cooperative Extension Service, University of Illinois, Urbana-Champaign, Illinois, U.S.A.

Guthrie, W. D. 1989. Advances in rearing the European corn borer on a meridic diet, pp. 46–59. *In* Proceedings of the International symposium of Methodologies for Developing Host Plant Resistance to maize Insects, March 9–14, 1987. CIMMYT, El Batan, Mexico

Hilbeck, A., M. Baumgartner, P. M. Fried, and F. Bigler. 1998. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environmental Entomology* 27: 480–487.

Hilbeck, A., W. J. Moar, M. Pusztai-Carey, A. Filippini, and F. Bigler. 1999. Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea*. *Entomologia Experimentalis et Applicata* 91: 305–316.

Koziel, M. G., G. L. Beland, C. Bowman, N. B. Carozzi, R. Crenshaw, L. Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Launis, K. Lewis, D. Maddox, K. McPherson, M. R. Meghji, E. Merlin, R. Rhodes, G. W. Warren, M. Wright, and S. V. Evola. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *BioTechnology* 11: 194–200.

Litell, R. C., R. J. Freund, and P. C. Spector. 1991. *SAS System for Linear Models*, 3rd ed. SAS Institute, Inc., Cary, North Carolina, U.S.A.

Maddox, J. V. 1973. The persistence of the microsporidia in the environment. *Miscellaneous Publications of the Entomological Society of America* 9: 99–104.

Onstad, D. W., J. P. Siegel, and J. V. Maddox. 1991. Distribution of parasitism by *Macrocentrus grandii* (Hymenoptera: Braconidae) in maize infested by *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Environmental Entomology* 20: 156–159.

Orr, D. B. and D. A. Landis. 1997. Oviposition of European corn borer (Lepidoptera: Pyralidae) and impact of natural enemy populations in transgenic versus isogenic corn. *Journal of Economic Entomology* 90: 905–909.

Ostlie, K. R., W. D. Hutchison, and R. L. Hellmich. 1997. *Bt Corn and European Corn Borer*. North Central Region Extension Publication NCR 602. University of Minnesota, St. Paul, Minnesota, U.S.A.

Pilcher, C. D. 1999. Phenological, physiological, and ecological influences of transgenic Bt corn on European corn borer management. Ph.D. dissertation. Iowa State University, Ames, Iowa, USA.

Pilcher, C. D., J. J. Obrycki, M. E. Rice, and L. C. Lewis. 1997. Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. *Environmental Entomology* 26: 446–454.

Rice, M. E. and K. R. Ostlie. 1997. European corn borer management if field corn: A survey of perceptions and practices in Iowa and Minnesota. *Journal of Production Agriculture* 10: 628–634.

Siegel, J. P., J. V. Maddox, and W. G. Ruesink. 1987. Survivorship of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) in central Illinois. *Environmental Entomology* 16: 1071–1075.

Zwahlen, C., W. Nentwig, F. Bigler, and A. Hilbeck. 2000. Tritrophic interactions of transgenic *Bacillus thuringiensis* corn, *Anaphothrips obscurus* (Thysanoptera: Thripidae), and the predator *Orius majusculus* (Heteroptera: Anthocoridae). *Environmental Entomology* 29: 846–850.