ABSTRACT

Title of dissertation: AN ECOLOGICAL RISK ASSESSMENT OF BT TRANSGENIC SWEET CORN ON NON-TARGET INVERTEBRATE COMMUNITIES

Robyn Ilene Rose, Doctor of Philosophy, 2005

Dissertation directed by: Professor Galen P. Dively
Department of Entomology

An ecological risk assessment evaluating potential adverse affects to non-target arthropods is necessary when releasing a novel transgenic crop. Field studies were conducted to assess tritrophic level effects of insecticide treated and untreated Bt and non-Bt sweet corn hybrids on the abundances, species diversity and functional processes of foliage-dwelling, aerial and soil surface arthropods. The diversity and abundance of decomposers, predators and parasitoids were determined by plant inspections, sticky card, and pitfall trap sampling for two years at two Maryland locations. Functional processes including predation, parasitism, reproduction and colonization were evaluated at one location over three years. Predation rates were estimated from sentinel egg masses of a lepidopteran; levels of parasitism were measured from naturally-occurring chrysopid eggs; emergence traps determined the recruitment rates of arthropods emerging from soil-litter; and litterbags measured arthropod colonization and litter degradation rates. Beneficial arthropod communities and functional processes were not significantly affected by exposure to Bt sweet corn. Insecticides significantly reduced arthropod diversity and abundance as well as the rate of predation, parasitism, colonization and recruitment of organisms. Less disruption occurred in Bt plots treated once than compared with non-Bt plots treated five times. Since the number of insecticide

applications are reduced, planting Bt sweet corn will result in benefits to non-target arthropods and ecological processes.

A second aspect of the ecological risk assessment focused on laboratory and field studies to evaluate the effects of Bt pollen on honey bee survival, brood development and foraging behavior. Results also were used to examine the statistical power of study protocols. Laboratory tests showed no effects of feeding on Bt pollen on survival or hypopharyngeal gland development measured indirectly by head weight gain of newly-emerged bees. In field studies conducted for two years at three locations, colonies foraging in sweet corn plots and fed Bt pollen cakes for 28 days showed no adverse effects on bee weight, hive strength, brood development and foraging behavior. Foraging bee weight, number of foragers returning with pollen loads, pollen load weight, and brood size were the most reliable endpoints to examine for non-target effects on the general fitness of colonies.

AN ECOLOGICAL RISK ASSESSMENT OF BT TRANSGENIC SWEET CORN ON NON-TARGET ARTHROPOD COMMUNITIES

by

Robyn Ilene Rose

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2005

Advisory Committee:

Professor Galen Dively, Chair
Professor Scott Angle
Professor David Hawthorn
Professor William Lamp
Professor Jeffery Pettis
Professor Michael Raupp

TABLE OF CONTENTS

Table of Contents……ii

List of Tables……..iii

List of Figures……iv

Chapter I: Community-Level Effects of Bt Transgenic Sweet Corn on the Abundances and Species Diversity of Arthropods

Abstract……1
Introduction……..2
Materials and Methods……4
Results……10
Discussion……..23
References Cited……28
Tables……34
Figures……48

Chapter II: Effects of Bt Transgenic Sweet Corn on Selected Functional Processes of Arthropod Communities

Abstract……..67
Introduction……67
Materials and Methods……...71
Results……77
Discussion……..83
References Cited……88
Tables……94
Figures……96

Chapter III: Effects of Bt Corn Pollen on Honey Bees with Emphasis on Protocol Development

Abstract……103
Introduction……..104
Materials and Methods……106
Results……..113
Discussion……118
References Cited……..129
Figures……..133

References Cites……...143

LIST OF TABLES

Table 1.1 Frequency of occurrence and abundance of arthropods found on sweet corn plants at the Upper Marlboro, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments……34

Table 1.2 Frequency of occurrence and abundance of arthropods found on sweet corn plants at the Salisbury, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments……36

Table 1.3 Frequency of occurrence and abundance of arthropods found on sticky traps at the Upper Marlboro, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments……38

Table 1.4 Frequency of occurrence and abundance of arthropods found on sticky traps at the Salisbury, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments……...41

Table 1.5 Frequency of occurrence and abundance of arthropods found in pitfall traps at the Upper Marlboro, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments……...43

Table 1.6 Frequency of occurrence and abundance of arthropods found in pitfall traps at the Salisbury, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments……...46

Table 2.1. Frequency of occurrence and abundance of arthropods emerging from the surface litter and colonizing litterbags after harvest at Upper Marlboro, MD. Taxa are listed by ecological function in order of overall abundance. Means were computed by pooling data over all sampling dates and treatments…….94

LIST OF FIGURES

Figure 1.1 Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots……..48

Figure 1.2 Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Salisbury, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots……..49

Figure 1.3 Principal response curves and taxon weights of plant-dwelling arthropod communities exposed to Bt and insecticide-treated sweet corn compared to the isogenic control. Responses of taxa with positive weights followed the PRC patterns, whereas those with negative weights showed the opposite pattern. Taxa with weights between -0.5 to 0.5 are not shown……...50

Figure 1.4. Mean number of aphids recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….51

Figure 1.5. Mean number of thrips recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….51

Figure 1.6. Mean number of fungus beetles (primarily Nitidulidae, Corylophidae and Phalacridae) recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)...52

Figure 1.7. Mean number of chrysopids recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….52

Figure 1.8. Mean number of anthocorids recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….53

Figure 1.9. Mean number of coccinellids recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….53

Figure 1.10. Mean number of spiders recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….54

Figure 1.11. Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots……..55

Figure 1.12. Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Salisbury, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots……..56

Figure 1.13. Principal response curves and taxon weights of aerial arthropod communities exposed to Bt and insecticide-treated sweet corn compared to the isogenic control. Responses of taxa with positive weights followed the PRC patterns, whereas those with negative weights showed the opposite pattern. Taxi with weights between -0.5 to 0.5 are not shown……...57

Figure 1.14. Mean number of parasitic hymenopterans recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….58

Figure 1.15. Mean number of dipterans recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….58

Figure 1.16. Mean number of fungus beetles recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….59

Figure 1.17. Mean number of predatory beetles recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….59

Figure 1.18. Mean number of predatory bugs recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….60

Figure 1.19. Mean number of cicadellids and mirids recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….60

Figure 1.20. Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots……..61

Figure 1.21. Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Salisbury, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots……..62

Figure 1.22. Principal response curves and taxon weights of surface-dwelling arthropod communities exposed to Bt and insecticide-treated sweet corn compared to the isogenic control. Responses of taxa with positive weights followed the PRC patterns, whereas those with negative weights showed the opposite pattern. Taxi with weights between -0.5 to 0.5 are not shown……...63

Figure 1.23. Mean number of ants recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)……...64

Figure 1.24. Mean number of spiders recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)……...64

Figure 1.25. Mean number of staphylinids recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….65

Figure 1.26. Mean number of predatory carabid beetles recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….65

Figure 1.27. Mean number of collembolans recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0)…….66

Figure 2.1. The fate of chrysopid eggs expressed as the percentage parasitized, predated, hatched and unhatched. Data were pooled over two sampling periods approximately one week after peak anthesis in 2002 and 2003. Upper Marlboro, MD……...96

Figure 2.2 Percentage portion of *O. nubilalis* egg masses consumed by chewing and piercing-sucking predators in Bt, non-Bt insecticide-treated, and non-Bt control plots in sweet corn. Data were averaged over six sampling dates in 2002 and 2003. Upper Marlboro, MD……96

Figure 2.3. Mean Shannon-Wiener diversity index of the arthropod community emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002…….97

Figure 2.4. Mean number of Collembola emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002…….97

Figure 2.5. Mean number of Diptera emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002……98

Figure 2.6. Mean number of fungivorous Coleoptera emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002……..98

Figure 2.7. Mean number of Psocoptera emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002…….....99

Figure 2.8. Mean number of parasitic Hymenoptera emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002……..99

Figure 2.9. Mean Shannon-Wiener diversity index of the arthropod community colonizing litterbags at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002……..100

Figure 2.10. Mean number of Collembola colonizing litterbags at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002……..100

Figure 2.11. Mean number of oribatid mites colonizing litterbags at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002……..101

Figure 2.12. Mean number of predatory mites colonizing litterbags at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002……..101

Figure 2.13. Mean number of fungivorous beetles colonizing litterbags at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002……..102

Figure 2.14. Percentage dry matter loss of Bt and non-Bt sweet corn plant material in litter bags removed at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002……...102

Figure 3.1. Percentage survival and pollen consumption of honey bees fed Bt and non-Bt sweet corn pollen and mixed pollen from natural sources for 35 days in a no-choice laboratory study conducted in 2001. ……...133

Figure 3.2. Average head weight of bees collected from colonies at test initiation (day 0) and removed at 10 days from treatment cages in 2001. Marked colony bees were the same age as laboratory exposed bees, whereas random bees were of mixed ages representative of the colonies. Newly-emerged bees collected day 0 were exposed only to hive pollen as larvae. Mean bars with the same letter are not significantly different at the 5% probability level……133

Figure 3.3 Percentage survival and pollen consumption of bees fed Bt, non-Bt and treated sweet corn pollen for 20 days in a no-choice laboratory study conducted in 2002. The treated pollen contained a 2 ppm concentration of imidacloprid…….134

Figure 3.4. Average number of foraging bees and the proportion carrying pollen loads returning to A) hives with established brood and food frames and B) hives that began with blank foundation frames. Data are shown for hives exposed during anthesis in plots of Bt and non-Bt sweet corn, and in control plots of non-Bt sweet corn treated with carbaryl. 2001……..134

Figure 3.5. Average number of foraging bees and the proportion carrying pollen loads returning to hives exposed during anthesis in plots of Bt and non-Bt sweet corn. Data are shown for hives fed Bt, non-Bt, and non-Bt pollen treated with imidacloprid in the respective plots. 2002……...135

Figure 3.6. The number of dead bees found on a 8' x 10' tarp around the hives located in the middle of Bt and nonBt (treated and untreated) cornfields during pollination in the 2001 and 2002 growing seasons. Non-Bt treated plots were sprayed with Sevin7 in 2001 and pollen cakes were mixed with imidacloprid in 2002……135

Figure 3.7. Average weight of foraging bees carry pollen loads to hives and the weight of their pollen loads for A) hives with established brood and food frames and B) hives that began with blank foundation frames. Data are shown for hives exposed during anthesis in plots of Bt and non-Bt sweet corn, and in control plots of non-Bt sweet corn treated with carbaryl. 2001……..136

Figure 3.8. Average weight of foraging bees carry pollen loads to hives and the weight of their pollen loads. Data are shown for hives exposed during anthesis in plots of Bt and non-Bt sweet corn, and in control plots of non-Bt sweet corn exposed to imidacloprid treated pollen cakes. 2002……136

Figure 3.9. Pre- and post-exposure bee strength, percent honey, percent pollen and percent capped brood. Data are shown for hives exposed during anthesis in plots of Bt and non-Bt sweet corn, and in control plots of non-Bt sweet corn treated with carbaryl. Number of bees and standard error for number of bees graphed are 100-fold less than actual numbers. 2001……...137

Figure 3.10. Pre- and post-exposure bee strength, percent honey, percent pollen and percent capped brood. Data are shown for hives exposed during anthesis in plots of Bt and non-Bt sweet corn, and in control plots of non-Bt sweet corn pollen treated with imidacloprid. Number of bees and standard error for number of bees graphed are 100-fold less than actual numbers. 2002……...137

Figure 3.11. Number of replicate cohorts of 50 bees required in laboratory tests to detect a range of relative differences in percent bee survival from the control at 80% statistical power. ……..138

Figure 3.12. Combinations of replicate cohorts and number of bees per cohort required in laboratory tests to detect a range of differences in bee head weight at 80% statistical power……138

Figure 3.13. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in pollen stores from the control at 80% statistical power……139

Figure 3.14. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in honey stores from the control at 80% statistical power……139

Figure 3.15. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in the bee population from the control at 80% statistical power……...140

Figure 3.16. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in the number of bees returning to hives from the control at 80% statistical power. Variance and mean values for power calculations are based on counts per 5 minutes at the entrance of the hive……...140

Figure 3.17. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in the number of foraging bees returning to hives with pollen loads from the control at 80% statistical power. Variance and mean values for power calculations are based on counts per 5 minutes at the entrance of the hive……..141

Figure 3.18. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in the weight of pollen loads carried back to hives by foraging bees relative to the control at 80% statistical power. Variance and mean values for power calculations are based on average weights of ten pollen loads combined per replicate……..141

Figure 3.19. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in the weight of foraging bees from the control at 80% statistical power. Variance and mean values for power calculations are based on average weights of cohorts of ten bees……142

CHAPTER I

Community-Level Effects of Bt Transgenic Sweet Corn on the Abundances and Species Diversity of Arthropods

Abstract

A field study was conducted over two years to determine the effects of transgenic sweet corn containing a gene from the bacterium *Bacillus thuringiensis* (Bt) on the community diversity and abundances of non-target arthropods. The transgenic hybrid was compared with isogenic and transgenic hybrids treated with a foliar insecticide, and with an isogenic hybrid without insecticides as a control. Direct plant inspections, yellow sticky cards, and pitfall traps were used to sample the foliage, aerial and surface-dwelling communities. A total of 573,672 arthropods were enumerated, representing 128 taxonomic groups in 95 families and 18 orders. By functional group, 39, 32, 24 and 5% of the arthropod community was comprised of decomposers, predators, herbivores and parasitoids, respectively. Overall biodiversity and community-level responses were not significantly affected by expression of the Cry1Ab protein. The Bt hybrid had no significant adverse effects on the population densities of non-target herbivores, decomposers, and natural enemies enumerated at the family level by the various sampling methods during the crop cycle. As expected, the insecticide lambda-cyhalothrin had broad negative impacts on many non-target arthropods. One insecticide application in the Bt plots reduced communities of natural enemies by 21 to 48%. Five applications in the non-Bt plots reduced natural enemy communities by 33 to 70%. A few taxa were also positively affected by the insecticide treatments. Non-target communities affected by Bt

treated plots exhibited some recovery, but communities exposed to five applications showed no trends toward recovery during or after the crop cycle. This study clearly showed that the non-target effects of Bt transgenic sweet corn on natural enemies and other arthropods were far less than the community-level disruptions of insecticide control, which have an accepted level of safety.

Introduction

Sweet corn containing a gene from the bacterium *Bacillus thuringiensis* (Bt) was introduced commercially in 1996, as Attribute hybrids by Syngenta Seeds. The plants were transformed with *cry1Ab* and *pat* marker genes inserted by traditional breeding with event Bt11 in transgenic field corn. Constitutive expression of the *cry1Ab* endotoxin provides exceptional control, preventing virtually 100% of the damage caused by European corn borer (*Ostrinia nubilalis* Hübner) and providing more than 90% control of the corn earworm (*Helicoverpa zea* Boddie) and fall armyworm (*Spodoptera frugiperda* Smith). Use of Bt sweet corn hybrids has resulted in significant reductions in the number of insecticide applications.

Like any insect control technology, transgenic expression of an insecticidal protein may present a risk to the non-target arthropod community. Although tiered laboratory tests indicate no acute adverse effects on a suite of individual non-target organisms (Sims 1995, 1997, EPA 2000), few studies have been conducted at the community level to assess tritrophic effects. Bt sweet corn can interact both directly and indirectly with target and non-target organisms at different trophic levels within the cropping system, as well as in habitats in the surrounding landscape (Schuler *et al.* 1999). A reduction in host or prey populations, indirect contact with endotoxin by feeding on

intoxicated organisms, feeding directly on plant parts (e.g., pollen), or changes in plant chemical cues could all have adverse effects on natural enemies. Conversely, reductions in insecticide use resulting from planting Bt sweet corn should be beneficial to natural enemies and reduce overall environmental impacts (Betz *et al.* 2000). Conservation of predators and parasitoids in Bt fields may aid in controlling secondary non-target pests. Thus, an assessment of the ecological risks of Bt sweet corn on non-target organisms should include a comparison with the risks of conventional control methods. Furthermore, risks should be assessed in at a spatial scale large enough to represent typical agronomic conditions and involve a broad taxonomic range of organisms at the community level (Jepson *et al.* 1994, Candolfi *et al.* 2000).

Sweet corn represents an ideal crop system for comparing potential non-target effects of transgenic Bt and conventional insecticide control. This crop is heavily treated with insecticides for lepidopteran pests, and has a shorter crop cycle than other transgenic crops. Thus, there is greater chance of ecological disruption, at least from insecticides, and less time for non-target populations to recover from these disturbances before the end of the crop cycle. Sweet corn provides a favorable habitat for many types of natural enemies and harbors a variety of prey or host organisms, if insecticides are not applied. Copious pollen, produced during anthesis, serves as a supplemental protein source for many beneficial organisms. Because sweet corn is harvested at a premature stage, endotoxin expression is consistently high throughout the crop cycle and generally higher in certain tissues than in Bt field corn.

Reported here is a field study to determine the community-level effects of Bt sweet corn on the abundance and diversity of non-target arthropods, with special

emphasis on natural enemies. This study is the first community-level assessment of non-target effects on sweet corn and differs from other reported field studies in several ways. First, it compared the potential ecological risks of Bt transgenic control with the risks of conventional insecticide control. Secondly, the study involved a comprehensive community analysis of a broad taxonomic range of arthropods and ecological guilds, which allowed for a more complete assessment of the potential hazard to all taxa and possible routes of exposure.

Materials and Methods

Plot design and treatments. Replicated field plot experiments were conducted over two growing seasons (2000 and 2001) and duplicated at two locations within each year. The sweet corn hybrid 'Attribute GSS0966' (Syngenta Seeds, Research Triangle Park, NC; event BT11) and its non-transgenic isoline ('Prime Plus') were planted at the University of Maryland Research and Education facilities in Upper Marlboro and Salisbury, Maryland. Plantings at both locations were irrigated and grown according to recommended commercial practices. Experiments at the two locations differed with respect to the tillage system. At Upper Marlboro, plots were planted into no-till fields consisting of old corn stubble with a rye cover crop. Each block was 16 rows, 0.75 m wide and 39 m long. Sweet corn plots at Salisbury were conventionally tilled and consisted of 24 rows, 0.75 m wide and 30 m long. At both locations, a 10 m non-cropped buffer separated each block.

In both experiments, plots were laid out in a 2 x 2 split plot design with four replicate blocks. The two hybrids (Bt and non-Bt) were arranged as whole plots, while the subplots were either untreated or treated with lambda-cyhalothrin (Warrior 1E,

Syngenta Crop Protection) at a rate of 237 ml/ha. Treatments were applied at Upper Marlboro with an airblast sprayer delivering 536 liters of spray volume/ha, and at Salisbury with a high clearance drop nozzle sprayer delivering 475 liters of spray volume/ha. Treated Bt plots received one insecticide application at 100% fresh silk, which represented the recommended treatment regime to provide additional protection against secondary pests (e.g, nitidulids) or high populations of corn earworm in a late season planting of Bt sweet corn. Non-Bt treated plots received five sprays starting at early silking and repeated every three days. This treatment regime represented a typical schedule of silk sprays for control of ear-invading insects in mid to late season plantings of fresh market sweet corn. All plots were rotary mowed one week after the primary ears reached peak 'roasting ear' maturity.

Data collection. Arthropod abundance and diversity were measured by four sampling methods: inspections of plants, yellow sticky cards and pitfall traps. Samples of whole plants randomly selected from the central rows of each plot were carefully examined to enumerate all arthropod taxa. Sampling started during the late whorl stage and continued weekly until the primary ears reached fresh market maturity. At each time, four and eight plants per plot were sampled in 2000 and 2001, respectively. Each plant was carefully examined starting at the base and working upward by individually removing the tassel, leaves and ear. All arthropods found on the tassel, leaf and stalk surfaces, silks and inside the ear were identified and recorded to the order or family level. A numerical code was assigned to each taxonomic group, and the directory of codes was continuously updated as new taxonomic groups were identified. Digital images of

representative arthropods in each group were used to develop pictorial reference guides to assist in identification.

Yellow sticky cards were used to measure relative abundance and diversity of aerially-active arthropods in the plant canopy. This technique measured communities of small heteropterans, hymenopterans, and dipterans that were not recorded by other sampling methods. Four sticky cards (7.5 cm x 12.5 cm size, Olson Products, Inc, PO Box 1043, Medina, OH 44258) were placed within the center four to five rows of each plot. Cards were supported on cane poles at canopy level up to the late tassel stages and then at ear level during anthesis until harvest. Cards were exposed for 7-d periods four times during July at both locations in 2000 and six times in 2001 from mid June through July at Upper Marlboro and Salisbury. After exposure, cards were placed in a clear plastic resealable bag and brought to the laboratory where captured taxa were identified under a stereo microscope and recorded to the order or family level.

Surface-dwelling arthropods were monitored over weekly intervals during the growing season with pitfall traps. Four pitfall traps were placed in each plot in close proximity to the yellow sticky traps. A 355 ml clear plastic cup was buried in the soil with the mouth level with the soil surface. This cup remained in place to prevent soil disturbance and reburial as traps were replaced. A second cup containing approximately 250 ml of ethylene glycol preservative was inserted inside the secured cup each week and covered with a heavy-duty 25 cm plastic plate that protected the samples from rain and debris. The rim of the inverted plastic plate was placed approximately 1 cm above the soil surface and secured in the ground with three 9 cm bolts that served as legs. The inside cup with its collected sample of organisms was removed and returned to the laboratory

for processing. At Upper Marlboro, pitfall samples were collected eight times during June through August 2000 and six times from mid June through July in 2001. At Salisbury, six samples were collected during July through mid August in 2000 and four samples were collected during July in 2001. Captured arthropods were vacuum filtered over a fine organdy screen to remove the ethylene glycol. Filtered organisms were stored in 70% ethanol and later identified to the order or family levels. Carabids were identified to the genus level, and voucher specimens were confirmed by a coleopteran taxonomist.

Statistical analyses. Before any analysis, data sets were modified to include missing zero counts. The overall community composition of arthropods sampled by each method was characterized by computing the frequency of occurrence and mean density per sample unit for each location and year. For univariate analysis, data were tested for normality and homogeneity of variances using Spearman's Rank Correlation and Shapiro-Wilk's W tests and by examining for non-random patterns in residual plots. Appropriate transformations were performed and/or variances were grouped prior to analysis (Russek-Russek-Cohen and Douglass 1999) to correct for skewness and heterogeneous variances. For density measurements of individual taxa, data over both years were considered repeated measurements since plots were exposed to the same treatments. To increase power of testing differences in population densities, data from both years for each location were averaged by matching sampling dates to corresponding weekly intervals before and after the first insecticide application (early silking). This effectively synchronized the two data sets on a plant phenological basis. A mixed model ANOVA (SAS Institute 1997) was used to test for treatment and interaction effects on selected arthropods or combined taxonomic groups for each location. Treatment and

weekly sampling time were considered fixed factors, while replicate was treated as a random block effect. The repeated measures option was used to correct for temporally correlated data. Contrast tests were performed on each treatment comparison. Interaction means were separated, following a significant F test, by using Tukey's adjustment for pairwise comparisons ($P \leq 0.05$).

Due to the complexity of the data sets, subtle changes in community composition and abundance may not be evident when examining specific populations using univariate statistics. Therefore, several multivariate approaches were used to examine and summarize simultaneously all taxa recorded by each sampling method, thus allowing for an evaluation of treatment effects on the behavior of the community as a whole. The Shannon-Wiener diversity index (Shannon and Weaver 1949) and number of taxa were used to compare the community diversity of the arthropod fauna. For each sampling method, indices for individual samples were computed at each sampling time. ANOVA (PROC MIXED, SAS Institute 1997) was used to test for time, treatment, and interaction effects.

A novel multivariate method, called the principal response curve method (PRC), was used to distill the time-dependent, community-level effects of the treatments into a graphical form (Van den Brink and Ter Braak 1999). This method is based on redundancy analysis (RDA) which is a constrained form of principal component analysis (PCA). Like PCA, RDA is widely used in community ecology to detect and represent the underlying structure of a data set and then relate that structure to explanatory or environmental variables. It differs from PCA in that the explanatory variables (treatment, time, and their interaction) are always fixed *a priori*, which allows the total variance to be

partitioned by multivariate regression into explained variance and residual variance. This makes it possible to compute an F statistic based on the percentage of variance explained. In contrast to multivariate analysis of variance (MANOVA), RDA does not assume an underlying distribution and has no restrictive upper limit on the number of taxa that can be analyzed simultaneously.

Results of RDA are normally summarized in ordination biplots, similar to PCA. However, biplots become very cluttered and difficult to visualize differences between treatments or how treatment effects develop in time, when time by treatment effects are included. PRC overcomes these difficulties by plotting the treatment effects against time in relation to a pre-assigned control. In this study, the arthropod community in the untreated, non-transgenic plots was designated the undisturbed control community and represented as a horizontal time trajectory. To compute the appropriate statistics, RDA was forced to describe a multiple regression model of the abundances of all taxa simultaneously as a linear combination of treatment and time explanatory variables. The model also included an additional term which represented the response of each taxon relative to the response of that taxon in the control community. Thus, effects were expressed as departures from the control community.

A PC-based program (Canoco) provided all the statistics of RDA necessary to generate the canonical coefficients for each treatment plotted over time. The line graph produced by these coefficients represented the principal response and reflected the weighted sum of the abundances of all taxa. Principal responses were produced and plotted over time for the Bt, insecticide-treated Bt, and insecticide-treated non-Bt treatments, thus allowing graphical comparisons of the behavior of each community

relative to the untreated control. The significance of the principal responses were tested by Monte-Carlo permutations. The null hypothesis was that the canonical coefficients equaled zero for all times and treatments. A shuffling sequence for generating permutations of the original data set was designed to remove blocking and repeated measures effects as sources of error from the residual. The permutation procedure generated 1,000 new sets of data that were equally likely under the null hypothesis while keeping the explanatory variables fixed. The significance level was calculated by the proportion of F values greater than or equal to the F value based on the original data set.

RDA also generated taxon-specific weights or scores, which are akin to the slope coefficient of simple regression. These weights are interpreted as relative contributions to the PRC and thus were used to identify which taxa were most or least affected by the hybrid treatments relative to the untreated non-Bt control. Populations of arthropods with high positive weights were inferred to likely follow the pattern shown by the principal response, whereas taxa with greater negative weights were inferred to show the opposite pattern. Taxa with weights near zero (-0.5 to 0.5) were arbitrarily considered to show either no response or one that was unrelated to the pattern shown by the principal response. The most abundant individual or pooled taxa that were most affected were further analyzed by mixed model ANOVA (see above).

Results

Diversity of taxonomic groups. Summed over both years and locations, 573,672 adult and immature arthropods were identified and counted, representing 128 taxonomic groups in 95 families and 18 orders. The diversity and abundance of sampled communities were grouped by ecological function into decomposers, herbivores,

parasitoids and predators. Tables 1.1-1.6 list the frequency of occurrence and mean density of the taxa recorded by each sampling method. Decomposers were the most abundant group comprising 39% of the arthropods recorded. Most of these organisms were found in surface litter residue or on the plant associated with degraded pollen, open wounds caused by caterpillar injury, or senescent plant tissue. The most abundant decomposers included broad mites (Tarsonemidae), oribatid mites (Oribatida), springtails (Sminthuridae, Entomobryidae, and Isotomidae), psocids (Psocoptera), flies (Diptera), and fungivorous beetles (Nitidulidae, Corylophidae, Phalacridae, Crytophagidae, Oedemeridae, Anthicidae, Mycetophagidae, Lathridiidae).

Foliage- and surface-dwelling predators were the second most abundant trophic group comprising 32% of all arthropods. Predominate taxa included insidious flower bugs (Anthocoridae), lady beetles (Coccinellidae), green lacewings (Chrysopidae), predaceous mites (Mesostigmata), ants (Formicidae), spiders (primarily Lycosidae), ground beetles (Carabidae), rove beetles (Staphylinidae), and centipedes (Chilopoda). Twenty four percent of the arthropods were herbivores, which were mainly recorded in the plant samples. Of these plant feeders, aphids (Aphididae), thrips (Thripidae), leafhoppers (Cicadellidae), leafminers (Agromyzidae), flea beetles (Chrysomelidae), and plant bugs (Miridae) were the most abundant. Only 5% of all arthropods identified in the study were parasitoids, and these insects were largely collected on sticky traps. The parasitoids were mostly hymenopterans (Scelionidae, Mymaridae, Trichogrammatidae, Ptermalidae, Charipidae, Encrytidae, Aphelinidae, Ceraphronidae, Eulophidae, Braconidae, Ichneumonidae, Chrysididae, Proctotrupidae, Pompilidae) and some dipterans (Tachinidae).

Plant-dwelling community. *Diversity.* A steady increase in the number and diversity of plant-dwelling arthropods occurred as corn biomass and structural diversity provided more habitat niches and resources. Diversity changed from simple communities of a few dominant taxa during June to more diverse communities of 25-30 taxa during July (Upper Marlboro, Fig. 1.1; Salisbury, Fig. 1.2). Changes in community diversity over time were less evident in 2000 because sampling commenced later in the crop season. The mean numbers of taxa per plant sample and diversity index were significantly reduced by the insecticide treatments. The treatment by time interaction effect on the number of taxa for each year was highly significant (Upper Marlboro 2000: $F_{(9,35.6)} = 10.81$, $P < 0.001$; Upper Marlboro 2001: $F_{(21,84)} = 15.27$, $P < 0.001$; Salisbury 2000: $F_{(6,24)} = 10.81$, $P < 0.001$; Salisbury 2001: $F_{(15,60)} = 6.9$, $P < 0.001$). The number of taxa found on plants after the insecticide applications was reduced by 63% and 30% in the non-Bt treated and Bt treated plots, respectively. Similarly, there was a significant treatment by time effect for the Shannon-Wiener diversity indices at Upper Marlboro (2000: $F_{(9,47)} = 3.55$, $P = 0.002$; 2001: $F_{(21,96)} = 9.74$, $P < 0.001$) and at Salisbury in 2001 ($F_{(15,72)} = 3.6$, $P = 0.001$), but only a significant treatment effect in 2000 ($F_{(3,36)} = 35.73$, $P < 0.001$). Moreover, the number of taxa and diversity in Bt communities exposed to the single application showed evidence of recovery, while the non-Bt treated communities continued to decline in most cases. For both locations and years, differences in the number of taxa and diversity index between Bt and non-Bt control plots were not significant (P values > 0.13), except for the Shannon-Wiener index at Salisbury in 2000 ($F_{(1,36)} = 10.23$, $P = 0.003$) and mean number of taxa in 2001 ($F_{(1,9)} = 10.3$, $P = 0.01$). The

overall number of plant-dwelling taxa recorded in the no-till plots at Upper Marlboro was 22% higher than the number of taxa recorded in the conventional plots at Salisbury.

Response curves. The PRCs of the plant-dwelling communities are shown in Fig. 1.3 with the response of the non-Bt control community indicated by the horizontal line set at zero. The first axis of canonical ordination explained 65 and 60% of the variation due to the treatment effects at the Upper Marlboro and Salisbury sites, respectively. Since this axis accounted for significantly more variation than the other axes, it was used to construct the response curves. The Monte-Carlo test indicated a significant treatment effect at Upper Marlboro (F = 34.9, P = 0.001) and Salisbury (F = 18.5, P = 0.001), which was clearly due to negative departures of the insecticide-treated communities from the control community. Perturbations in community structure inferred by the departures coincided with insecticide applications and were much greater in the non-Bt plots receiving five insecticide applications compared to the Bt plots treated once. Responses of the untreated Bt community fluctuated slightly around the zero line and were not statistically different from the control community (F = 2.74; P = 0.08). The PRC analysis provided taxon weights, which were interpreted as the relative contributions of each taxonomic group to the overall responses depicted in Fig. 1.3. Taxa with weights ranging from -0.5 to 0.5 are not listed because they were arbitrarily considered to show no effect or an unrelated response (personal communication, P. Van den Brink). The most affected taxa had positive weights greater than 0.5, indicating that their abundance was reduced by the insecticide treatments. The higher the positive weight, the greater the negative effect on the indicated taxonomic group. The PRC analysis also indicated that no arthropod

group was positively affected by the treatments, since none of the taxon weights were negative.

Effects on specific taxa. Sixty seven percent of the arthropods found on plants were herbivores, of which aphids and thrips were the most abundant at both locations. Aphid populations were generally higher at Upper Marlboro prior to silking and then declined due to predation in all plots before insecticides were applied (Fig. 1.4). After silking commenced, aphids were differentially affected at both locations by the treatments as indicated by the significant interaction effects (Upper Marlboro: $F_{(18,72)} = 4.77$, $P < 0.001$; Salisbury: $F_{(12,48)} = 3.56$, $P < 0.001$). Aphid densities were significantly reduced in the non-Bt and Bt treated plots but appeared to recover or resurge in the latter, mainly at Salisbury. Aphid numbers in untreated Bt and control plots were statistically the same at Salisbury. However, unexpected responses in the Bt plots were noted at Upper Marlboro but in opposite directions at the beginning and end of the sampling period. Thrips were significantly more abundant at both locations during the 14-21 days before silking but populations naturally declined prior to any insecticide treatment (Fig. 1.5). Interaction differences in thrips activity were indicated by the ANOVA results but these effects were not relevant because the insecticide treatments were not yet applied. Other common herbivores, including chrysomelids (flea beetles), cicadellids, and mirids, were probably under-estimated due to their small size and escapes from plants. Even so, densities of these insects grouped together were reduced in the Bt treated and non-Bt treated plots by 55 and 84% but not affected in the Bt plots. Interaction effects on this pooled group were significant at both locations (Upper Marlboro: $F_{(18,72)} = 3.17$, $P < 0.001$; Salisbury: $F_{(12,48)} = 3.56$, $P < 0.001$).

Eleven percent of the plant-dwelling community in terms of total abundance consisted of decomposing organisms. Of these, nitidulids and other fungivorous beetles (mainly Corylophidae and Phalacridae) were the predominate group. The pooled density of this group was significantly reduced by 22-34% in the Bt treated plots and by 76-83% in the non-Bt treated plots (Fig. 1.6) (Upper Marlboro: $F_{(18,72)} = 7.84$, $P < 0.001$; Salisbury: $F_{(12,48)} = 3.61$, $P < 0.001$). Differences were not significant between Bt and non-Bt untreated plots, although most decomposers were numerically less abundant in Bt plots at the end of the growing season.

Predators, primarily chrysopids, anthocorids, coccinellids and spiders, accounted for 22% of the plant-dwelling arthropods and increased during anthesis as pollen and other food sources became available. The treatment by time interaction effect was significant at both locations for chrysopids (Fig. 1.7) (Upper Marlboro: $F_{(18,72)} = 3.11$, $P < 0.001$; Salisbury: $F_{(12,48)} = 4.19$, $P < 0.001$); anthocorids (Fig. 1.8) (Upper Marlboro: $F_{(18,72)} = 21.4$, $P < 0.001$; Salisbury: $F_{(12,48)} = 1.19$, $P = 0.03$); and coccinellids (Fig. 1.9) (Upper Marlboro: $F_{(18,72)} = 6.02$, $P < 0.001$; Salisbury: $F_{(12,48)} = 2.09$, $P = 0.036$). These major predators were all adversely affected by the insecticide treatments, averaging 27 and 58% less overall in the Bt and non-Bt treated plots, respectively. Spiders also were noticeably affected by the insecticide (Fig. 1.10) but differences were only significant for the treatment effect at Upper Marlboro ($F_{(3,12)} = 8.01$, $P < 0.003$). Population levels of all plant-dwelling predators were statistically the same in the Bt plots compared with levels in the non-Bt control.

Aerial community. *Diversity.* More than 80 taxonomic groups were identified from sticky card captures at both locations. In terms of overall abundance, herbivores

were the most common (50%), followed by decomposers (31%), parasitoids (13%), and predators (7%). The number and diversity of arthropods in the aerial community were significantly affected by the treatments but differences were smaller than and not as consistent as those recorded by plant inspections. At Upper Marlboro, there were significant treatment by time interaction effects on the number of taxa (2000: $F_{(9,29.8)}$ = 2.99, P = 0.01; 2001: $F_{(15,60.1)}$ = 2.19, P = 0.02)) and S-W diversity indices (2000: $F_{(9,48)}$ = 2.01, P = 0.06; 2001: $F_{(15,72)}$ = 2.65, P = 0.003) (Fig. 1.11). These effects were not significant at Salisbury (Fig. 1.12), except for the treatment by time interaction for the S-W indices in 2000 ($F_{(9,48)}$ = 2.25, P = 0.034). Generally, the number and diversity of taxa were the lowest on the last sampling dates in non-Bt plots that received five insecticide applications. Moreover, there was no consistent trend of any reduction in community diversity in the Bt plots.

Response curves. The PRC analysis showed no differences between aerial communities in the Bt and non-Bt plots at both locations but significant negative departures from the control community when insecticides were applied (Fig. 1.13). The majority of the variation due to the treatment effect was explained by the first ordination axis (43.9% for Upper Marlboro; 34% for Salisbury). The overall treatment effect was significant as indicated by negative changes in the weighed abundances of taxa over time relative to the control community (Upper Marlboro: F = 8.8; P = 0.001; Salisbury: F = 5.6; P = 0.001). Reductions were greatest in non-Bt plots receiving five insecticide treatments compared with Bt plots treated once. The analysis indicated that 17 taxa with positive weights followed the same pattern of negative departures depicted by the response curves, thus their populations were reduced in the insecticide-treated plots. Of

these taxa, dipterans (Chloropidae, Otitidae), fungus beetles (Phalacridae, Corylophidae), plant bugs (Miridae), leafhoppers (Cicadellidae), coccinellids, and parasitic wasps (Mymaridae, Scelionidae) were the most adversely affected. Negative weights of collembolans, mites, aphids, and thrips contributed in the opposite direction, thus they were positively affected by the insecticide treatments. More than half of the taxa with weights >0.5 were the same at both locations, indicating that the same sensitive arthropods were consistently affected.

Effects on specific taxa. Sticky traps provided the most accurate assessment of parasitic Hymenoptera, which were too small and mobile to count directly on plants. Egg and larval parasitoids in the families Scelionidae, Mymaridae, Trichogrammatidae, Pteromalidae, Encyrtidae, Braconidae, Ceraphronidae, and Aphelinidae comprised 45% of all hymenopterans recorded and averaged eight per sticky card. Since the PRC weights (Fig. 1.13) indicated that these insects responded similarly to the treatment effects, densities of all families were pooled as a functional group and plotted in Fig. 1.14. There was a significant main effect on the number of parasitic hymenopterans at Upper Marlboro ($F_{(3,12)} = 4.5$, $P = 0.02$) and a significant treatment by date effect at Salisbury ($F_{(12,7.3)} = 9.31$, $P = 0.003$). The overall parasitoid populations in the Bt plots treated once and non-Bt plots treated five times were reduced by 17 and 47%, respectively. At both locations, however, the numbers of parasitoids captured on sticky cards in Bt plots were not significantly different from captures in the control plots ($P > 0.3$).

The sticky cards also provided the most reliable data on adult dipterans that were active in the corn canopy. The majority of the flies captured were decomposers, although some species could be direct plant feeders or parasitoids as larvae. Frit flies

(Chloropidae), picture-winged flies (Otitidae), and humpbacked flies (Phoridae) were the most abundant with mean densities ranging from 8.75 to 50.28 flies per card. PRC weights of all dipteran families were positive and thus responded in the same direction as the treatment effects. As a pooled group, overall densities were significantly higher and increased over time at Upper Marlboro compared to lower, declining populations at Salisbury (Fig. 1.15). Post-treatment densities of flies were significantly reduced by 31 to 56% in the insecticide-treated plots but unaffected in the Bt plots compared to the control (Upper Marlboro treatment by date effect: $F_{(12,12)} = 3.43$, $P = 0.02$; Salisbury main effect: $F_{(3,28.1)} = 7.07$, $P = 0.001$). Fungus beetles (mainly Corylophidae and Crytophagidae), the second most abundant group of decomposers, were also reduced by insecticide treatments at both locations but not affected by Bt exposure (Upper Marlboro: $F_{(12,12)} = 5.18$, $P = 0.004$; Salisbury $F_{(12,12)} = 4.0$, $P = 0.01$) (Fig. 1.16).

The composition of predatory arthropods captured by sticky cards was comparable to the composition of predators active on the corn plants. However, relative numbers captured were the opposite of the population trends in the plant-dwelling community (Fig. 1.17). Sticky cards captured declining numbers of predators during and after silking whereas the plant inspections recorded expanding populations during the same period. Differences were due to the life stages of predators sampled by each method, since the sticky card captured mainly adults. Mean densities of predatory beetles, pooled over the families Coccinellidae, Staphylinidae, Lampyridae, and Cantharidae, were reduced by 27% after insecticides were applied at Upper Marlboro ($F_{(3,8.1)} = 22.22$, $P < 0.001$) but were not significantly affected at Salisbury. Predatory bugs, primarily insidious flower bugs (Anthocoridae) and big-eyed bugs (Lygaeidae), were numerically

less abundant in the insecticide-treated plots at both locations but differences were not significant (Fig. 1.18). None of the predatory arthropods recorded on sticky cards displayed density changes over time that would indicate an adverse effect due to Bt exposure.

Thrips, leafhoppers, aphids, flea beetles and plant bugs were consistently the most abundant herbivores captured on sticky cards. In accordance with the high positive PRC weights, populations of plant bugs (Miridae) and leafhoppers (Cicadellidae) were significantly reduced by the insecticide treatments at Upper Marlboro ($F_{(3,12)} = 8.25$, $P = 0.003$; $F_{(3,11.3)} = 4.08$, $P = 0.03$; respectively) and Salisbury ($F_{(3,31)} = 7.57$, $P < 0.001$; $F_{(3,33.3)} = 3.14$, $P = 0.04$; respectively) (Fig. 1.19). Overall densities of flea beetles also were 38% less in the insecticide-treated plots compared with the control but differences were not significant. In contrast, sticky card captures of thrips and aphids generally increased after the insecticides were applied. However, this response was only statistically significant for thrips at Upper Marlboro ($F_{(12,8.93)} = 5.86$, $P = 0.006$). Captures of collembolans also showed signs of increased aerial activity associated with the insecticide applications but counts were too variable to test for significance.

Surface-dwelling community. *Diversity.* The pitfall traps captured 73 taxonomic groups of surface-dwelling arthropods, of which decomposers and predators comprised 90 to 98% of the total community on the basis of abundance. The number and diversity of taxa did not change significantly over the growing season at both locations (Figs. 1.20 and 1.21). The number of taxa recorded in 2001 was generally higher than the number recorded the previous year, particularly at Salisbury where more carryover residue left by previous year's corn crop and the winter cover crop may have influenced diversity. There

were no significant treatment or time effects on the mean number and S-W indices in either year at both locations, except for a treatment by time interaction effect in 2001 at Salisbury ($F_{(9,36)} = 3.98$, $P = 0.001$). Although it was not possible to statistically test for differences in diversity between locations, the total abundance of arthropods captured in pitfall traps in no-tilled plots at Upper Marlboro was 2.2-fold higher than the total captured in conventional-tilled plots at Salisbury.

Response curves. The PRC curves in Fig. 1.22 show how the surface-dwelling communities collected in pitfall traps responded over time in the Bt and insecticide-treated plots. At Upper Marlboro, the PRC analysis captured 26.6% of the variation due to the treatment effect in the first axis, which depicted overall treatment responses that were significantly different from the control community ($F = 5.27$, $P = 0.009$). At Salisbury, the first axis of the PRC curves explained 42.0% of the treatment effect and also depicted significant departures of the treated communities ($F = 7.66$, $P = 0.001$). Monte-Carlo permutations testing only the Bt exposed communities showed no significant departures in the weighed abundances of taxa compared with the control community. Community responses in the Bt treated plots were also not significant when tested against the control, although negative departures occurred after the single insecticide application at both locations. It is noted that the *P* value was 0.06 for the Salisbury results, which would be significant if a one-directional response was assumed. Significant perturbations of arthropod communities in the non-Bt plots treated five times with insecticides were clearly visualized by PRC. At both locations, the insecticide-treated communities showed trends towards recovery after about two weeks elapsed from the first application.

As indicated by higher positive weights, populations of staphylinids, crickets, spiders, and coccinellids were the most adversely affected by the insecticide treatments. The carabid genera *Pterostichus*, *Stenolophus* and *Amara* also contributed significantly to changes in community responses, but these beetles increased in the treated plots when insecticides were applied, according to the negative weights. Collembolans consistently had the largest negative weights at both locations and thus also increased in numbers when insecticides were applied.

Effects on specific taxa. Pitfall traps provided the best estimations of the major surface-dwelling predators, namely ants, spiders, carabid beetles, and staphylinids. As the taxon weights indicated, these predatory groups either were not affected or responded in different directions to the treatment effects. For instance, ants did not contribute significantly to the PRCs, yet they were abundant in the surface-dwelling community, particularly in the no-tilled plots at Upper Marlboro (Fig. 1.23). ANOVA results indicated no significant main or interaction effects on ants at Upper Marlboro. In contrast, ant captures in pitfall traps at Salisbury were 10-fold less due to the absence of surface residue, but were significantly higher in the non-Bt treated plots ($F_{(33,9)} = 6.79$, $P = 0.01$). Spiders, primarily lycosids, were consistently captured at both locations, reaching peak densities (15 to 25 per pitfall trap) several weeks prior to silking (Fig. 1.24). Spider activity declined naturally after silking in all plots but at a significantly greater rate in the insecticide-treated plots (Upper Marlboro: $F_{(21,84)} = 2.26$, $P = 0.005$; Salisbury: $F_{(12,48)} = 3.78$, $P = 0.0005$). Similar to ants, staphylinid populations were 10-fold higher in the no-tilled plots at Upper Marlboro compared to overall populations at Salisbury (Fig. 1.25). At both locations, mean densities were reduced by 39 and 67% in

the Bt and non-Bt insecticide-treated plots, respectively, but this effect was only significant at Upper Marlboro ($F_{(21,84)} = 2.26$, $P = 0.005$). As indicated by the negative PRC weights, certain carabid genera exhibited positive responses to the insecticide treatments; however, data for these taxa were too variable to test for significance. The seed-feeding carabids in the genus *Amara* were captured primarily during the first sampling date at Upper Marlboro (averaging 5.6 per trap in 2000), but were rare at Salisbury. Pooled densities of all carabids that function chiefly as predators were not significantly affected by the treatments at either location (Fig. 1.26). None of the predatory taxa described above were significantly affected by exposure to Bt corn, nor was any population trends evident that might suggest an adverse effect.

Collembolans and oribatid mites were by far the predominant decomposing organisms recorded in pitfall samples, followed by field crickets and a variety of dipterans and fungivorous beetles. Mite abundance was 64-fold higher in the no-tilled plots at Upper Marlboro compared to densities at Salisbury. Despite their high abundance, mites did not contribute to the overall community responses described by the PRCs and were not significantly affected by treatments. Collembolans accounted for 53% of the total pitfall captures and contributed significantly to changes in the overall community responses as indicated by the larger negative PRC weights. Similar to mites, densities of collembolans were about 5-fold higher at Upper Marlboro in response to food sources provided by the surface residue in the no-tilled plots (Fig. 1.27). The combined densities of all families of Collembola responded positively to the insecticide treatments (Upper Marlboro: $F = 8.14$, df = 3,9, $p = 0.006$; Salisbury: $F = 4.52$, df = 3,9, $P = 0.03$). Overall activity in pitfall traps after insecticides were applied increased by 8% in the Bt

and non-Bt treated plots. Crickets were relatively abundant in the no-tilled plots at Upper Marlboro and responded significantly to a treatment by time interaction effect ($F = 2.72$, df = 21,84, $P < 0.001$). Generally, fewer crickets were captured in the treated plots after insecticides were applied. Numerical trends in dipteran and fungus beetle captures also showed evidence of negative effects due to insecticides but data were too variable to test for significance.

Discussion

This study was the first community-level assessment of the potential non-target effects of Cry1Ab expression in Bt sweet corn compared with conventional insecticide control. The sampling methods provided time-specific information on 128 taxonomic groups. By ecological role, 39, 32, 24 and 5% of the arthropod community was comprised of decomposers, predators, herbivores and parasitoids, respectively. Overall biodiversity and community-level responses depicted by the PRCs were not significantly affected by expression of the Cry1Ab protein. Results enumerated by all sampling methods clearly established that the expression of the Cry1Ab protein had no adverse impact on any beneficial predatory group and seem to only directly impact the targeted pest species. These results agree with laboratory and other field studies that found no significant non-target effects from the Cry1Ab protein (Orr and Landis 1997, Pilcher *et al.* 1997, Lozzia 1999, 2002, Head *et al.* 2001, Hagerty *et al.* 2001, Candolfi *et al.* 2002, Naranjo 2002, Wilson and Fitt 2002, French 2004, Men *et* al. 2004, Sisterson *et al.* 2004). The study also found no direct or indirect adverse effects on parasitoids from Bt sweet corn, which was expected given the known spectrum of activity of the Cry1Ab protein.

These results agree with other Bt corn studies that showed no effect on parasitic Hymenoptera (Lozzia 1999, Candolfi *et al*. 2003, Jasinski *et al*. 2003).

Since it is very labor-intensive to examine all potential non-target organisms associated with transgenic corn, this study provided a database for selecting the most consistent and sufficiently abundant non-target beneficial taxa that could serve as bioindicators for future work. The major foliage-dwelling predators were anthocorids (mainly *Orius insidiosus* (Say)), chrysopids (*Chrysoperla carnea* (Stephens)), and coccinellids (predominately *Coleomegilla maculata* (DeGeer)). Populations of these insects peaked during the maximum exposure of pollen and are known to be important predators of many insect pests in corn agroecosystems (Coll and Bottrell 1992). Anthocorids, chrysopids, and coccinellids are also good candidates as bioindicators because they are potentially exposed to the expressed Bt proteins via three routes of exposure - feeding on pollen, secondary exposure by feeding on herbivorous insects that have fed on the Bt plant, or direct contact with Bt toxins by feeding on the plant (only in the case of *O. insidiosus).* Plant inspections provided the most consistent data on the life stages of the three predator groups, whereas the sticky cards captured primarily adult predators and were vulnerable to exaggerated captures of excited arthropods after insecticide applications. Also, changes in predator populations over time determined by plant inspections were more representative of the actual population densities.

Pitfall trapping has become the standard method in non-target studies for sampling epigeal predators in the soil-litter community. In this study, the prominent surface-dwelling predators were ants, spiders (mainly lysocids), staphylinids, and carabids. Of these predators, selection of the most appropriate bioindicators depends

upon whether the non-target study system is conventionally or no-tilled. Several species of ants (*Aphaenogaster rudis* Emery and *Myrmica americana* Weber) were commonly found in relatively high numbers at Upper Marlboro but were scarce in the conventionally-tilled plots at Salisbury. However, ants may not be reliable bioindicators because of their non-random spatial distribution in cornfields, which can lower the statistical power for detecting differences. Ants also exhibit variable feeding habits, so their ecological role in food web interactions may be difficult to interpret without identification to the species level. Carabids and staphylinids have been recommended as bioindicators to assess the ecological risks of the rootworm-resistant Bt corn (EPA 2002). The composition of carabid beetles at the genus level varied by sampling date and tillage system but consistently included *Harpalus, Stenolophus, Chlaenius,* and *Pterostichus.* Carabid densities in the no-tilled plots were two-fold greater than the densities in conventionally-tilled plots. The species *Pterostichus lucublandus, Chlaenius nemoralis,* and *Harpalus pennsylvanicus* were common predatory species that occurred during silking and any one of these species would be appropriate indicators of potential treatment effects. Like ants, staphylinid populations were favored by the presence of surface litter. Pitfall captures of rove beetles were ten times more abundant in the no-tilled plots and four times more numerous than carabids at Upper Marlboro. In the conventionally-tilled plots at Salisbury, rove beetles were relatively low and ranked second to carabids. The taxonomy of Staphylinidae is difficult, so the predominate species could not be determined. However, the majority of rove beetles belonged to the genera *Aleochara, Leptusa,* and *Myllaena. Aleochara* are parasitoids of root maggots and

the species *Aleochara bilineata* Gyll. has been used as a standard test organism for testing non-target effects.

The families Mymaridae, Trichogrammatidae, and Scelionidae were the most frequently captured parasitoids on sticky cards, which provided the most reliable method for assessing changes in these tiny, mobile wasps. The majority of parasitoids in these families parasitize eggs of insects and other arthropods, thus they would probably not be appropriate indicators for measuring effects of the Bt toxins, because they are relatively unexposed and not affected by density changes in target pest populations. Tachinid flies and braconid wasps have been studied as specific non-targets in Bt crops (Orr and Landis 1997, Venditti and Steffey 2003). However, these insects were not abundant enough in this study to statistically test for treatment effects. For beneficial surface-dwelling decomposers, springtails have been a standard indicator group for testing non-target effects of pesticides and Bt crops (Jepson 1994, EPA 2000, 2002). Pitfall captures of collembolans were 2.6 times greater at Upper Marlboro where the decomposing litter provided fungal growth and moist micro-environmental conditions for population growth. Thus, non-target field studies that focus specifically on Collembola should be conducted in a no-tillage system to maximize the population exposed and reduce the sampling variance.

The pyrethroid insecticide applied to the Bt and non-Bt corn plots had a significant negative impact on many arthropods. In all cases, PRC responses of the pyrethroid-treated communities showed significant negative departures from the non-Bt control, which coincided with insecticide applications. One insecticide application in the Bt plots reduced communities of natural enemies by 21 to 48%. Five applications in the

non-Bt plots reduced natural enemy communities by 33 to 70%. All plant-dwelling predators, parasitoids, decomposers, and most herbivores were sensitive in varying degrees to the pyrethroid insecticide. The surface-dwelling arthropods were generally less affected because of the shelter afforded by the surface litter and the fact that less insecticide residue settled on the litter surface. Other field studies have reported significant reductions in the diversity and abundance of non-target communities in conventionally sprayed fields compared with more diverse communities in Bt cotton and potato fields (Reed *et al*. 2001, Head *et al*. 2001 Head *et al*. 2001, Naranjo *et al.* 2002, Fitt and Wilson 2003, Men *et al*. 2004). The adverse effects on specific taxonomic groups were predictable based on the available literature on the non-target effects of pesticides (Croft 1990, Jepson 1989, Theiling and Croft 1988). A few taxa were also positively affected by the insecticide treatments. Collembola, mites, and aphids tended to increase in numbers in the insecticide-treated plots. Resurgence of mites and aphids often occurs after applications of pyrethroid insecticides as a result of disruption of their natural enemy populations. Non-target communities in Bt plots treated once with an insecticide exhibited some recovery depending upon the particular non-target group, but communities exposed to five applications showed no trends toward recovery during the crop cycle. Whether these ecological disturbances carryover to the following season or have landscape-scale consequences remains unknown.

In summary, this study clearly showed that the non-target effects of Bt transgenic sweet corn on natural enemies and other arthropods were significantly far less than the community-level disruptions of insecticide control, which have an accepted level of safety. The weight of evidence supports the general consensus that there are no

unexpected ecological risks caused by transgenic lepidopteran-resistant corn on nontarget organisms. Bt sweet corn is expected to result in significant reductions in conventional insecticide use and thus fewer disruptions to natural enemies and other non-target organisms. Beneficial insects allowed to persist in Bt sweet cornfields may aid in controlling secondary pests (Betz *et al.* 2000).

References Cited

Al-Deeb, M.A., G.E. Wilde and R.A. Higgins. 2001. No effect of Bacillus thuringiensis corn and Bacillus thuringiensis on the predator *Orius insidiosus* (Hemiptera : Anthocoridae). Environ. Entomol. 30: 625-629.

Andow, D.A. 1990. Characterization of predation on egg masses of *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 83: 482-486.

Betz, F.S., B.G. Hammond, and R.L. Fuchs. 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. Reg. Tox. Pharmacol. 32: 156-173.

Candolfi M, Bigler F, Campbell P, Heimbach U, Schmuck R, Angeli G, Bakker F, Brown K, Carli G, Dinter A, Forti D, Forster R, Gathmann A, Hassan S, MeadBriggs M, Melandri M, Neumann P, Pasqualini E, Powell W, Reboulet JN, Romijn K, Sechser B, Thieme T, Ufer A, Vergnet C, Vogt H. 2000. Principles for regulatory testing and interpretation of semifield and field studies with nontarget arthropods. J. Pest Sci. 73: 141-147.

Candolfi, M., K. Brown, and B. Reber. 2003. A faunistic approach to assess potential side-effects of genetically modified *Bt*-corn on non-target arthropods under field conditions. Environ. Biocontrol Science and Technology 14: 129-170.

Coll, M. and D.G. Bottrell. 1991. Microhabitat and resource selection of the European corn borer (Lepidotpera: Pyralidae) and its natural enemies in Maryland field corn. Environ. Entomol. 20: 526-533.

Coll, M. and D.G. Bottrell. 1992 Mortality of European corn borer larvae by natural enemies in different corn microhabitats. Bio. Con. 2: 95-103.

Doanne Marketing Research, Inc. 1998. Multi Client Studies.

Dogan, E.B. R.E. Berry, G.L. Reed, and P.A. Rossignol. 1996. Biological parameters of convergent lady beetle (Coleoptera: Coccinellidae) feeding on aphids (Homoptera: Aphididae) on transgenic potato. J. Econ. Entomol. 89: 1105-1108.

Donegan, K.K., D.L. Schaller, J.K Stone, L.M. Ganio, G. Reed, P.B. Hamm, and R.J. Seidler. 1996. Microbial populations, fungal species diversity and plant pathogen levels in field plots of potato plants expressing the *Bacillus thuringiensis* var. *tenebrionis* endotoxin. Transgenic Research. 5:25-35.

Dutton, A., H. Klein, J. Romeis and F. Bigler. 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. Ecol. Entomol. 27:441-447.

Environmental Protection Agency. Scientific Advisory Panel. 2000. Characterization and non-target organism data requirements for protein plant-pesticides. SAP Report No. 99-06A dated February 4, 2000, of December 8, 1999, FIFRA SAP meeting, Arlington, VA.

Environmental Protection Agency. Scientific Advisory Panel. 2002. Corn rootworm plant-incorporated protectant insect resistance management and non-target insect issues. Transmittal of meeting minutes of the FIFRA Scientific Advisory Panel Meeting held August 27-29 at the Marriott Crystal City Hotel, Arlington, VA.

Fitt, G. and L. Wilson. 2003. Non-Target Effects of Bt Cotton: A Case Study from Australia. In R. J. Akhurst, C. E. Beard and P. Hughes (eds.). *Biotechnology of Bacillus thuringiensis and its environmental impact : Proceedings of the 4th Pacific Rim conference*, Canberra, AU, CSIRO Entomology.

French, B.W., L.D. Chandler, M.M. Ellsbury, B.W. Fuller and M. West. 2004. Ground beetle (Coleoptera: Carabidae) assemblages in a transgenic corn-soybean cropping system. Environ Entomol. 33: 554-563.

Gill, S.S., E.A. Cowles, and P.V. Pietrantonio. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Ann. Rev. Entomol. 37: 615-636.

Hagerty, A., S.G. Turnipseed and M.J. Sullivan. 2001. Impact of predaceous arthropods in cotton IPM. In Proceedings of the Beltwide Cotton Conferences, National Cotton Council, Memphis, TN, pp. 812-815.

Head, G., C.R. Brown, M.E. Groth and J.J. Duan. 2001a. Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. Entomol. Exp. Applic. 99: 37-45.

Head, G., B. Freeman, W. Moar, J. Ruberson and S. Turnipseed. 2001b. Natural enemy abundance in commercial Bollgard and conventional cotton fields. In Proceedings of the Beltwide Cotton Conferences, National Cotton Council, Memphis, TN, pp. 796-798.

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

Hilbeck, A., W.J. Moar, M. Pusztai-Carey, A. Filippini, and F. Bigler. 1998b. Toxicity of *Bacillus thuringiensis* CryIAb toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 27:1255-1263.

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*. Entomol. Exper. Appl. 91: 305-316.

Jasinski, J.R., J.B. Eisley, C.E. Young, J. Kovach and H. Willson. 2003. Select nontarget arthropod abundance in transgenic and nontransgenic field crops in Ohio. Environ. Entomol. 32: 407-413.

Jepson, P.C. 1989. The temporal and spatial dynamics of pesticide side-effects on non-target invertebrates. Pesticides and non-target invertebrates. editor: Paul C. Jepson. Wimborne, Dorset, England : Intercept. pp. 95-127.

Jepson, P.C., B.A. Croft, and G.E. Pratt. 1994. Test systems to determine the ecological risks posed by toxin release from *Bacillus thuringiensis* genes in crop plants. Mol.Ecol. 3: 81-89.

Johnson, M.T. and F. Gould. 1992. Interaction of genetically engineered host plant resistance and natural enemies of *H. virescens* (Lepidoptera: Noctuidae) in tobacco. Environ. Entomol. 21: 586-597.

Johnson, M.T., F. Gould, and G.C. Kennedy. 1997. Effects of natural enemies on relative fitness of *Heliothis virescens* genotypes adapted and not adapted to resistant host plants. Entomiol. Exper. Appl. 82: 219-230.

Lozzia, G.C. 1999. Biodiversity of ground beetle assemblages (Coleoptera: Carabidae) in Bt corn and its effects on non target insects. Bollentino di Zoologia agraria e di Bachicoltura. 31: 37-50.

Manachini, B., M. Agosti and M. Rigamonti. 1999. Environmental impact of Bt-corn on non target entomofauna: synthesis of field and laboratory studies. XI Symposium Pesticide Chemistry.

Men, X., F. Ge, C.A. Edwards and E.N. Yardim. 2004. Influence of pesticides applications on pest and predatory arthropods associated with trangenic *Bt* cotton and nontransgenic cotton plants. Phytoparasitica. 32: 246-254.

Naranjo, S.E. and P.C. Ellsworth. 2002. Arthropod communities and transgenic cotton in the western United States: implications for biological control. In Proceedings of the First International Symposium of Biological Control of Arthropods, U.S. Forest Service, Amherst, MA.

Orr, D.B. and D.A. Landis. 1997. Oviposition of European corn borer (Lepidoptera: Pyralidae) and impact on natural enemy populations in transgenic versus isogenic corn. J. Econ. Entomol. 90: 905-909.

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. Environ. Entomol. 26: 446-454.

Raps, A., J. Kehr, P. Gugerli, W.J. Moar, F. Bigler and A. Hilbeck. 2001. Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. Molec. Ecol. 10: 525-533.

Reed, G. L., A. S. Jensen, J. Riebe, G. Head, and J.J. Duan. 2001. Transgenic Bt potato and conventional insecticides for Colorado potato beetle management: Comparative efficacy and non-target impacts. Entomologia Experimentalis et Applicata 100: 89-100.

Riddick, E.W. and P. Barbosa. 1998. Impact of Cry3Aintoxicated *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and pollen on consumption, development, and fecundity of *Coleomegilla maculata* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 91: 303-307.

Riddick, E.W., G. Dively and P. Barbosa. 1998. Effect of a seedmix deployment of Cry3A transgenic and nontransgenic potato on the abundance of *Lebia grandis* (Coleoptera: Carabidae) and *Coleomegilla maculata* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 91: 647-653.

Romeis, J., A. Dutton and F. Bigler. 2004. *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). J Insect Physiol. 50: 175-183.

Russek-Cohen, E. and L.W. Douglass. 1999. Mixed models data analysis: using PROC MIXED for biological research. Mixed model shortcourse manual. P. 89-103. Jan. 11-14, 1999. Beltsville, MD.

SAS Institute. 1997. SAS user=s guide. SAS Institute, Raleigh, NC. 494 pp.

Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D.R. Zeigler, and D.H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Molec. Biol. Rev. 62: 775-806.

Schuler, T.H. , G.M. Poppy, B.R. Kerry, and I. Denholm. 1999. Potential side effects of insect-resistant transgenic plants on arthropod natural enemies. Trends in Biotechnology 17: 210-216.

Shannon, C. E. and W. Weaver. 1949. The mathematical theory of communication. Urbana IL: University of Illinois Press.

Sims, S.R. 1997. Host activity spectrum of the CryIIA *Bacillus thuringiensis* subsp. *kurstaki* protein. Effects on Lepidoptera, Diptera, and nontarget arthropods. Southwest. Entomol. 22: 395-404

Sims, S.R. and J.W. Martin. 1996. Effect of the *Bacillus thuringiensis* insecticidal proteins on CryIA(b), CryIA(c), CryIIA, and CryIIIA on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola). Pediobiologia. 41: 412-416.

Sims, S.R. 1995. *Bacillus thuringiensis* var. *kurstaki* [CryIA (C)] protein expressed in transgenic cotton: effects on beneficial and other nontarget insects. Southwestern Entomol. 20: 493-500.

Sisterson, M.S., R.W. Biggs, C.O. Olson, Y. Carriere, T.J. Dennehy and B.E. Tabashnik. 2004. Arthopod abundance and diversity in Bt and non-Bt cotton fields. Environ. Entomol. 33: 921-929.

Sparks, A.N., H.G. Chiang, C.C. Burkhardt, M.L. Fairchild, and G.T. Weekman. 1966. Evaluation of the influence of predation on corn borer populations. J. Econ. Entomol. 59(1): 104-107.

Theiling, K.M. and B.A. Croft. 1988. Pesticide side effects on arthropod natural enemies. Agric. Ecosys. Environ. 21: 191-218.

Van den Brink, P.J. and C.J.F Ter Braak. 1999. Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. Environ. Tox. Chem. 18: 138-148.

Venditti, M.E. and K.L. Steffey. 2002. Field effects of Bt corn on the impact of parasitoids and pathogens on European corn borer in Illinois. In Proceedings of the First International Symposium of Biological Control of Arthropods, U.S. Forest Service, Amherst, MA.

Wilson and Flint. 2002. Presentation at the National Biological Control Conference.

Wold, S.J., E.C. Burkness, W.D. Hutchison and R.C. Venette. 2001. In-field monitoring of beneficial insect populations in transgenic corn expressing a *Bacillus thuringiensis* toxin. J. Entomol. Sci. 36: 177-187.

Xia. J.Y., J.J. Cui, L.H. Ma, S.X. Dong and X.F. Cu. 1999. The role of transgenic Bt cotton in integrated insect pest management. Acta Gossypii Sinica. 11: 57-64.

Yu, L., R.E. Berry and B.A. Croft. 1997. Effects of *Bacillus thuringiensis* toxins in transgenic cotton and potato on *Folsomia candida* (Collembola: Isotomidae) and *Oppia nitens* (Acari: Orbatidae). J Econ Entomol. 90: 113-118.

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

Table 1.1 Frequency of occurrence and abundance of arthropods found on sweet corn plants at the Upper Marlboro, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments.

		2000		2001	
Common name	Taxonomic group	Mean density per plant	Percentage occurrence	Mean density per plant	Percentage Occurrence
Decomposers					
Sap beetles[1]	Nitidulidae	1.95	81.0	1.28	52.3
Minute fungus beetles[2]	Corylophidae	NR	NR	0.54	35.2
Psocids	Psocoptera	0.76	52.4	0.44	27.3
Flies	Diptera	0.11	7.9	0.29	50.8
Hairy fungus beetles[2]	Mycetophagidae	NR	NR	0.23	14.1
Shining mold beetles[2]	Phalacridae	0.27	34.9	0.11	28.9
Slugs	Agrolimacidae	0.23	7.9	NR	NR
Silken fungus beetles[2]	Crytophagidae	0.15	12.7	NR	NR
Herbivores					
Thrips	Thripidae	NR	NR	23.35	81.3
Aphids	Aphididae	4.01	63.5	2.05	80.5
Blotch leaf miners	Agromyiidae	0.03	3.2	0.53	57.8
Flea beetles	Chrysomelidae	0.58	61.9	0.34	63.3
Plant bugs	Miridae	0.13	30.2	0.15	42.2
Leafhoppers	Cicadellidae	0.04	12.7	0.14	43.0
European corn borers[3]	Crambidae	NR	NR	0.06	14.1
Japanese beetles	Scarabaeidae	0.02	4.8	0.04	15.6
Cucumber beetles	Chrysomelidae	0.08	12.7	0.05	13.3
Click beetles	Elateridae	0.06	15.9	0.03	10.9
Weevils	Curculionidae	0.03	7.9	0.03	8.6
Planthoppers	Delphacidae	NR	NR	0.02	7.0
Corn earworms[3]	Noctuidae	NR	NR	0.02	3.9
Parasitoids					
Chalcidoid wasps	Chalcidoidea	0.79	58.7	0.04	14.8
Predators					
Insidious flower bugs	Anthocoridae	2.09	81.0	2.59	80.5
Green lacewings[4]	Chrysopidae	1.08	90.5	2.52	93.8
Lady beetles	Coccinellidae	0.78	58.7	0.53	69.5
Spiders[5]	Araneae	0.12	33.3	0.38	76.6
Predaceous mites	Mesostigmata	NR	NR	0.26	40.6
Ants[6]	Formicidae	0.27	9.5	0.08	22.7
Soldier beetles	Cantharidae	NR	NR	0.10	21.9
Hover fly larvae	Syrphidae	NR	NR	0.10	12.5
Long-legged flies	Dolichopodidae	NR	NR	0.09	11.7
Rove beetles[7]	Staphylinidae	0.06	7.9	0.08	10.2
Big eyed bugs	Lygaeidae	0.08	14.3	NR	NR

Predaceous thrips	Phlaeothripidae	NR	NR	0.06	14.1
Damselbugs	Nabidae	0.05	14.3	0.04	14.1
Big eyed bugs	Lygaeidae	NR	NR	0.07	13.3
Stink bugs[8]	Pentatomidae	NR	NR	0.02	4.7

Percentage occurrence per sampling unit of four plants per plot.
NR = not recorded
[1] Primarily decomposers but also invade and feed directly on developing kernels
[2] Fungivores
[3] Predominantly eggs found on plants
[4] Based on both egg and larval stages; adults feed on pollen and nectar
[5] >90% wolf spiders (Lycosidae)
[6] Mostly predators but some species (e.g., harvester ants) feed on plant material
[7] Primarily predators but will also feed on decaying vegetation
[8] Primarily spined soldier bugs; some species are phytophagous

Table 1.2 Frequency of occurrence and abundance of arthropods found on sweet corn plants at the Salisbury, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments.

		2000		2001	
Common name	Taxonomic group	Mean density per plant	Percentage occurrence	Mean density per plant	Percentage Occurrence
Decomposers					
Sap beetles[1]	Nitidulidae	0.80	75.0	1.54	55.2
Psocids	Psocoptera	1.22	58.3	1.14	34.4
Silken fungus beetles[2]	Crytophagidae	0.52	18.8	NR	NR
Shining mold beetles[2]	Phalacridae	0.19	41.7	0.21	46.9
Minute fungus beetles[2]	Corylophidae	NR	NR	0.21	39.6
Flies	Diptera	NR	NR	0.05	18.8
Herbivores					
Thrips	Thripidae	11.96	91.7	1.25	42.6
Aphids	Aphididae	7.76	83.3	0.66	61.5
Leafhoppers	Cicadellidae	0.44	20.8	0.02	11.5
Plant bugs	Miridae	0.13	25.0	0.11	24.0
Click beetles	Elateridae	0.17	27.1	0.03	14.6
Flea beetles	Chrysomelidae	0.13	25.0	0.02	7.3
Blotch leafminer	Agromyzidae	NR	NR	0.07	18.8
Caterpillars	Lepidoptera	NR	NR	0.06	15.6
Stink bugs[3]	Pentatomidae	NR	NR	0.04	4.2
European corn borer egg masses	Crambidae	NR	NR	0.03	10.4
Planthoppers	Delphacidae	NR	NR	0.03	8.3
Japanese beetles	Scarabaeidae	NR	NR	0.02	7.3
Parasitoids					
Parasitic wasps	Hymenoptera	NR	NR	0.02	6.3
Predators					
Green lacewings[4]	Chrysopidae	4.10	100.0	1.45	89.6
Insidious flower bugs	Anthocoridae	0.95	70.8	1.30	79.2
Lady beetles	Coccinellidae	0.38	52.1	0.48	62.5
Spiders[5]	Araneae	0.27	58.3	0.43	77.1
Ground beetle larvae	Carabidae	NR	NR	0.05	8.3
Thrips	Phlaethripidae	NR	NR	0.03	10.4
Big eyed bugs	Lygaeidae	NR	NR	0.02	7.3
Ground beetles	*Leptotrachelus*	NR	NR	0.02	3.1
Rove beetles[6]	Staphylinidae	NR	NR	0.02	4.2

Percentage occurrence per sampling unit of four plants per plot in 2000 and eight plants per plot in 2001.
NR = not recorded
[1] Primarily decomposers but also invade and feed directly on developing kernels.

[2] Fungivores
[3] Primarily spined soldier bugs; some species are phytophagous.
[4] Based on both egg and larval stages; adults feed on pollen and nectar.
[5] >90% wolf spiders (Lycosidae)
[6] Primarily predators but will also feed on decaying vegetation

Table 1.3 Frequency of occurrence and abundance of arthropods found on sticky traps at the Upper Marlboro, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments.

		2000		2001	
Common name	Taxonomic group	Mean density per plant	Percentage occurrence	Mean density per plant	Percentage Occurrence
		Decomposers			
Frit flies[1]	Chloropidae	36.89	25.4	48.19	98.7
Picture winged flies[1]	Otitidae	9.06	16.5	1.43	48.8
Dark winged fungus gnats[2]	Sciaridae	6.05	25.0	1.62	58.2
Shining mold beetles[2]	Phalacridae	2.66	18.5	0.91	40.2
Psocids	Psocoptera	2.64	21.4	0.42	28.5
Midges	Chironomidae	2.41	17.7	2.36	47.8
Oribatid mites	Oribatida	1.75	9.7	1.17	20.1
Gall gnats[1]	Cecidomyiidae	0.88	9.7	1.08	54.0
Minute fungus beetles[2]	Corylophidae	1.14	13.3	0.96	40.5
Springtails	Collembola	NR	NR	0.94	19.6
Pomace flies	Drosophilidae	1.03	14.9	0.25	18.0
Humpbacked flies[3]	Phoridae	0.63	5.2	0.66	38.1
Biting midges[4]	Ceratopogonidae	NR	NR	0.45	28.2
Face flies	Muscidae	NR	NR	0.28	18.5
Flesh flies	Sarcophagidae	NR	NR	0.22	9.9
Spear winged flies	Loncopteridae	0.20	4.4	0.08	4.7
Sap beetles[2]	Nitidulidae	0.19	2.0	NR	NR
Silken fungus beetles[2]	Crytophagidae	NR	NR	0.12	8.4
False blister beetles	Oedemeridae	NR	NR	0.09	6.0
Crickets	Gryllidae	NR	NR	0.09	1.3
Sap beetles[5]	Nitidulidae	NR	NR	0.04	3.4
Hairy fungus beetles[2]	Mycetophagidae	NR	NR	0.04	1.8
Fungus gnats[2]	Mycetophilidae	NR	NR	0.03	1.6
Antlike stone beetle	Scydmaenidae	NR	NR	0.02	1.6
		Herbivores			
Thrips	Thripidae	12.72	23.0	44.52	98.4
Aphids	Aphididae	56.78	22.2	3.38	71.0
Leafhoppers	Cicadellidae	6.50	25.0	21.81	98.2
Flea beetles	Chrysomelidae	3.02	19.8	2.90	65.5
Plant bugs	Miridae	0.80	9.3	2.73	68.4
Froghoppers	Cercopidae	1.55	10.5	0.08	3.7
Planthoppers	Delphacidae	0.50	5.6	0.71	40.5
Blotch leaf miners	Agromyzidae	0.83	13.7	0.20	11.7
Negro bugs	Corimelaenidae	NR	NR	0.38	6.5
Cucumber beetles	Chrysomelidae	NR	NR	0.22	15.9

Non-carabid beetles	Coleoptera	NR	NR	0.17	11.7
Click beetles	Elateridae	0.19	1.2	0.11	8.6
Weevils	Curculionidae	NR	NR	0.06	5.0
		Parasitoids			
Chalcidoids[6]	Chalcidoidea	37.63	25.4	NR	NR
Scelionids	Scelionidae	3.19	3.6	3.24	84.9
Fairyflies	Mymaridae	NR	NR	2.61	84.3
Trichogrammatids	Trichogrammatidae	NR	NR	1.22	55.6
Braconids	Braconidae	0.36	6.5	1.22	48.8
Tachinids	Tachinidae	0.58	8.9	0.82	43.3
Pteromalids	Pteromalidae	NR	NR	0.66	37.6
Misc wasps	Hymenoptera	NR	NR	0.45	21.9
Charipids	Charipidae	NR	NR	0.43	25.8
Aphelinids	Aphelinidae	NR	NR	0.28	12.0
Encyrtids	Encyrtidae	NR	NR	0.25	14.9
Ceraphronids	Ceraphronidae	NR	NR	0.14	11.0
Ichneumonids	Ichneumonidae	NR	NR	0.10	7.3
Eulophids	Eulophidae	NR	NR	0.09	7.8
Spider wasps	Pompilidae	NR	NR	0.04	1.6
Proctotrupids	Proctotrupidae	NR	NR	0.04	1.3
Cuckoo wasps	Chrysididae	NR	NR	0.03	1.0
		Predators			
Insidious flower bugs	Anthocoridae	7.52	23.8	4.93	84.1
Lady beetles	Coccinellidae	0.66	10.1	1.10	48.3
Thrips	Phlaeothripidae	1.31	14.5	0.60	31.6
Long-legged flies	Dolichopodidae	0.25	4.4	0.84	28.5
Rove beetles	Staphylinidae	0.17	4.0	0.59	32.9
Big eyed bugs	Lygaeidae	0.30	6.0	0.52	30.0
Spiders[7]	Araneae	NR	NR	0.45	26.9
Damselbugs	Nabidae	0.69	2.4	0.02	1.0
Lightening bugs[8]	Lampyridae	NR	NR	0.44	11.2
Ants[9]	Formicidae	0.15	2.8	0.34	15.7
Soldier beetles	Cantharidae	NR	NR	0.22	11.0
Ground beetles	Carabidae	0.33	7.3	0.04	1.0
Predaceous mites	Mesostigmata	NR	NR	0.21	10.4
Green lacewings[8]	Chrysopidae	NR	NR	0.14	3.7
Hover flies	Syrphidae	NR	NR	0.14	9.7
Ground beetles	*Leptotrachelus*	NR	NR	0.07	3.7

Percentage occurrence per sampling unit of four sticky cards per plot.
NR = not recorded
[1] Primarily decomposers but will also feed on plant material
[2] Fungivores
[3] Some species are parasitic
[4] Also forms galls on plants

[5] Primarily decomposers but will also feed on plant material.
[6] All chalcidoid wasps were recorded as a group in 2000.
[7] >90% were wolf spiders (Lycosidae)
[8] Larvae are predators but adults feed on plant material such as nectar
[9] Some species are herbivores (e.g., harvester ants)

Table 1.4 Frequency of occurrence and abundance of arthropods found on sticky traps at the Salisbury, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments.

		2000		2001	
Common name	Taxonomic group	Mean density per plant	Percentage occurrence	Mean density per plant	Percentage Occurrence
		Decomposers			
Frit flies	Chloropidae	13.91	23.3	7.91	94.7
Minute fungus beetles[1]	Corylophidae	2.38	10.3	1.50	62.3
Shining mold beetles[1]	Phalacridae	4.17	18.6	0.73	44.1
Psocids	Psocoptera	0.91	13.0	0.99	54.6
Dark winged fungus gnats[1]	Sciaridae	0.80	13.0	0.99	57.0
Picture winged flies[2]	Otitidae	2.09	8.3	0.43	27.7
Flesh flies	Sarcophagidae	NR	NR	0.49	28.0
Long-legged flies	Dolichopodidae	NR	NR	0.42	30.9
Humpbacked flies[3]	Phoridae	0.31	5.1	0.41	33.2
Pomace flies	Drosophilidae	1.46	11.5	0.04	4.0
Midges	Chironomidae	0.73	11.1	0.03	2.1
Biting midges[4]	Ceratopogonidae	NR	NR	0.33	23.0
Springtails	Collembola	NR	NR	0.23	8.4
Silken fungus beetles[1]	Crytophagidae	NR	NR	0.18	14.5
Face flies	Muscidae	NR	NR	0.17	14.0
Flies	Diptera	NR	NR	0.15	12.9
Antlike flower beetles	Anthicidae	NR	NR	0.07	5.0
False blister beetles	Oedemeridae	NR	NR	0.04	3.4
Sap beetles[2]	Nitidulidae	NR	NR	0.01	1.1
		Herbivores			
Thrips	Thripidae	2.58	20.2	32.41	98.4
Leafhoppers	Cicadellidae	4.34	23.7	21.50	94.7
Aphids	Aphididae	10.66	20.2	1.82	72.3
Plant bugs	Miridae	1.69	15.8	3.23	59.1
Gall gnats	Cecidomyiidiae	1.02	13.8	0.24	19.8
Planthoppers	Delphacidae	0.50	8.3	0.40	14.0
Non-carabid beetles	Coleoptera	NR	NR	0.35	28.2
Click beetles	Elateridae	0.34	2.4	0.22	11.6
Cucumber beetles	Chrysomelidae	NR	NR	0.15	10.8
Flea beetles	Chrysomelidae	0.52	4.7	0.06	5.0
Froghoppers	Cercopidae	NR	NR	0.11	8.2
Blotch leaf miners	Agromyzidae	0.34	7.5	NR	NR
Weevils	Curculionidae	NR	NR	0.07	5.5
Negro bugs	Corimelaenidae	NR	NR	0.06	5.0
Caterpillars	Lepidoptera	NR	NR	0.02	1.8
Long-horned	Tettigoniidae	NR	NR	0.01	1.1

grasshoppers					
Grasshoppers	Acrididae	NR	NR	0.00	0.3
		Parasitoids			
Fairyflies	Mymaridae	NR	NR	4.74	89.7
Pteromalids	Pteromalidae	NR	NR	1.85	65.4
Trichogrammatids	Trichogrammatidae	NR	NR	1.28	57.8
Scelionids	Scelionidae	0.91	5.9	1.23	59.6
Aphelinids	Aphelinidae	NR	NR	0.61	20.1
Braconids	Braconidae	0.22	5.1	0.45	29.8
Misc. wasps	Hymenoptera	NR	NR	0.37	26.6
Encyrtidae	Encyrtidae	NR	NR	0.25	16.9
Charipids	Charipidae	NR	NR	0.21	18.5
Tachinids	Tachinidae	0.61	7.1	0.04	2.1
Eulophids	Eulophidae	NR	NR	0.13	12.1
Ceraphronids	Ceraphronidae	0.25	2.8	0.10	7.9
Ichneumonids	Ichneumonidae	NR	NR	0.05	4.7
		Predators			
Insidious flower bugs	Anthocoridae	2.73	22.5	4.42	81.8
Lady beetles	Coccinellidae	0.38	7.5	0.50	28.2
Spiders[5]	Araneae	NR	NR	0.36	30.1
Rove beetles	Staphylinidae	NR	NR	0.25	19.8
Thrips	Phlaeothripidae	0.89	13.4	0.24	18.7
Ants[6]	Formicidae	NR	NR	0.17	14.2
Ground beetles	Carabidae	0.36	6.7	0.10	7.7
Big eyed bugs	Lygaeidae	0.25	5.1	0.09	7.1
Hover flies	Syrphidae	0.17	3.2	0.08	7.1
Aphid flies	Chamaemyiidae	0.23	4.0	0.02	1.6
Long-legged flies	Dolichopodidae	0.19	2.8	NR	NR
Green lacewings[7]	Chrysopidae	NR	NR	0.03	2.6
Ground beetles[8]	*Stenolophus*	NR	NR	0.02	1.6

Percentage occurrence per sampling unit of four sticky cards per plot.
NR = not recorded
[1] Fungivores
[2] Primarily decomposers but will also feed on plant material
[3] Some species are parasitic
[4] Some species are ectoparasites and some are predators of other insects
[5] >90% were wolf spiders (Lycosidae)
[6] Some species are herbivores (e.g., harvester ants)
[7] Larvae are predators but adults will feed on plant material such as nectar.
[8] Some species will also feed on seeds.

Table 1.5 Frequency of occurrence and abundance of arthropods found in pitfall traps at the Upper Marlboro, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments.

		2000		2001	
Common name	Taxonomic group	Mean density per plant	Percentage occurrence	Mean density per plant	Percentage Occurrence
		Decomposers			
Springtails	Collembola	46.55	89.0	176.86	99.5
Mites[1]	Oribatida	11.84	72.1	24.50	90.9
Crickets	Gryllidae	10.87	88.2	1.99	66.6
Flies	Diptera	6.86	65.5	1.75	55.1
Slugs	Agromilacidae	3.18	52.6	0.20	11.0
Sap beetles[2]	Nitidulidae	2.05	49.5	0.54	26.7
Shining mold beetles[3]	Phalacridae	0.58	8.9	0.19	10.7
Antlike flower beetles	Anthicidae	0.43	22.8	NR	NR
Humpbacked flies[4]	Phoridae	NR	NR	0.47	19.0
Hairy fungus beetles[3]	Mycetophagidae	NR	NR	0.33	16.3
Dark-winged fungus gnats[3]	Sciaridae	NR	NR	0.21	9.9
False blister beetles	Oedemeridae	NR	NR	0.16	9.6
Sowbugs	Isopoda	0.07	4.9	0.03	1.6
Silken fungus beetles[3]	Crytophagidae	NR	NR	0.14	8.3
Minute fungus beetles[3]	Corylophidae	NR	NR	0.08	5.6
Psocids	Psocoptera	NR	NR	0.20	4.8
Millipedes	Diplopoda	0.02	1.1	0.03	1.3
Antlike stone beetle	Scydmaenidae	NR	NR	0.10	2.1
Hister beetles	Histeridae	NR	NR	0.04	1.3
		Herbivores			
Ground beetles[5]	*Amara*	5.63	16.1	0.09	4.0
Click beetles	Elateridae	0.70	22.0	0.27	19.0
Flea beetles	Chrysomelidae	0.32	16.5	0.27	22.5
Plant bugs	Miridae	0.17	12.9	0.68	21.7
Weevils	Curculionidae	0.12	9.5	0.03	2.1
Thrips	Thripidae	NR	NR	0.28	12.8
Leafhoppers	Cicadellidae	0.17	11.2	0.13	9.6
Aphids	Aphididae	0.07	4.7	0.05	3.7
Negro bugs	Corimelaenidae	0.06	4.0	NR	NR
Scarab beetles	Scarabeidae	0.06	3.0	0.06	5.9
Cucumber beetles	Chrysomelidae	0.04	2.1	0.13	4.3
Caterpillars	Lepidoptera	0.02	1.7	0.09	4.8
Grasshoppers	Acrididae	0.03	1.7	0.03	2.4
Froghoppers	Cercopidae	NR	NR	0.04	3.7
Japanese beetles	Scarabaeidae	NR	NR	0.04	1.3

		Parasitoids			
Chalcidoids	Chalcididae	2.31	47.1	3.63	68.2
Misc. wasps	Hymenoptera	0.10	6.1	1.00	41.2
Braconids	Braconidae	NR	NR	0.14	11.0
Trichogrammatids	Trichogrammatidae	NR	NR	0.26	9.9
Fairyflies	Mymaridae	NR	NR	0.10	6.7
Ichneumonids	Ichneumonidae	NR	NR	0.06	2.4
Scelionids	Scelionidae	NR	NR	0.06	2.4
		Predators			
Ants[6]	Formicidae	11.99	94.5	21.41	98.9
Rove beetles[7]	Staphylinidae	8.51	72.5	13.93	95.5
Spiders[8]	Araneae	6.43	83.3	18.55	96.8
Ground beetles[9]	*Harpalus*	1.45	52.2	0.89	60.4
Ground beetle larvae	Carabidae	0.84	27.5	1.60	46.8
Predaceous mites	Mesostigmata	NR	NR	9.80	77.3
Stink bugs[10]	Pentatomidae	0.39	7.4	0.05	2.1
Centipedes	Geophilomorpha	0.33	15.4	0.23	13.4
Ground beetles	*Chlaenius*	0.32	15.6	0.04	3.7
Soldier beetles	Cantharidae	NR	NR	0.40	20.1
Ground beetles	*Scarites*	0.14	9.9	0.19	14.7
Lady beetles	Coccinellidae	0.13	8.5	0.15	11.2
Ground beetles	*Pterostichus*	0.12	9.7	0.06	4.5
Ground beetles[9]	*Stenolophus*	0.06	3.2	0.20	15.0
Ground beetles	*Agonum*	0.09	6.3	NR	NR
Lightening bugs[11]	Lampyridae	0.08	4.7	0.03	1.3
Ground beetles[9]	*Stenocellus*	0.07	5.3	NR	NR
Ground beetles	*Dyschurius*	0.06	2.3	0.04	1.6
Ground beetles[9]	*Anisodactylus*	0.04	3.4	NR	NR
Ground beetles	Carabidae	0.04	2.7	NR	NR
Minute pirate bugs	Anthocoridae	NR	NR	0.12	5.1
Tiger beetles	Cicindelidae	0.02	1.5	0.09	4.8
Ground beetles	*Clivinia*	0.01	1.5	0.03	1.3
Big eyed bugs	Lygaeidae	NR	NR	0.04	1.9
Ground beetles	*Bembideon*	NR	NR	0.09	1.9
Damselbugs	Nabidae	0.01	1.3	0.05	2.4
Green lacewings[11]	Chrysopidae	NR	NR	0.05	1.6
Ground beetles	*Colliuris*	0.01	1.1	NR	NR

Percentage occurrence per sampling unit of four pitfall traps per plot.
NR = not recorded
[1] Primarily oribatid mites
[2] Primarily decomposers but will also feed on plant material.
[3] Fungivores
[4] Some species are parasitic
[5] Feeds on seeds
[6] Some species are herbivores (e.g., harvester ants)

[7] Primarily predators but will also feed on decaying vegetation.
[8] >90% were wolf spiders (Lycosidae)
[9] Some species will also feed on seeds.
[10] Some species are herbivores
[11] Larvae are predators but adults will feed on plant material such as nectar.

Table 1.6 Frequency of occurrence and abundance of arthropods found in pitfall traps at the Salisbury, Maryland location. Taxa are listed by ecological function in order of most abundant. Means were computed by pooling data over all sampling dates and treatments.

		2000		2001	
Common name	Taxonomic group	Mean density per plant	Percentage occurrence	Mean density per plant	Percentage Occurrence
		Decomposers			
Springtails	Collembola	17.67	64.3	67.71	98.4
Mites [1]	Oribatida	5.65	68.4	0.52	23.8
Sap beetles [2]	Nitidulidae	3.56	53.5	1.09	34.0
Flies	Diptera	NR	NR	4.25	71.9
Hairy fungus beetles[3]	Mycetophagidae	NR	NR	0.69	32.4
Silken fungus beetles[3]	Crytophagidae	NR	NR	0.36	18.4
Humpbacked flies[4]	Phoridae	NR	NR	0.32	14.1
Shining mold beetles [3]	Phalacridae	NR	NR	0.31	14.8
Antlike flower beetles	Anthicidae	0.20	15.7	0.04	2.7
Frit flies	Chloropidae	NR	NR	0.22	9.0
Psocids	Psocoptera	NR	NR	0.22	8.2
False blister beetles	Oedemeridae	NR	NR	0.19	10.9
Minute fungus beetles[3]	Corylophidae	NR	NR	0.17	4.7
Crickets	Gryllidae	0.09	6.5	0.09	7.0
Sowbugs	Isopoda	0.09	3.8	0.07	2.7
Millipedes	Diplopoda	0.10	4.7	0.10	4.7
Dark winged fungus gnats[3]	Sciaridae	NR	NR	0.10	5.9
Lower fly larvae	Nematocera	NR	NR	0.08	3.5
Higher fly larvae	Cyclorrhapha	NR	NR	0.08	2.0
Minute brown scavenger beetles[3]	Lanthridiidae	NR	NR	0.04	2.3
		Herbivores			
Click beetles	Elateridae	4.12	47.6	0.52	35.5
Plant bugs	Miridae	0.11	6.8	1.25	44.1
Thrips	Thripidae	0.16	1.4	1.25	42.6
Non-carabid beetles	Coleoptera	0.71	9.5	0.22	9.4
Leafhoppers	Cicadellidae	0.05	4.9	0.54	33.2
Japanese beetles	Scarabeidae	0.17	14.6	0.16	14.5
Flea beetles	Chrysomelidae	0.04	3.8	0.20	12.5
Grasshoppers	Acrididae	0.10	3.5	0.05	2.0
Caterpillars	Lepidoptera	NR	NR	0.08	7.4
Aphids	Aphididae	NR	NR	0.08	6.3
Cucumber beetles	Chrysomelidae	NR	NR	0.06	4.3
Froghoppers	Cercopidae	NR	NR	0.05	2.7
Seed-eating ground beetle [4]	*Amara* spp.	0.03	1.4	0.03	2.7

Weevils	Curculionidae	0.02	1.1	0.03	3.1
		Parasitoids			
Misc. wasps	Hymenoptera	0.96	4.6	2.06	55.1
Chalcidoids	Chalcididae	1.21	18.9	1.82	54.3
Fairyflies	Mymaridae	NR	NR	0.16	12.5
Trichogrammatids	Trichogrammatidae	NR	NR	0.13	5.9
Braconids	Braconidae	NR	NR	0.05	5.1
		Predators			
Spiders [6]	Araneae	1.93	63.0	22.86	98.0
Rove beetles [7]	Staphylinidae	1.25	53.5	2.59	80.9
Ants [8]	Formicidae	0.99	41.4	1.24	50.0
Lady beetles	Coccinellidae	0.20	16.2	1.23	41.0
Ground beetles[9]	*Stenolophus*	0.34	24.3	1.05	41.8
Predaceous mites	Mesostigmata	NR	NR	0.81	28.9
Centipedes	Geophilomorpha	0.37	18.6	0.13	9.0
Ground beetles[9]	*Harpalus*	0.32	26.2	0.45	36.3
Tiger beetles	Cicindelidae	0.14	13.5	0.45	30.9
Ground beetle larvae	Carabidae	NR	NR	0.42	17.2
Ground beetles	*Agonum*	0.03	1.1	0.26	16.4
Insidious flower bugs	Anthocoridae	NR	NR	0.22	12.9
Ground beetles	*Pterostichus*	0.06	4.1	0.15	10.2
Soldier beetles	Cantharidae	NR	NR	0.14	3.5
Big eyed bugs	Lygaeidae	NR	NR	0.12	5.1
Damselbugs	Nabidae	0.03	1.9	0.07	6.6
Ground beetles[9]	*Anisodactylus*	NR	NR	0.07	3.1
Ground beetles	*Chlaenius*	0.04	1.9	0.04	3.5
Ground beetles	*Lebia* spp.	0.03	1.4	0.05	2.0
Ground beetles	*Leptotrachelus*	NR	NR	0.05	2.3
Ground beetles	*Scarites*	0.02	1.1	0.04	3.5

Percentage occurrence per sampling unit of four pitfall traps per plot.
NR = not recorded
[1] Primarily Orabatid mites
[2] Primarily decomposers but will also feed on plant material.
[3] Fungivores
[4] Some species are parasitic
[5] Feeds on seeds
[6] >90% were wolf spiders (Lycosidae)
[7] Primarily predators but will also feed on decaying vegetation
[8] Some species are herbivores (e.g., harvester ants)
[9] Some species will also feed on seeds

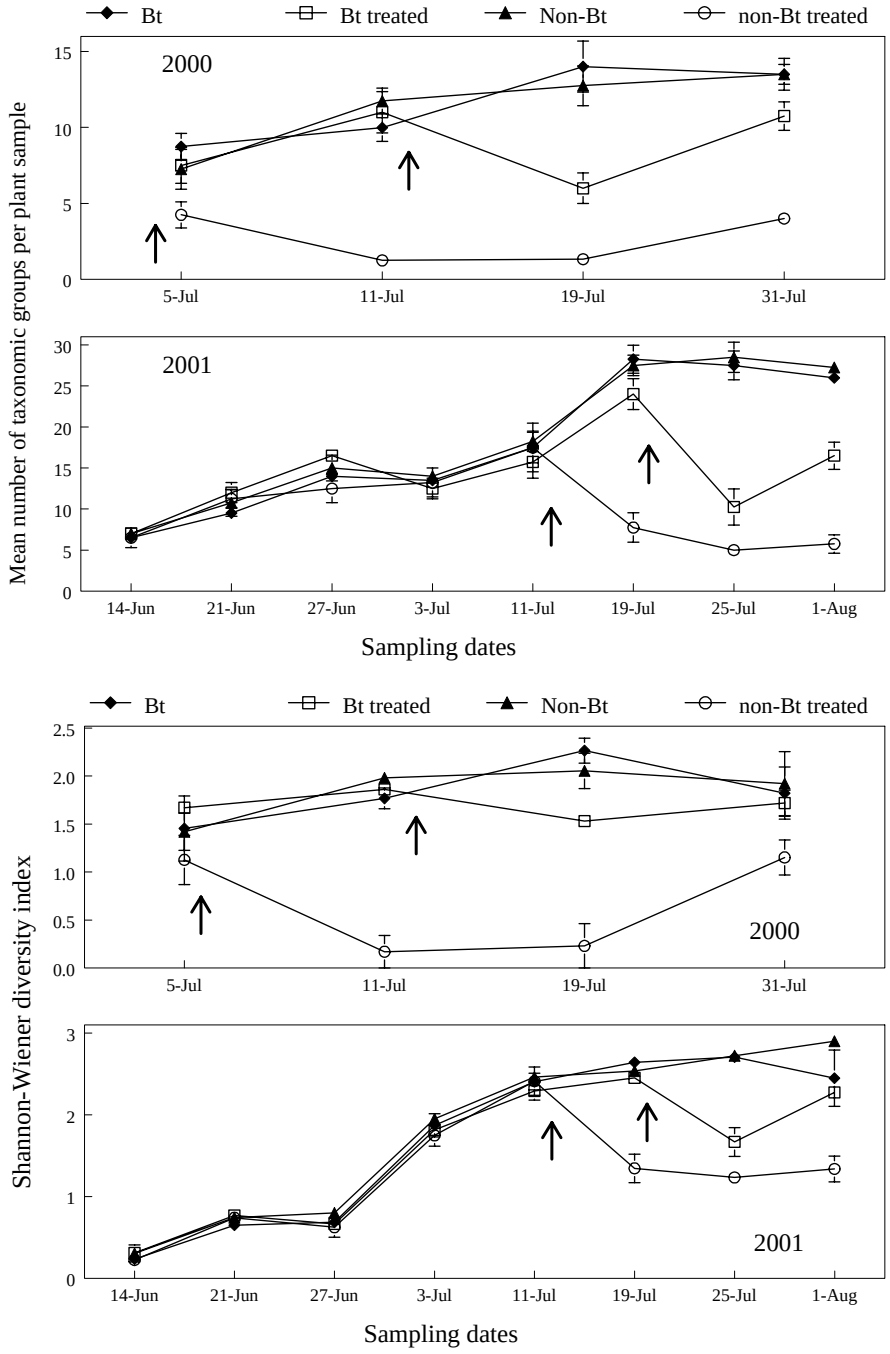

Figure 1.1 Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots.

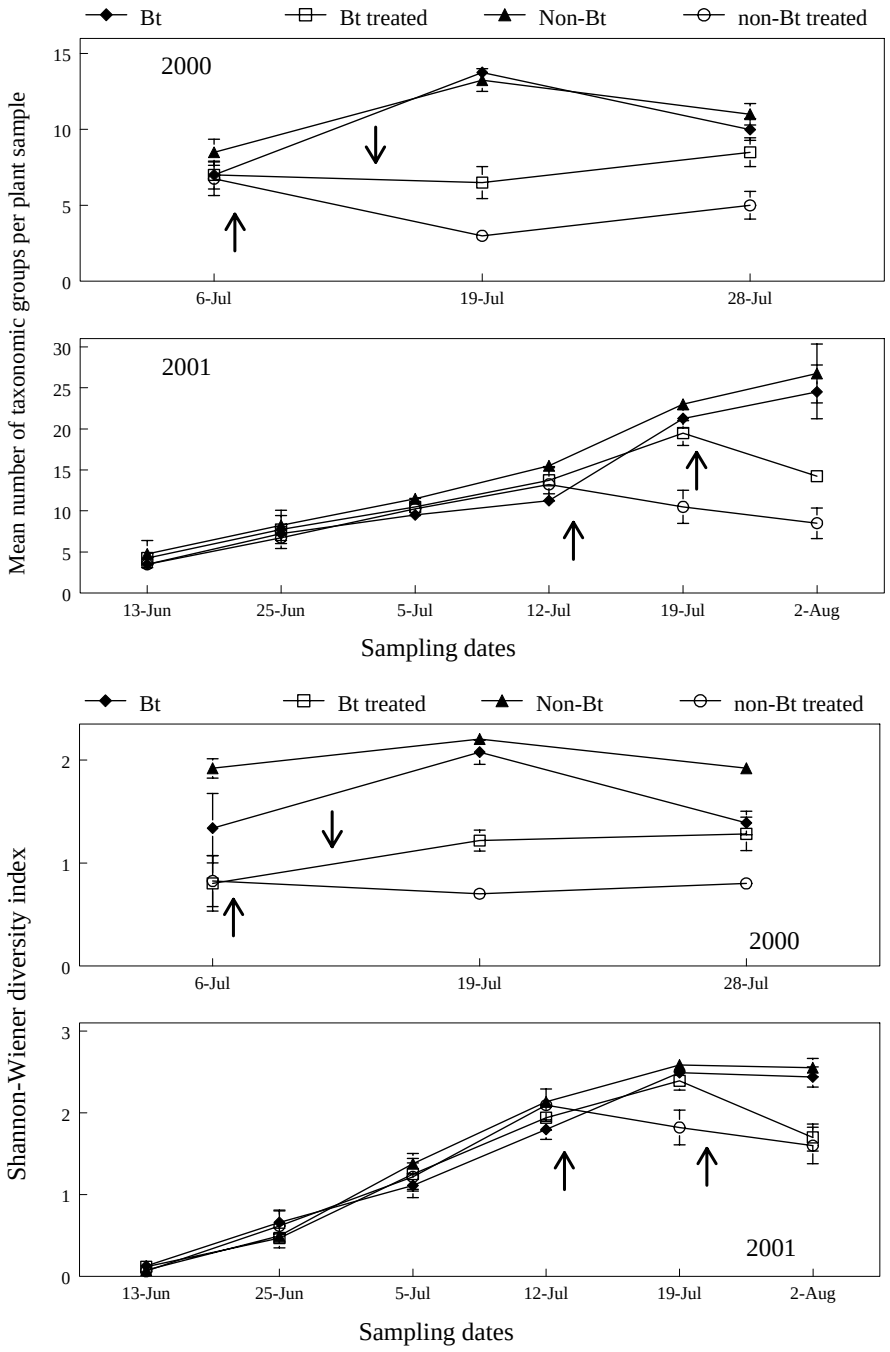

Figure 1.2 Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Salisbury, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots.

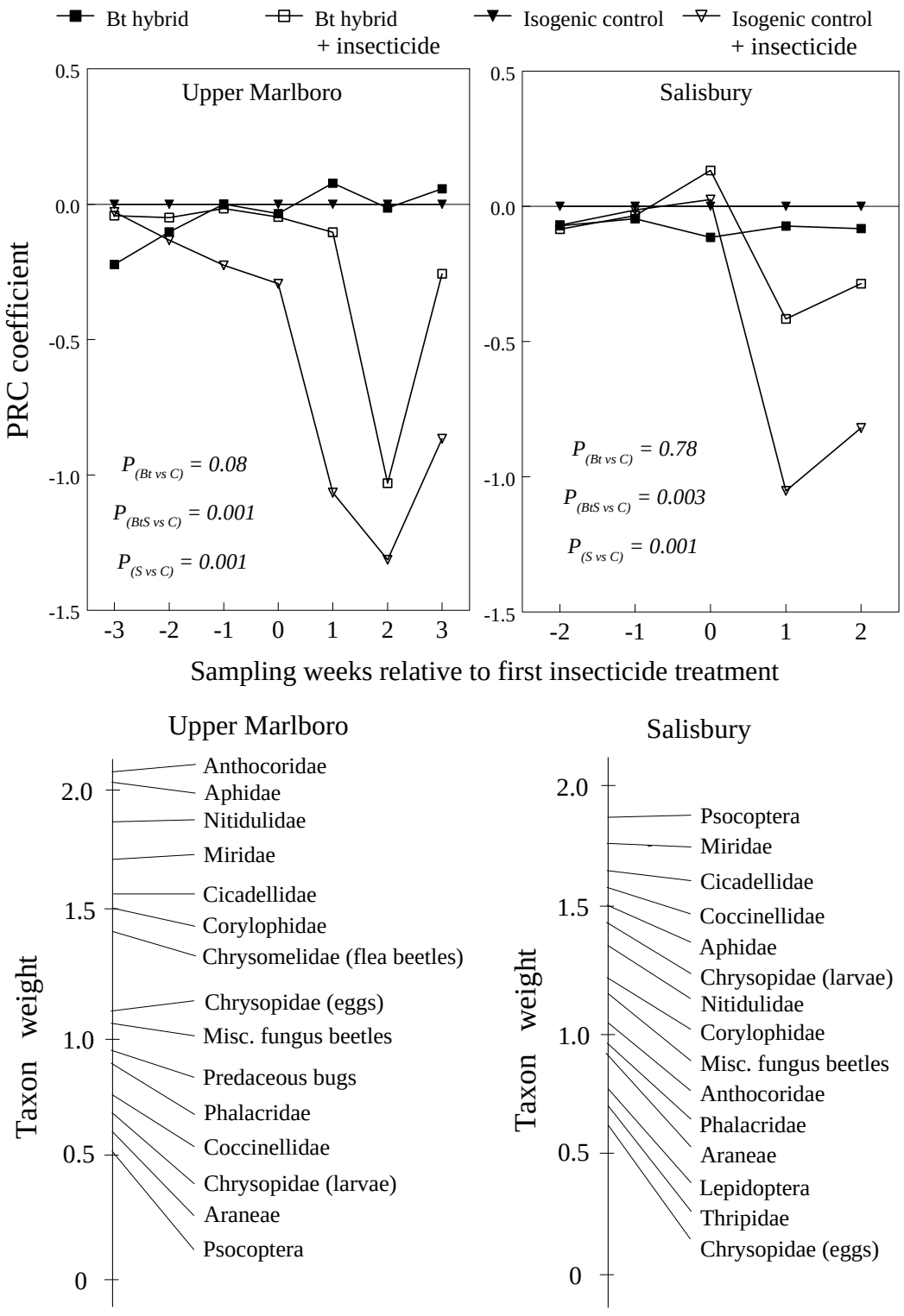

Figure 1.3 Principal response curves and taxon weights of plant-dwelling arthropod communities exposed to Bt and insecticide-treated sweet corn compared to the isogenic control. Responses of taxa with positive weights followed the PRC patterns, whereas those with negative weights showed the opposite pattern. Taxa with weights between -0.5 to 0.5 are not shown.

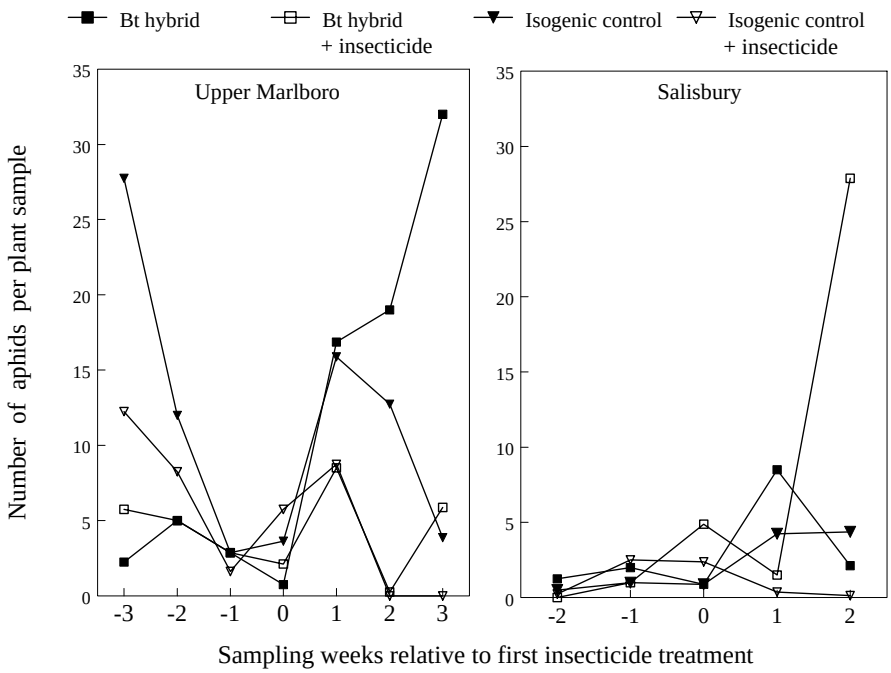

Figure 1.4. Mean number of aphids recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

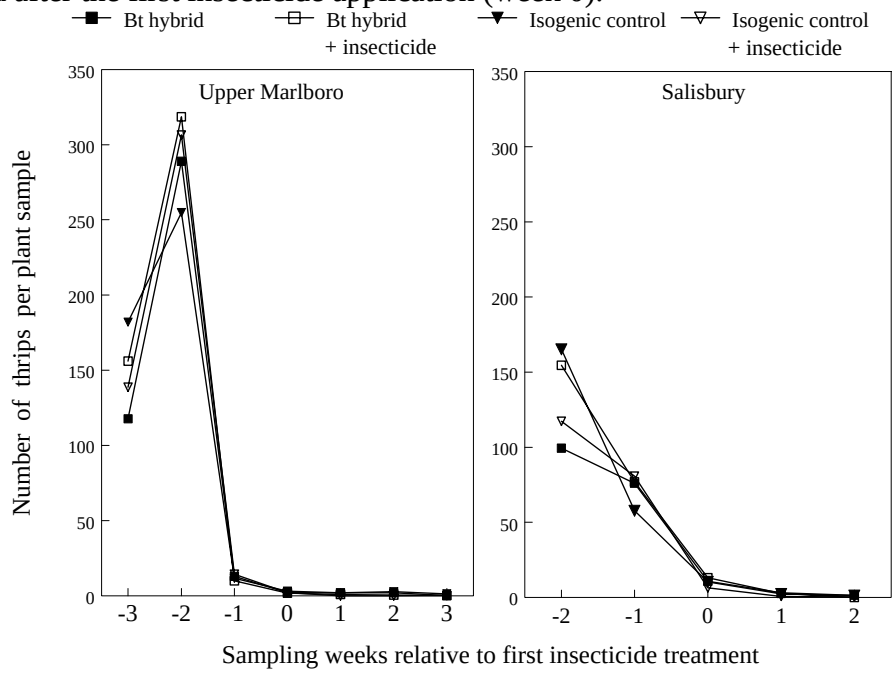

Figure 1.5. Mean number of thrips recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

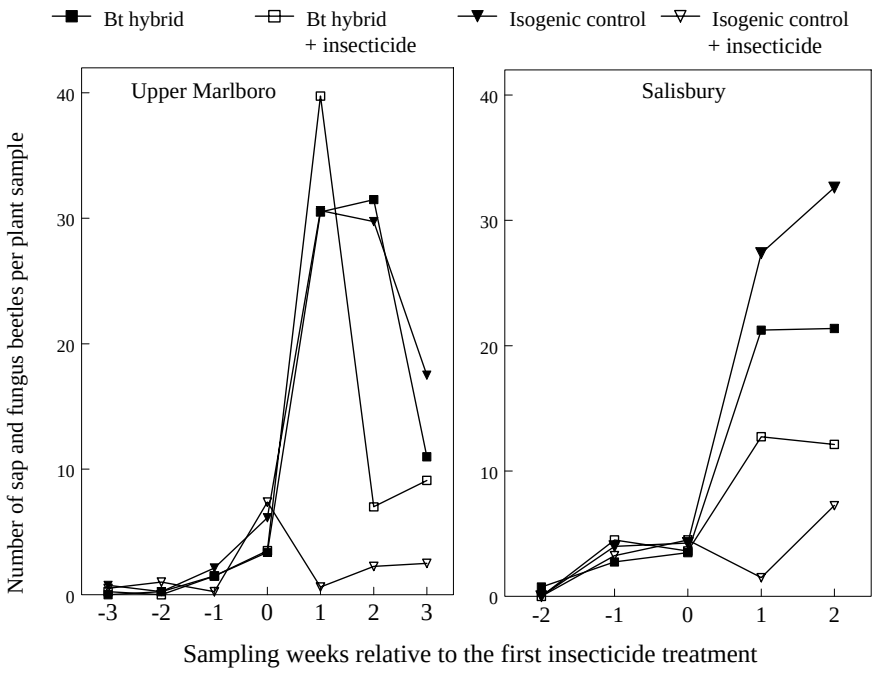

Figure 1.6. Mean number of fungus beetles (primarily Nitidulidae, Corylophidae and Phalacridae) recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

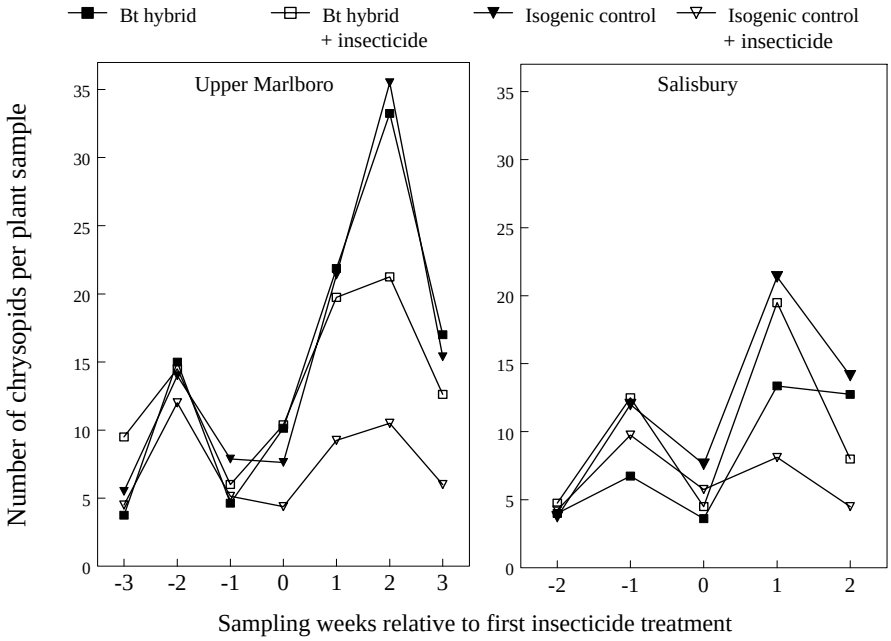

Figure 1.7. Mean number of chrysopids recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

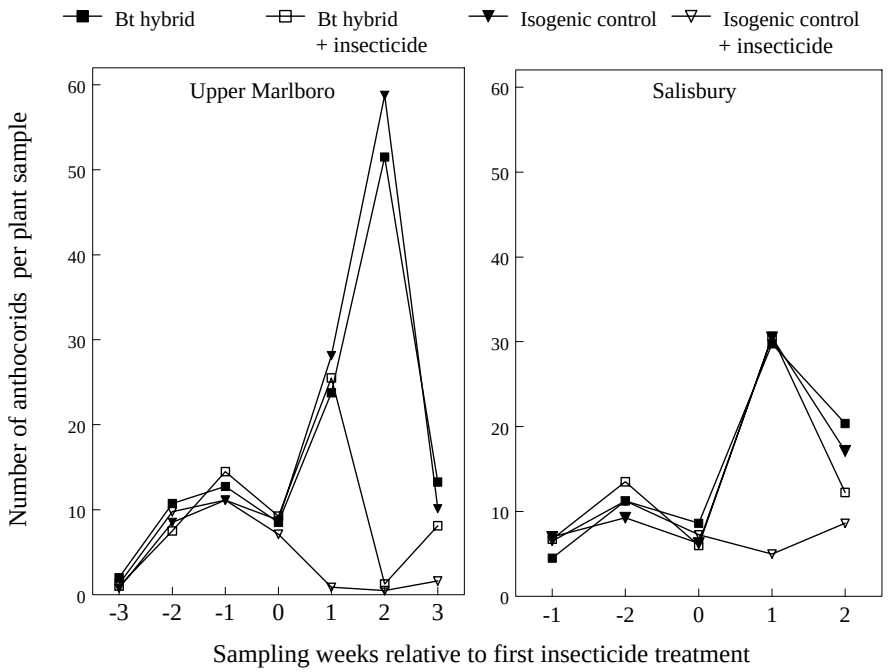

Figure 1.8. Mean number of anthocorids recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

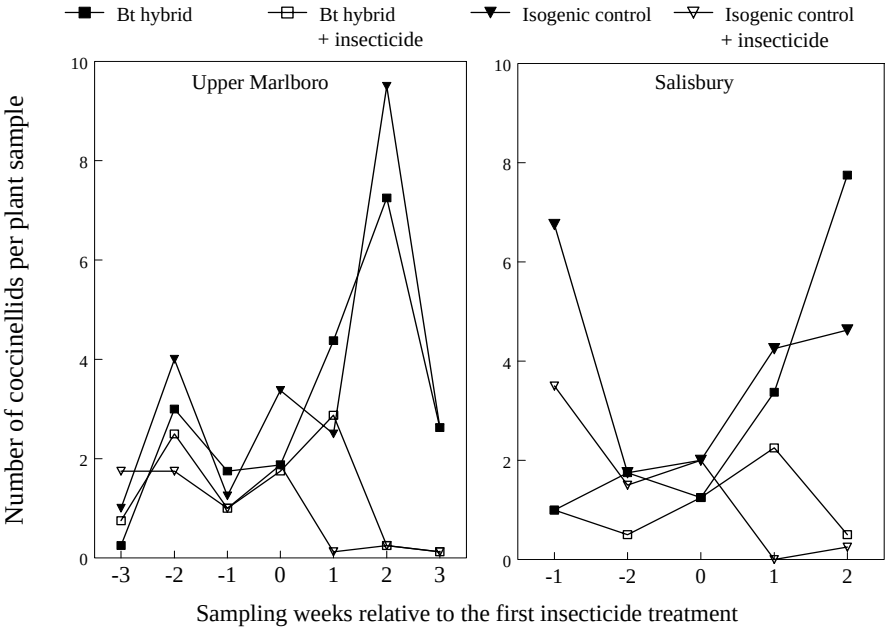

Figure 1.9. Mean number of coccinellids recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

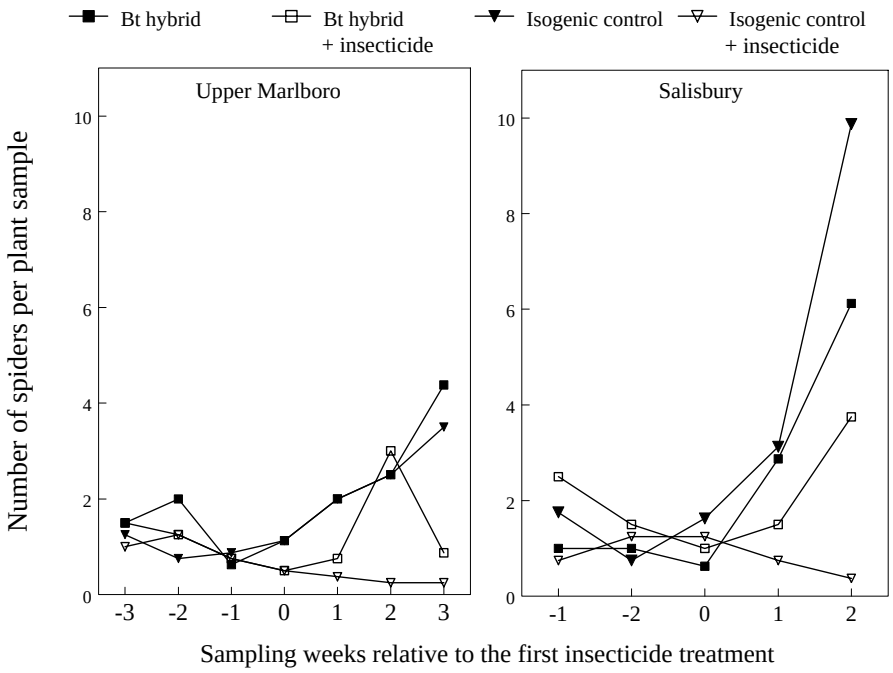

Figure 1.10. Mean number of spiders recorded by plant inspections in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

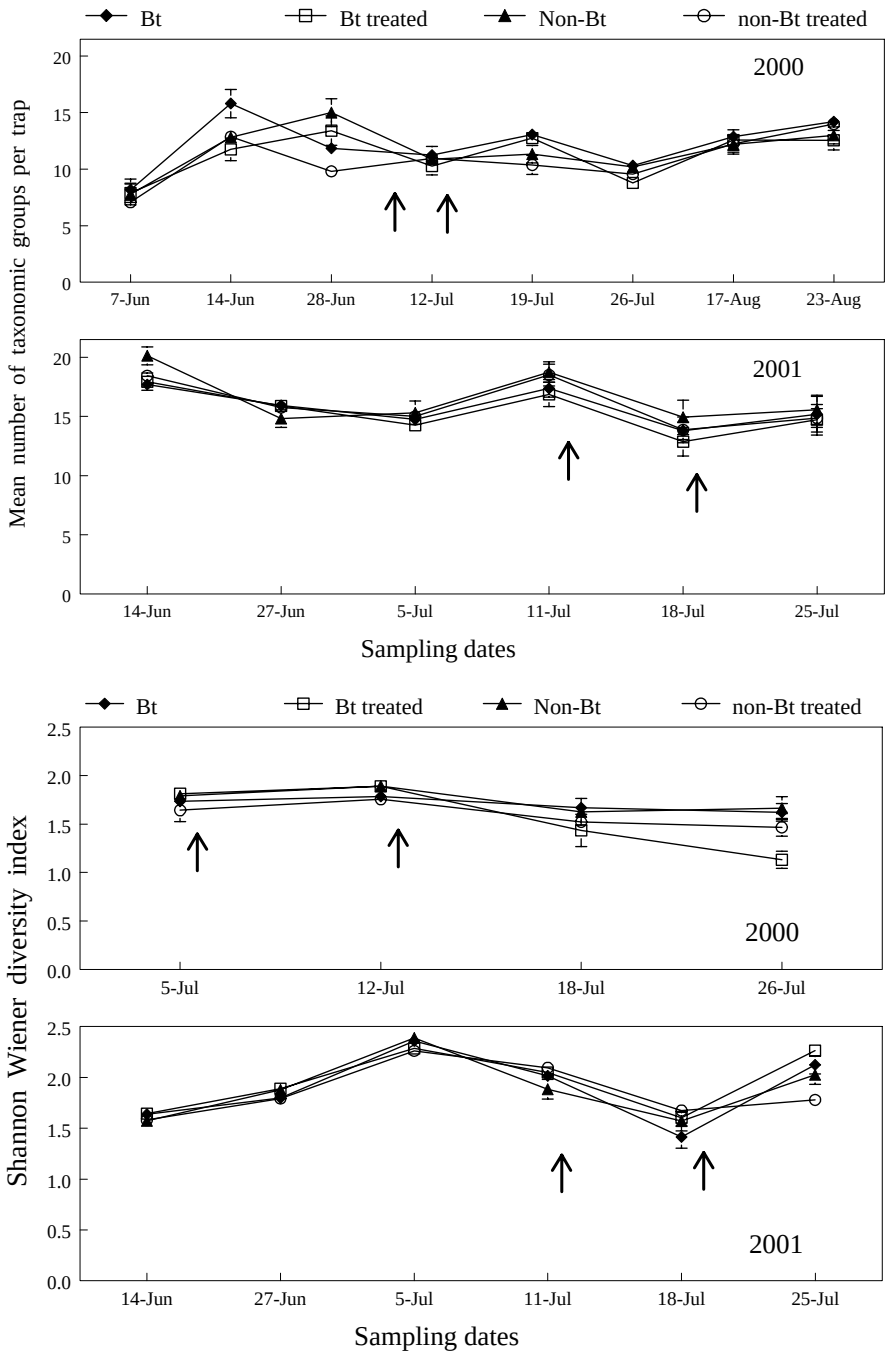

Figure 1.11. Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots.

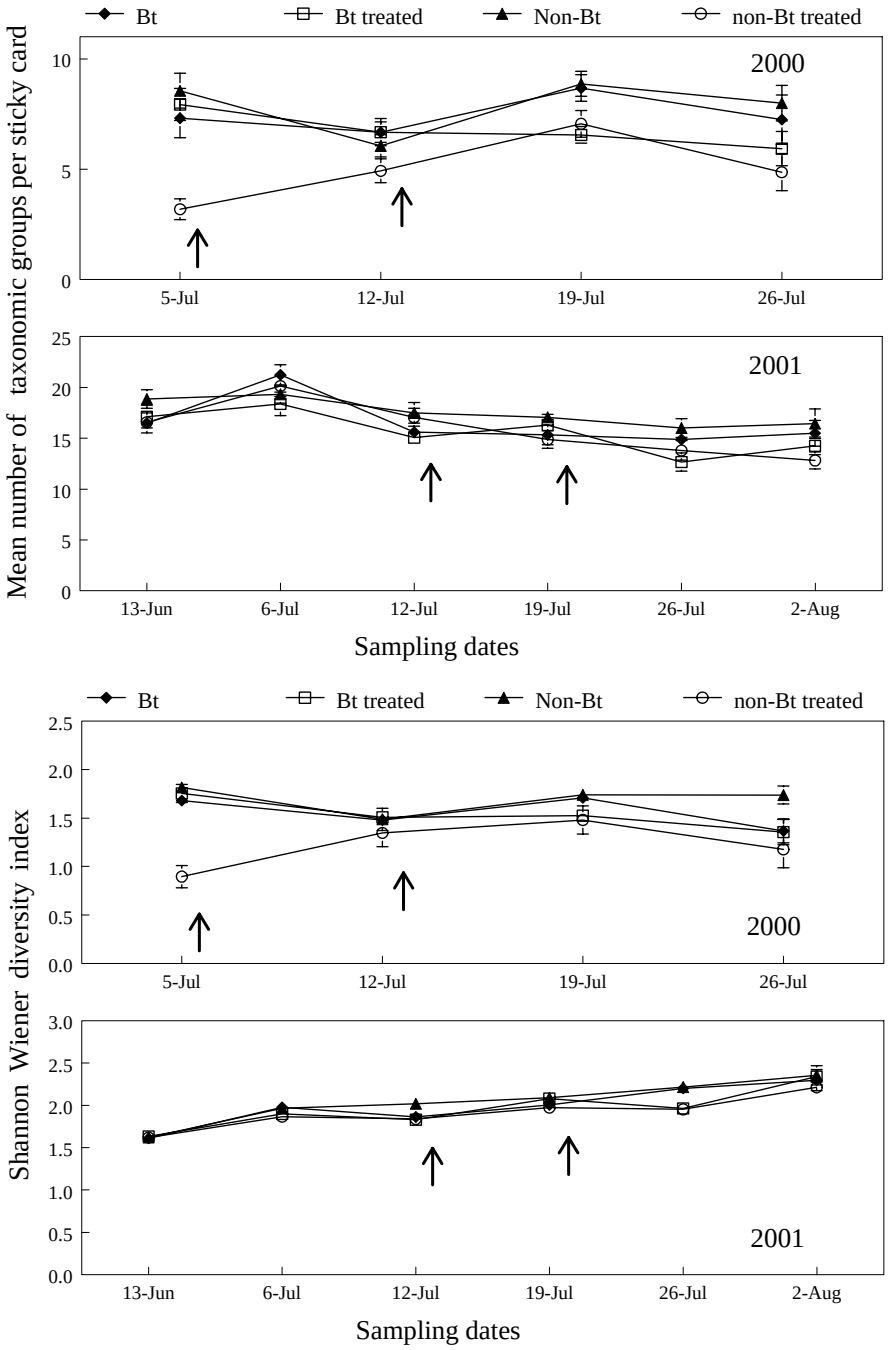

Figure 1.12. Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Salisbury, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots.

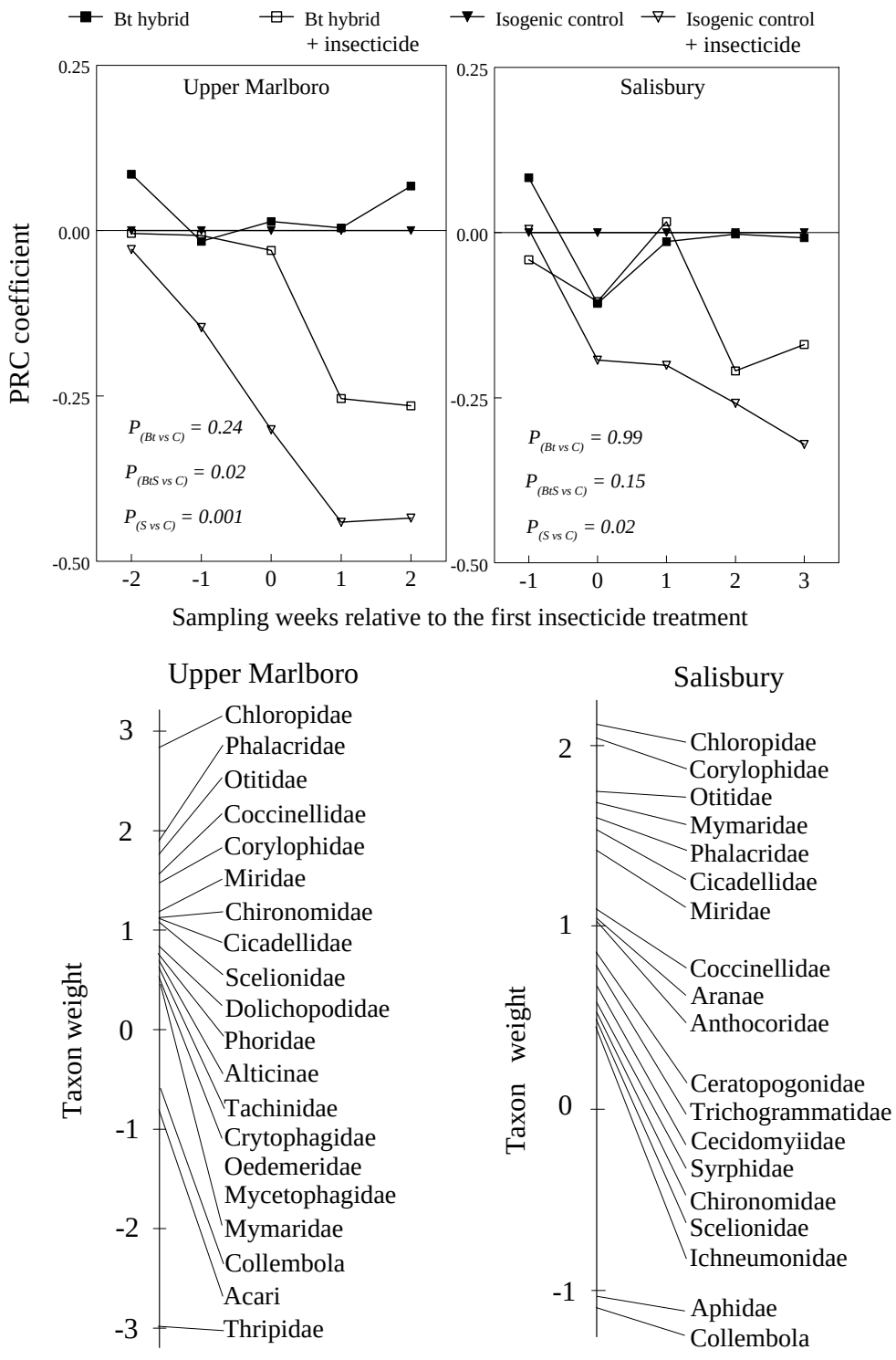

Figure 1.13. Principal response curves and taxon weights of aerial arthropod communities exposed to Bt and insecticide-treated sweet corn compared to the isogenic control. Responses of taxa with positive weights followed the PRC patterns, whereas those with negative weights showed the opposite pattern. Taxi with weights between -0.5 to 0.5 are not shown.

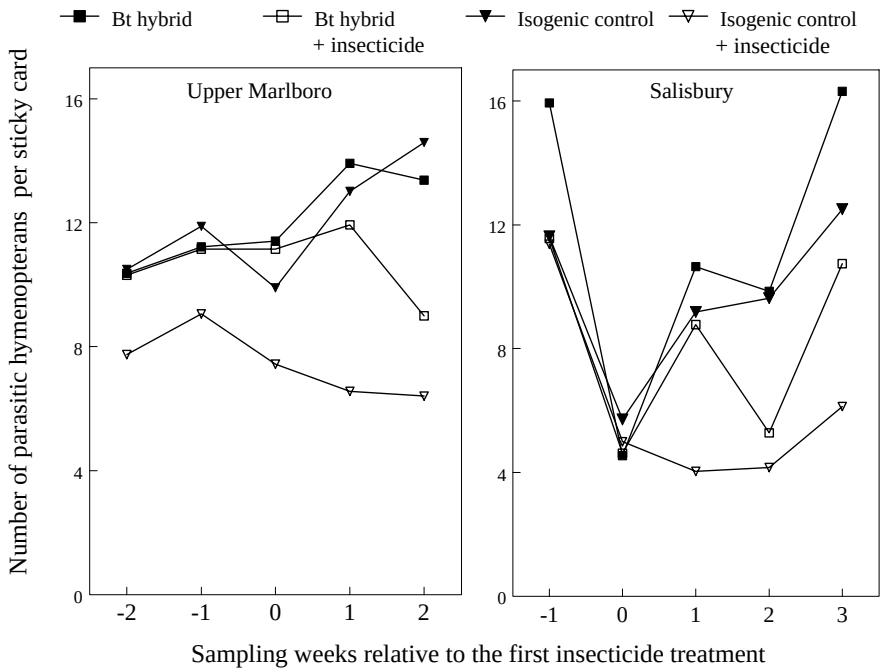

Figure 1.14. Mean number of parasitic hymenopterans recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

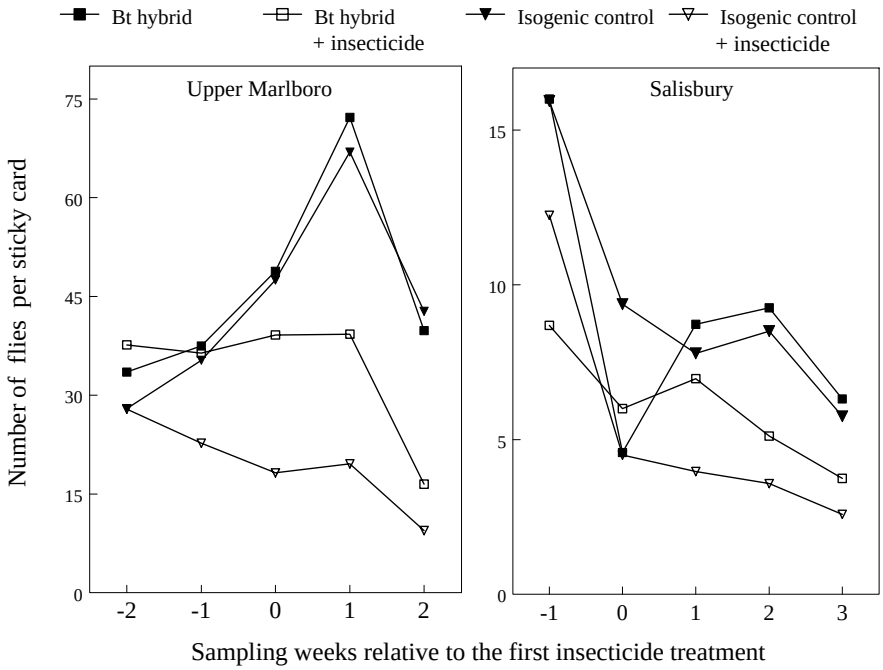

Figure 1.15. Mean number of dipterans recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

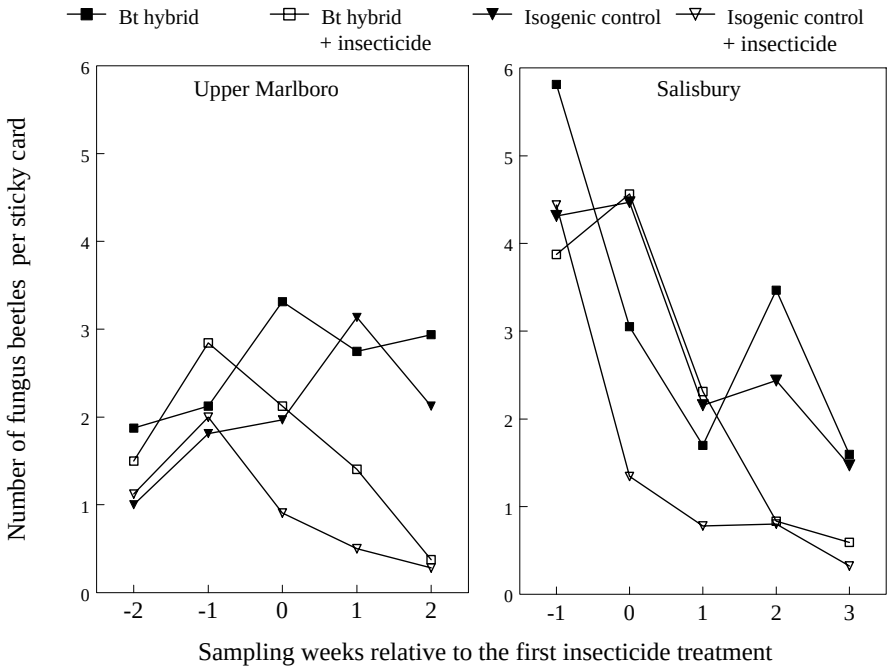

Figure 1.16. Mean number of fungus beetles recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

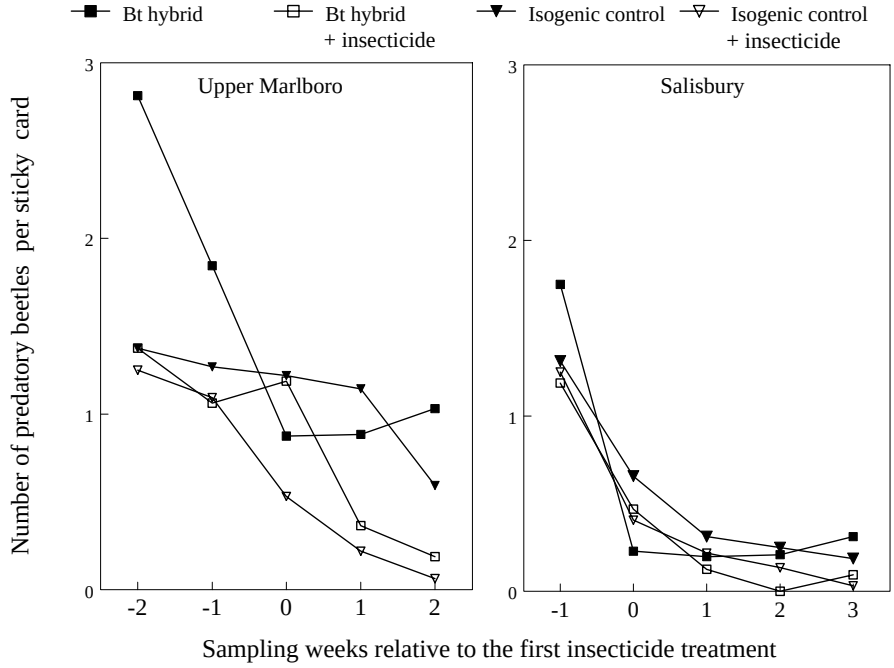

Figure 1.17. Mean number of predatory beetles recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

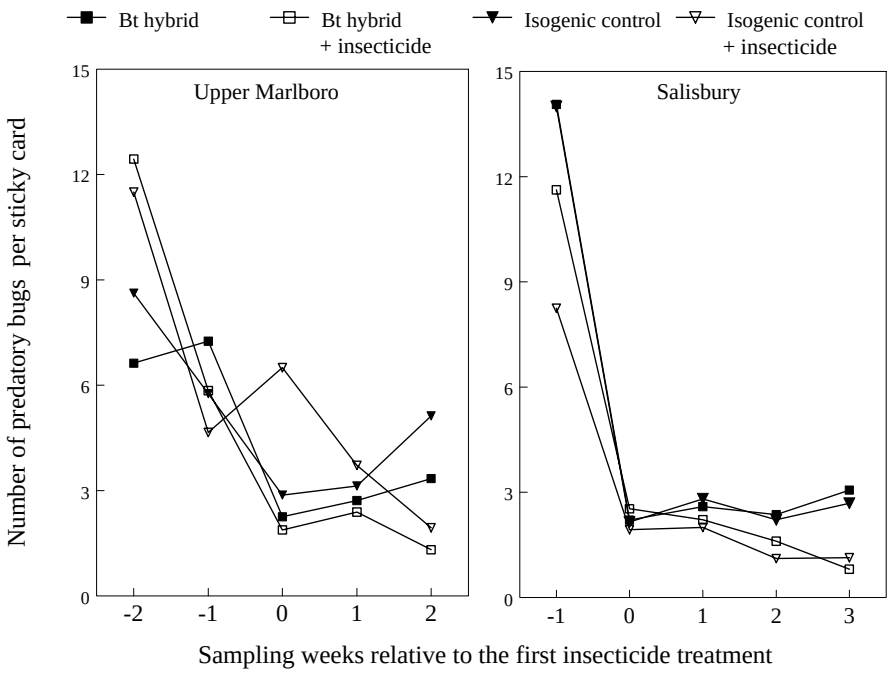

Figure 1.18. Mean number of predatory bugs recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

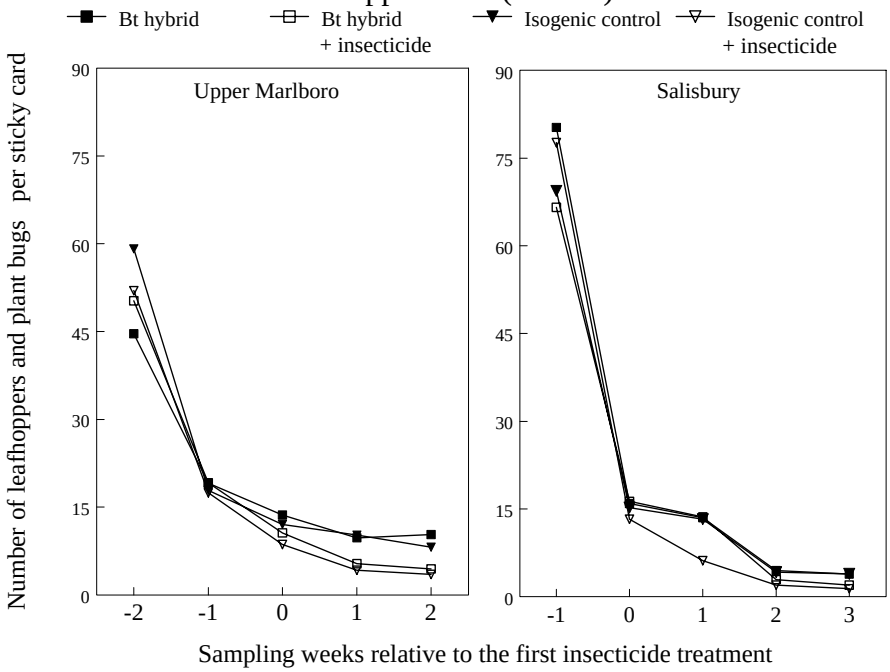

Figure 1.19. Mean number of cicadellids and mirids recorded by sticky cards in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

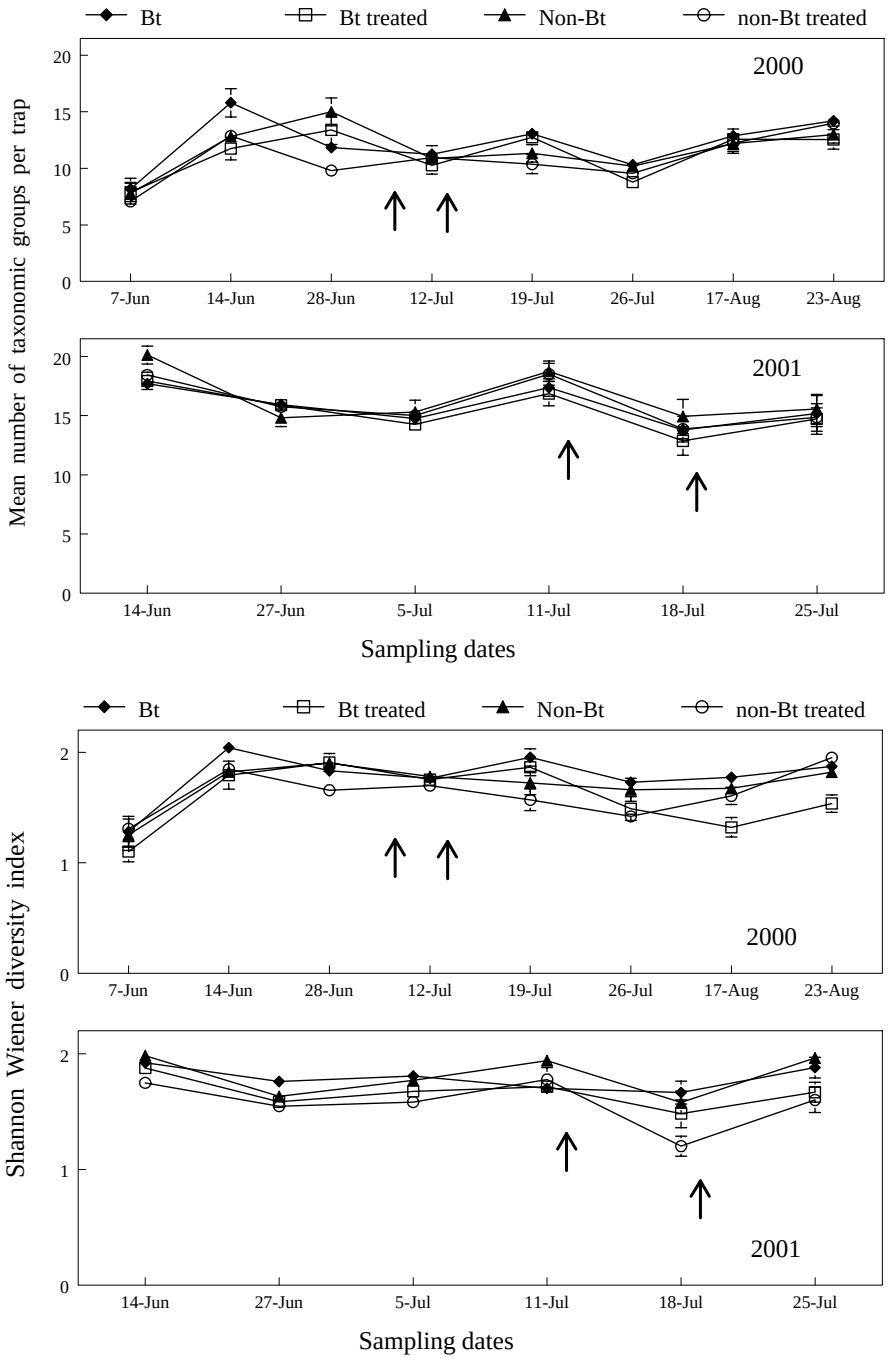

Figure 1.20. Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots.

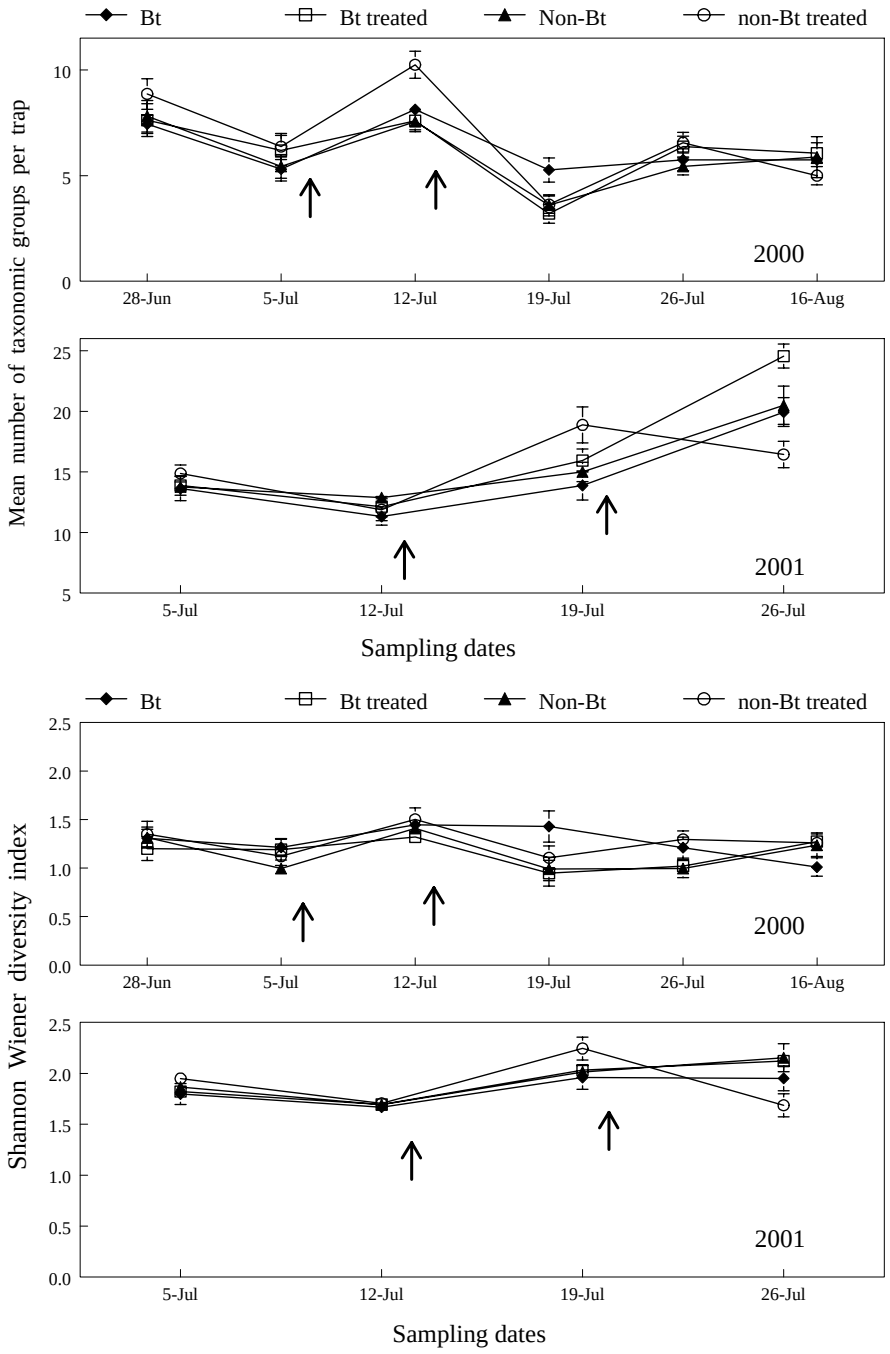

Figure 1.21. Mean number of taxonomic groups and Shannon-Wiener diversity indices of arthropod communities recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Salisbury, Maryland. 2000 and 2001. First arrow indicates first insecticide application in the non-Bt treated plots, while second arrow indicates timing of the single application in the Bt treated plots.

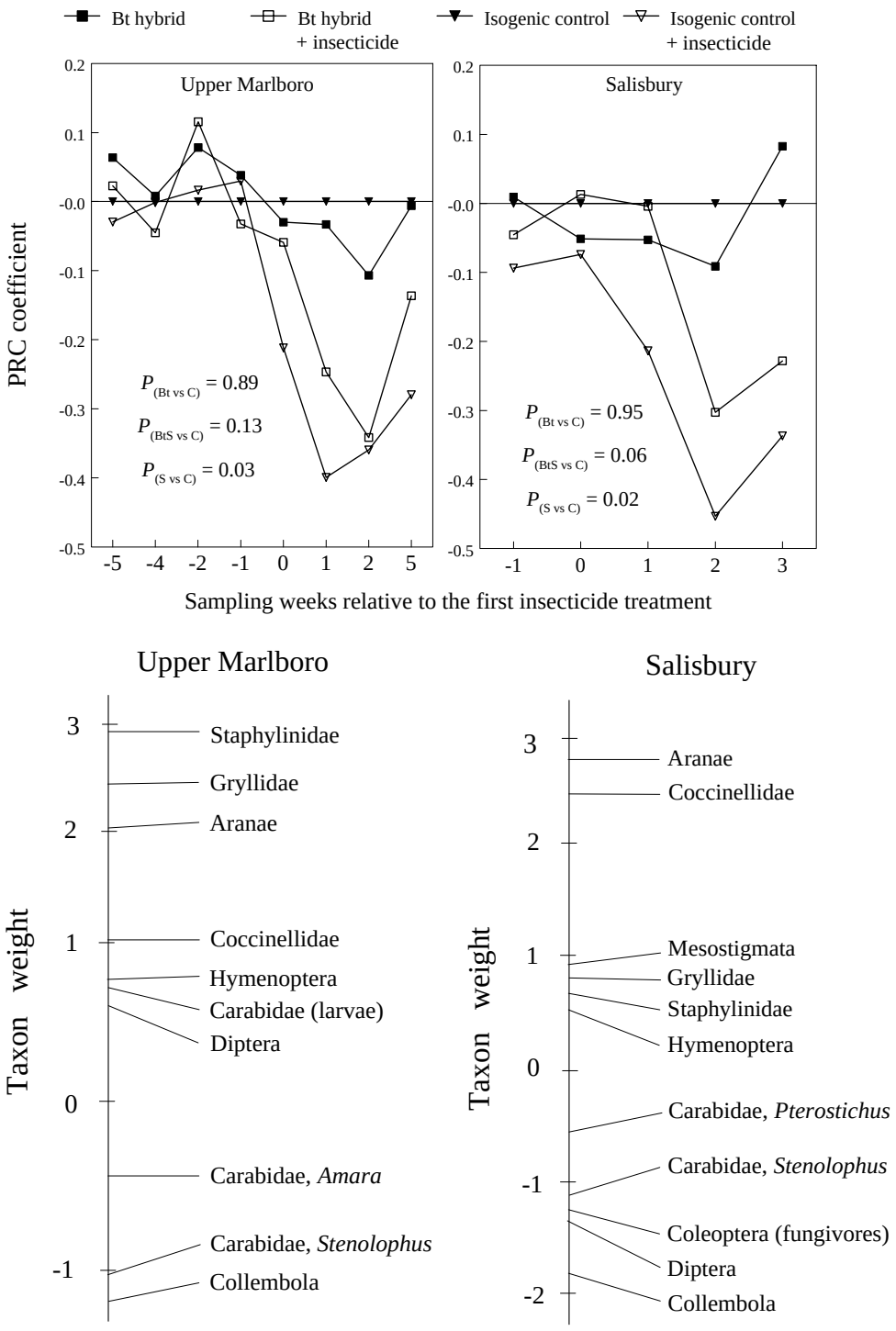

Figure 1.22. Principal response curves and taxon weights of surface-dwelling arthropod communities exposed to Bt and insecticide-treated sweet corn compared to the isogenic control. Responses of taxa with positive weights followed the PRC patterns, whereas those with negative weights showed the opposite pattern. Taxi with weights between -0.5 to 0.5 are not shown.

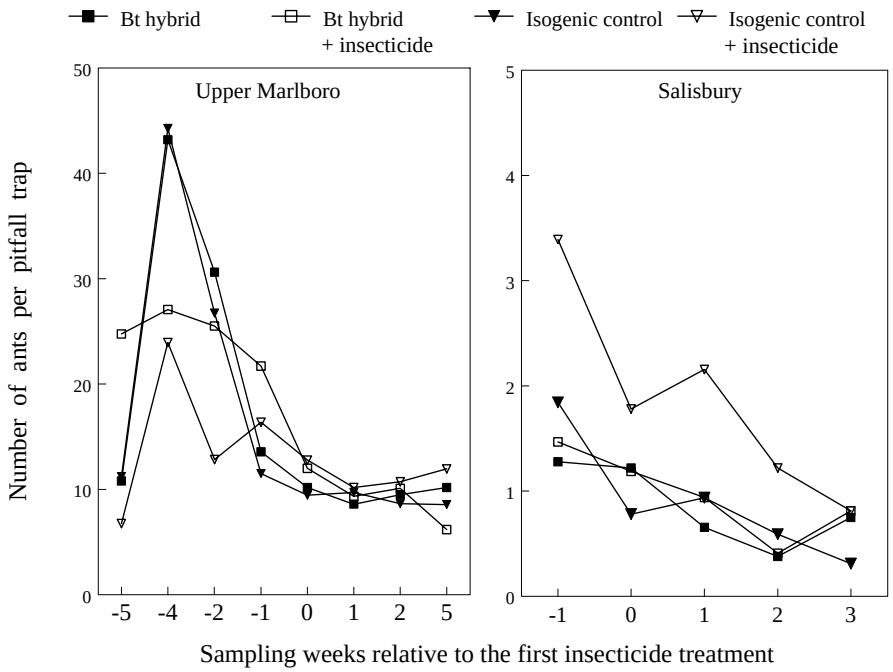

Figure 1.23. Mean number of ants recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

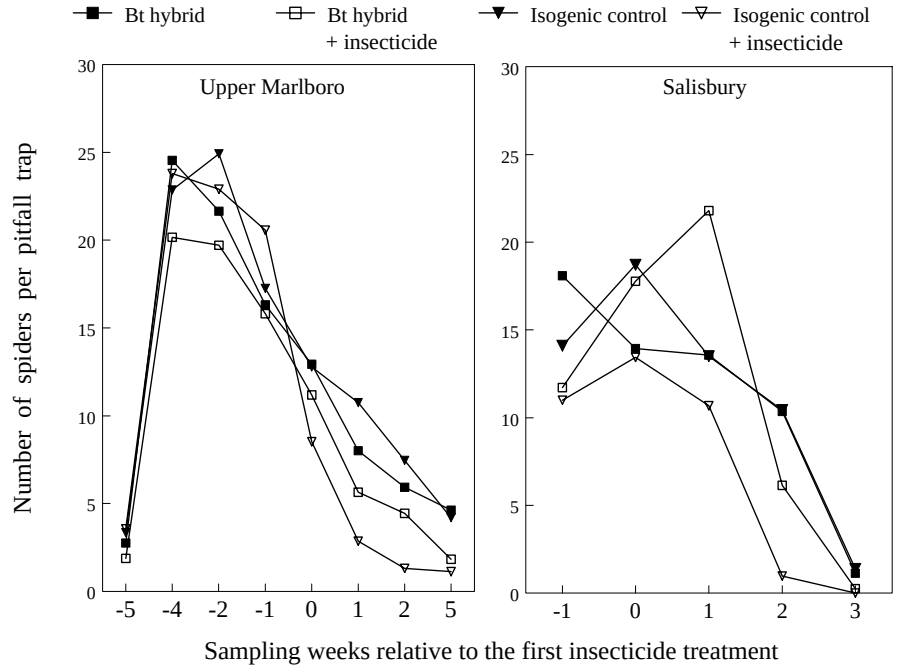

Figure 1.24. Mean number of spiders recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

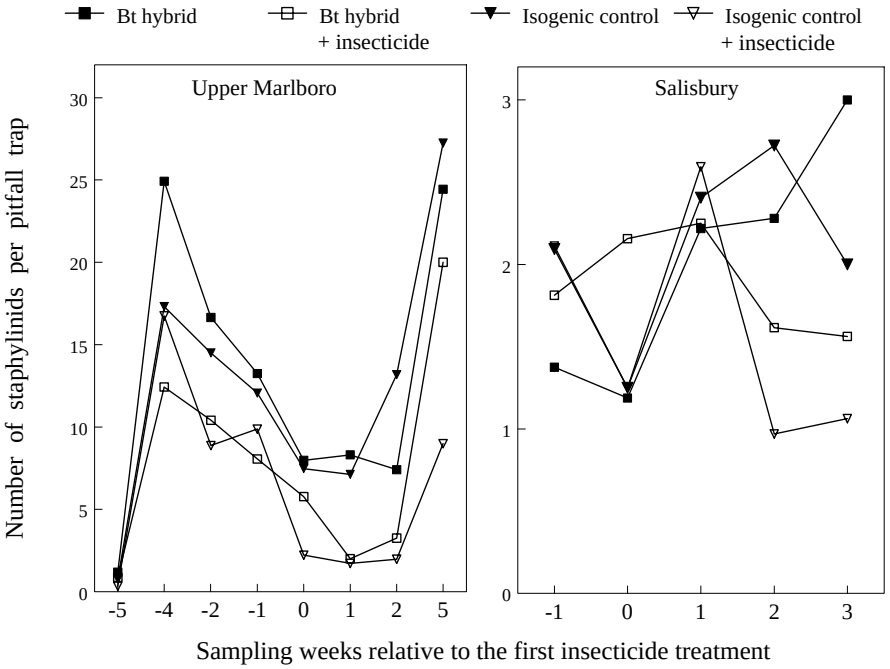

Figure 1.25. Mean number of staphylinids recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

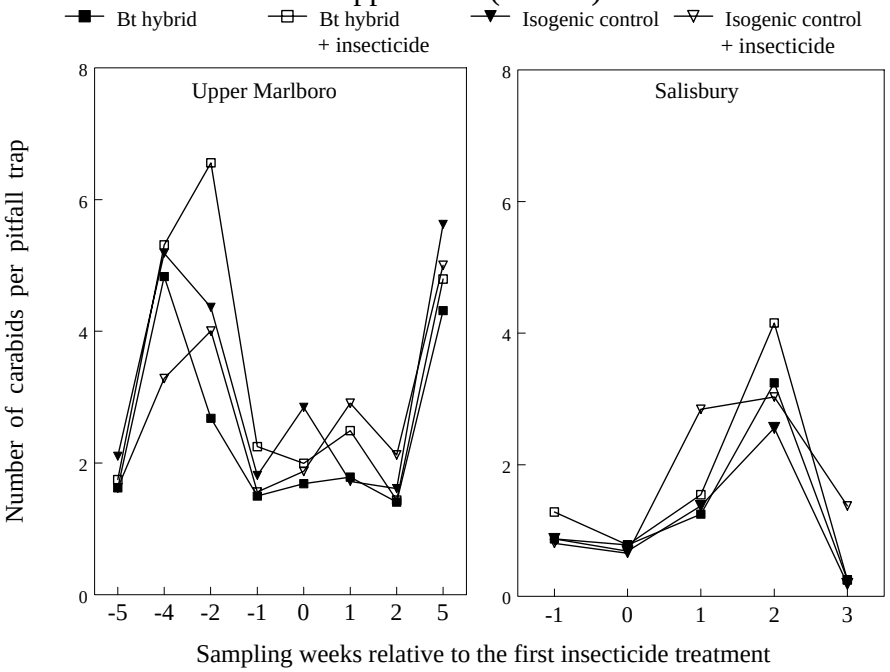

Figure 1.26. Mean number of predatory carabid beetles recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

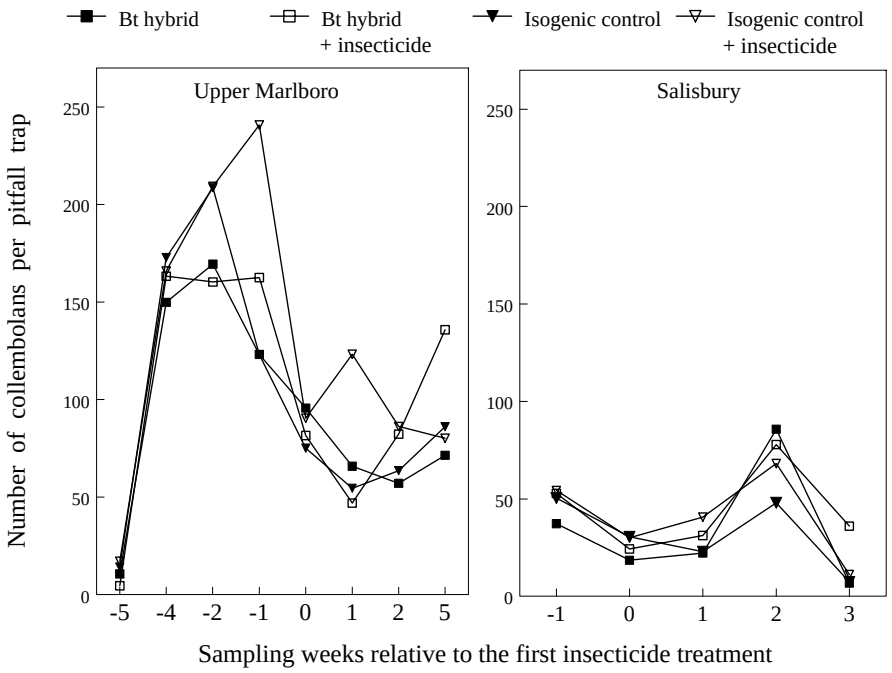

Figure 1.27. Mean number of collembolans recorded by pitfall traps in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro and Salisbury, Maryland. Data pooled and averaged over years relative to weekly intervals prior to and after the first insecticide application (week 0).

CHAPTER II

Effects of Bt Transgenic Sweet Corn on Selected Functional Processes of Arthropod Communities

Abstract

Field studies were conducted to assess the non-target effects on selected functional processes in Bt transgenic sweet corn in comparison to conventional insecticide control. Naturally-occurring chrysopid eggs were used to assess parasitism, which was not significantly affected by exposure to the Bt hybrid. However, applications of a pyrethroid insecticide significantly reduced the level of egg parasitism and permitted a greater percentage of chrysopid eggs to hatch. Sentinel egg masses of the European corn borer showed that predation of eggs was not affected by their placement in Bt plots but exposure in insecticide-treated plots significantly reduced the incidence of predation by more than 60%. Exposure in Bt plots had no effect on the overall community diversity and numbers of arthropods captured in emergence traps placed over surface litter. Litterbags placed after harvest and collected over a nine month period determined that the abundance of colonizing arthropods and rate of dry matter loss were not affected by the type of litter, or whether it came from insecticide-treated or untreated plots.

Introduction

Delta-endotoxins from *Bacillus thuringiensis* (Bt) have been used for more than 40 years in bioinsecticide formulations. However, their use in commercial agriculture has been limited due to the short residual action, narrow spectrum of activity, and route of entry that requires ingestion. The genetic engineering of plants that express Bt proteins

constitutively in all tissues and continuously throughout the growing season has overcome many of the limitations of Bt bioinsecticides. Adoption of Bt crops has resulted in fewer insecticide applications and thus lower management costs (Mara *et al.* 1998, Schnepf *et al.* 1998, NFAP 2002, Langrock *et al.* 2003). Another notable advantage of transgenic insecticidal crops over conventional insecticides is their high specificity, such that potential toxic effects on non-target beneficial insects should be minimal (Betz *et al.* 1999, MacIntosh *et al.* 1990).

An ecological risk assessment is a necessary process to address the acceptability of releasing a novel transgenic crop in the environment (Jepson *et al.* 1994). Most studies conducted thus far have focused on laboratory studies (Sims 1995, 1997) to assess the acute toxicity of Bt microbially derived proteins or on field studies to assess changes in abundance and diversity of non-target populations (Orr and Landis 1997, Lozzia 1999, Manachini *et al.* 1999, Pilcher *et al.* 1997, 2002, Head *et al.* 2001, Hagerty *et al.* 2001, Candolfi *et al.* 2003, Naranjo 2002, Wilson and Fitt 2002, Sisterson *et al.* 2004). Only a few studies have investigated the potential effects of plant-incorporated Bt proteins on the functional processes at work in a cornfield (Pilcher *et al.* 1997, Saxena and Stotzky 2001, Bourguet *et al.* 2002, Dutton *et al.* 2002, Groot and Dicke 2002, Shuler *et al.* 2003, Zwahlen *et al.* 2003). Two important ecological processes in a cornfield include trophic interactions, such as predation and parasitism, and recycling of organic matter via decomposition of plant material left on the soil surface after harvest. Understanding how Bt crops impact these processes is basic knowledge for evaluating the dynamics of non-target effects and predicting the ecological consequences of changes in non-target community structure and abundance.

The reproductive success of beneficial arthropods can be affected in several ways by large-scale commercial plantings of Bt corn. Predation and parasitism may be affected by a reduction in host or prey populations, indirect contact with Cry protein by feeding on intoxicated organisms, feeding directly on plant parts (e.g., pollen), or by changes in plant volatile chemicals (Schuler *et al*. 1999). Whether these changes will have any adverse effects on the functional sustainability of a particular ecological guild, community, or the corn agroecosystem as a whole is yet to be determined. Studies conducted thus far suggest that parasitoids and predators are not repelled by Bt-intoxicated prey (Groot and Dicke 2002, Schuler *et al*. 2003). Prey fitness costs such as delayed development time or a reduction in host defense mechanisms may be beneficial to parasitoids and predators (Johnson and Gould 1992, Groot and Dicke 2002). However, fewer natural enemies may be attracted to Bt cornfields due to a reduction in pest damage and volatile chemical cues (Groot and Dicke 2002, Schuler *et al*. 1999). A lack of chemical cues attracting natural enemies to Bt plants may lead to movement to more favorable habitats (Johnson 1997).

Few field studies have investigated the impacts on parasitoids or the level of parasitism in Bt cornfields. Changes in the quality and behavior of hosts may adversely or beneficially affect rates of parasitism (Schuler *et al* 1999, 2003). A reduction in host size may adversely affect the survival and growth of immature parasitoids, adult size, and fecundity. Also, more male eggs are typically laid in smaller hosts, thus sublethal effects could indirectly lead to changes in sex ratio. Indirect effects could result from secondary exposure to expressed proteins when parasitoids feed on herbivore hosts that have fed upon a Bt crop plant. Since the Cry1Ab protein is highly specific to certain lepidopteran insects, it is unlikely that parasitoids feeding on intoxicated hosts would be adversely

affected. Conversely, the immune response of hosts could be diminished by the toxin resulting in increased levels of parasitism (Schuler *et al.* 1999). Also, sublethal doses of Bt proteins consumed by the host larvae could delay development and thus increase exposure to parasitoid attack.

Because adult and immature predators are typically mobile and often have a wide host range, the potential population effects of a reduction in a particular prey species are less likely. Egg and larval stages of the European corn borer (*Ostrinia nubilalis* Hübner) are prey items for many predators in cornfields (Sparks *et al.* 1966). Studies conducted thus far have focused on survival and development of predators feeding on microbially derived Bt delta-endotoxin or Bt intoxicated prey in the laboratory (Melin and Cozzi 1990, Sims 1995, 1997, Pilcher *et al.* 1997, Zwahlen *et al.* 2000, Al-Deeb *et al.* 2001, Dutton *et al.* 2002) or abundance and diversity in the field (Pilcher *et al.* 1997, Manachini *et al.* 1999, Wold *et al.* 2001, Bourguet *et al.* 2002, Candolfi *et al.* 2003, Naranjo 2002, French *et al.* 2004, Sisterson *et al*. 2004). In general, these studies suggest that planting Bt crops will not result in adverse effects on predators. However, these studies only provide a quantitative assessment of the changes in community structure occurring in Bt fields. Whether these changes will have any adverse effects on the functional sustainability of a particular predator has received limited attention.

Degradation of organic matter in cornfields is an important process that can be impacted by the release of Bt proteins. Bt plant residue left in the field after harvest may take longer to decompose resulting in impacts on the soil ecosystem (Saxena and Stotzky 2001, Zwahlen *et al.* 2003, Flores *et al*. 2005). Ground-dwelling organisms that are important in the breakdown of organic matter and contribute to nutrient cycling and soil

structure may be affected by a change in the decomposition rate of plant material. Cry proteins are known to bind to soil particles particularly clays and humic acids which may slow the rate of microbial degradation of these toxins (Crecchio and Stotzky 1998, Koskella and Stotzky 1997, Tapp and Stotzky 1995, Tapp and Stotzky 1998, Stotzky 2000). However, field deposition of Cry protein from plant material (pollen or crop residue) or plant root exudates (e.g. carbohydrates and amino acids) typically stimulates microbial activity and reproduction (Cheng and Coleman 1990, Jensen and Soerensen 1994, Meharg 1994, Griffiths *et al.* 1999).

Several reviews of ecological risk assessment for Bt crops have emphasized the importance of measuring impacts of various ecological processes (Schuler *et al*. 1999, Jepson *et al.* 1994, EPA 2002). Reported here are field studies that assess the non-target effects on selected functional processes in Bt transgenic sweet corn in comparison to conventional insecticide control. Specifically, outsourcing of sentinel egg masses of *O. nubilalis* was used to estimate predation rates; naturally-occurring chrysopid eggs were used to measure levels of parasitism of a specific parasitoid; emergence traps were used to determine the recruitment rates of arthropods emerging from the soil-litter habitat; and litterbags were used to measure arthropod colonization and litter degradation rates.

Materials and Methods

Plot design and treatments. This study was conducted at the University of Maryland Research and Education facility at Upper Marlboro, Maryland. The sweet corn hybrid ‘Attribute GSS0966' (Syngenta Seeds, Golden Valley, MN; event BT11) and its non-transgenic isoline (‘Prime Plus’) were planted in early May and managed according to recommended commercial practices in the same no-tilled plots during the growing

seasons of 2001, 2002 and 2003. Plots were planted with the same hybrids into a rye cover crop with the previous year's corn residue. The experiment each year was arranged in a 2 x 2 split plot design with four replicate blocks. The two hybrids (Bt and non-Bt) were arranged as whole plots and insecticide treatments were applied to the subplots. Each block was 16 rows, 0.75 m wide and 39 m long and separated by a 10 m non-cropped buffer area. Repeated placement of treatments in the same plots allowed for the assessment of non-target effects from the cumulative exposure of Bt and conventional insecticide methods over three years.

Whole plots were divided into insecticide treated and untreated subplots. Treatments of lambda-cyhalothrin (Warrior 1E, Syngenta Crop Protection, Research Triangle Park, NC) at a rate of 237 ml/ha were applied with an airblast sprayer delivering 536 liters of spray volume/ha at a rate of 3.2 oz per acre. The Bt treated plots received one spray at 100% fresh silk, which represented a recommended treatment regime for a late planting to provide additional protection against non-target secondary pests or high populations of corn earworms (*Helicoverpa zea* Boddie). Non-Bt treated plots received five insecticide applications starting at early silk and repeated every three days. This treatment regime represented a typical schedule for control of ear-invading insects during mid to late season for fresh market sweet corn. All plots were rotary mowed one week after the primary ears reached peak 'roasting ear' maturity.

Chrysopid egg mortality. Naturally occurring chrysopid eggs were used as bio-indicators to measure parasitism in Bt, non-Bt treated, and non-Bt control plots. Since the slender stalk and chorion of a lacewing egg remained attached to the leaf for a relatively long period after hatch or parasite emergence, examination of cohorts of eggs over time

allowed for an estimation of the apparent parasitism rate. On 30 July 2001 and 4 August 2002, approximately one week after peak anthesis, samples of 50 to 100 lacewing eggs were randomly collected from each plot. All eggs were taken regardless of whether they appeared hatched or not. Due to low egg populations and time limitations, smaller samples of 50 eggs were collected in the insecticide-treated plots. Eggs were brought to the laboratory and examined under a stereomicroscope to determine their condition. Eggs were categorized as 1) unhatched – eggs were intact on stalk with normal shape, smooth surface, and coloration; 2) hatched – pale white chorion remained at apex of stalk, with swirled tears and the appearance of being ripped open from the inside; 3) parasitized – egg intact on stalk, normal shape but light brown coloration throughout and the appearance of a granulated surface; and 4) consumed – eggs either shriveled with the chorion intact by a sucking predator or partially consumed by a chewing predator. Some residue of the dried egg contents was usually associated with the remains of predated egg. Data were summarized as the percentage of eggs hatched, unhatched, predated and parasitized.

Predation. Sentinel egg masses of *O. nubilalis* manually placed on plants were used to estimate predation rates in each treatment plot. Wax paper sheets with two-day-old European corn borer egg masses attached were obtained from the USDA/ARS laboratory at Ames, IA. Masses of 20 to 30 eggs were selected and individually cut out of the waxed paper on a rectangular section (approximately 1 cm x 3 cm). Ten egg masses were placed on sweet corn plants in the center four rows of each plot on four dates in 2002 (18, 23, 26 and 31 July) and two dates in 2003 (6 and 25 August). Egg masses were attached by using an insect pin to stitch the wax paper section to the undersides of the

leaf below the primary ear. Egg placement dates occurred after all insecticide treatments were applied. After two days, the egg masses were removed and brought to the laboratory where they were examined under a stereomicroscope. Since eggs were approximately two days old when placed in the field, few egg masses hatched by the time they were collected. Eggs were categorized according to Andow (1990) as 1) hatched – most of the egg chorions remained and appeared ripped open, with a brown residue usually associated with the egg remains; 2) unhatched – no signs of physical feeding injury or abnormal discoloration due to parasitism; 3) consumed by a chewing predator – partially or completely consumed eggs characterized by pieces of jagged edges of the chorions remaining. 3) consumed by a piercing-sucking predator – contents of the eggs appeared sucked out, leaving the chorions intact but collapsed and showing small holes produced by piercing stylets; and 4) parasitized – a characteristic blackening of the vitelline membranes of eggs that occurs during the parasite prepupal and pupal stages. The condition of each egg mass was characterized by estimating the portion representing each category to the nearest 10%. Data were summarized as the percentage of eggs predated by chewing and sucking predators and percentage parasitized.

Recruitment. The community recruitment rate of various decomposers, parasitoids and predators emerging from soil and surface litter was estimated by emergence traps in 2002. Traps consisted of an inverted black plastic tub (0.5 m x 0.75 m) with a clear inverted funnel glued over an opening in the center of the top. Another clear funnel attached to sealed plastic cup was attached securely to the funnel on the tub. The plastic cup-funnel arrangement contained ethylene glycol and thus functioned as an inverted pitfall trap. Organisms emerging from the enclosed area were attracted to the

light through the opening and clear funnel and were captured in the cup. Four emergence traps were randomly placed in the interspace of the center rows of each plot for two consecutive weeks. Trapping was conducted two weeks prior to planting (initiated on 30 May), every 4 weeks during the crop cycle (initiated on 3 and 31 July), and twice after the crop was harvested and rotary mowed (initiated on 4 and 18 September). The contents of the collection cups were brought to the laboratory and vacuum filtered over a fine organdy screen to remove the ethylene glycol. Filtered organisms were stored in 70% ethanol and later identified and enumerated to the order or family level. Carabid adults were identified to genus level.

Litterbags. The effects of Cry1Ab protein expressed in sweet corn plant residue on colonization and decomposition were determined by litterbag sampling over time (Crossley and Hoglund 1962, Siedentop 1995, Cortet *et al.* 2002). When ears reached peak 'roasting ear' maturity in 2002, 20 plants were randomly selected and removed at ground level (excluding roots and ears) from each of the 16 plots. Each sample, including stalks, leaves, and tassels, were mulched into coarse pieces by a gasoline-powered mulcher. The mulched samples were allowed to partially dry in a vented greenhouse for 24 h to simulate conditions in the field and to minimize initial molting. Forty subsamples of the plant material (roughly 100 g) from each plot were placed in 26 x 14 cm mesh bags (1 mm × 1.5 mm mesh) and weighed. After the plots were rotary mowed, the bags were placed flat on the ground surface in their respective plot and covered with plant residue to shade from direct sunlight. The sheltered bags provided more uniform microenviromental conditions for microfauna and mesofauna to colonize the plant material. A subsample of the bags from both Bt and non-Bt plots were weighed, placed in a drying oven, and then

weighed again as dry weight. Using a wet-to-dry weight ratio, the initial dry weights of the bags placed in the field were calculated.

Litterbags were collected at intervals following their placement. Eight bags were removed from each plot in September, October and November, 2002, and April and May 2003. Due to a limited number of Berlese funnels, random pairs of bags from each plot were opened at one end and placed together in funnels in the greenhouse for two days to extract arthropods from the plant material. After extraction, each bag was dried to absolute dry weight. The difference in dry weights of bags placed in the field and weights when extracted was used to calculate percentage decomposition. Extracted arthropods in 70% ethanol were taken to the laboratory, where they were identified under a stereomicroscope and recorded to order or family.

Statistical analyses. Data generated on emergence and litterbag communities were modified to include missing zero counts. The Shannon-Wiener index (Shannon and Wiener 1949) was calculated for each sample to measure the relative community diversity of the arthropod fauna. Parasitism and predation data for each plot were averaged over multiple sampling times before analysis. All data were tested before analysis for normality and homogeneity of variances using Spearman's Rank Correlation and Shapiro-Wilk's W test. An appropriate transformation was used and variances were grouped (Russek-Cohen and Douglas 1999), if data did not meet the assumptions of ANOVA. The mixed model procedure (SAS Institute 1996) was used to test for treatment effects. Hybrid treatment and sampling time were considered fixed factors, while replicate was treated as a random block effect. The repeated measures option was used to correct for temporally correlated data. Contrast tests were performed on specific

treatment comparisons. Means were separated following a significant F test by using the Tukey's multiple comparison adjustment ($P < 0.05$).

Results

Chrysopid egg mortality. A total of 3,526 chrysopid eggs were collected and examined for parasitism and other sources of mortality. Thirty-nine % of the eggs collected in the non-Bt control plots were parasitized by an unidentified scelionid parasitic wasp. Significantly fewer eggs were parasitized in insecticide-treated non-Bt plots compared to levels of parasitism in the Bt or non-Bt control plots ($F_{(2,28)} = 4.02$, $P = 0.03$) (Fig. 2.1). The percentage hatched was correspondingly the highest in the insecticide-treated plots. The small difference in egg parasitism in Bt and control plots was not statistically significant ($P = 0.74$). Predation was very low based on the number of eggs remaining on the plants. However, eggs consumed by chewing insects were probably underestimated because eggs that were near completely consumed were not easily seen in the field.

Predation. A total of 960 *O. nubilalis* egg masses were placed in plots over a series of six sampling times. Relative differences among treatments were similar during both years, so data were averaged across sampling dates. The treatment effect on egg predation was significant for chewing predators ($F_{(3, 9)} = 7.17$, $p = 0.009$) and piercing-sucking predators ($F_{(3, 9)} = 6.46$, $p = 0.013$) (Fig. 2.2). The insecticide treatment in both Bt and non-Bt plots significantly reduced predation by chewing predators. Although the difference between treated plots was not significant, percentage predation was 67% less in non-Bt plots treated five times compared to predation in Bt plots treated once. Piercing-sucking predators caused less overall predation and relative responses to the

hybrid treatments was different from those observed for chewing predators. Significantly more eggs in the Bt treated plots were consumed. For both types of predation, rates did not differ significantly between the Bt and non-Bt control plots ($P > 0.28$). The most frequent predators found feeding on the sentinel eggs included adults and larvae of *Coleomegilla maculata* (DeGeer), adults of *Orius insidiosus* (Say), and larvae of *Chrysoperla carnea* (Stephens).

Recruitment. Seventy nine % of all arthropods emerging from the surface litter were decomposers, with springtails (Collembola) as the dominate group (67% of total abundance) (Table 2.1). Other major decomposers included humpbacked flies (Phoridae, 5%), darkwinged fungus flies (Sciaridae, 3.4%), frit flies (Chloropidae, 2.9%), picture-winged flies (Otitidae, 2.5%), minute scavenger flies (Scatopsidae, 1.3%), Psocids (Psocoptera, 1.7%), and many fungivorous Coleoptera (1.4% as a group, Nitidulidae, Corylophidae, Phalacridae, Crytophagidae, Mycetophagidae, Lathridiidae, Oedemeridae, Anthicidae, Scydmaenidae, Pselaphidae).

The diversity of emerging arthropods based on the Shannon-Wiener index changed significantly over time ($F_{(4,60)} = 45.72$, $P < 0.001$), showing the highest abundance and number of taxa before and after the crop season when the biomass of surface litter was greater (Fig. 2.3). The hybrid treatments had no significant effect on diversity when the analysis included the five sampling dates. However, there was a significantly lower diversity index for communities emerging in the non-Bt treated plots for emergence trapping initiated on 31 July ($F_{(1,12)} = 7.18$, $P = 0.02$). This period of emergence directly followed the schedule of five insecticide treatments applied to these

plots. Exposure to Bt, either via carryover surface litter or pollen that fell to the surface, had no significant effect on the overall community diversity of emerging arthropods.

The number of collembolans captured in emergence traps was highly variable but yet significantly affected by time ($F_{(4,43.7)} = 66.61$, $P < 0.001$) and hybrid treatment ($F_{(3,4.8)} = 6.18$, $P = 0.04$). Collembolans emerging from litter in Bt, Bt treated, non-Bt control and non-Bt treated plots averaged 351, 590, 570, and 588 per emergence trap, respectively (Fig. 2.4). Statistically, the numbers emerging in Bt plots were significantly the lowest compared to the other hybrid treatments. A contrast test also indicated that overall collembolan densities were significantly higher by 28% in plots treated with insecticides compared to plots without insecticides ($F_{(1,6.6)} = 7.91$, $p = 0.03$). This trend was particularly evident during the pre-season and in late July - early August after insecticides were applied.

Many dipterans used the decaying organic matter on the ground surface as a food source for recruitment. Five major families of dipterans were captured in more than 80% of emergence traps and together accounted for 12.3% of the total emerging community (Table 2.1). In accordance with the diversity indices, fly emergence was the highest before and after the crop season (Fig. 2.5). As a combined group, the recruitment rates in the Bt and non-Bt control plots were statistically the same. However, exposure to insecticides reduced the overall mean number of dipterans emerging by 9.6% and 29.8% in the Bt and non-Bt plots treated one and five times, respectively. The contrast test comparing fly emergence from treated versus untreated plots was significant ($F_{(1,9)} = 5.75$, $P = 0.04$). This response apparently carried over from the previous year because fly

recruitment was numerically lower during the pre-season and in early July before insecticides were applied.

A diverse guild of fungivorous beetles and psocids inhabited the surface litter and apparently utilized the fungus growth associated with decaying organic matter. Recruitment of the beetles was unaffected by exposure to Bt but significantly reduced in the non-Bt plots treated five times with insecticides ($F_{(1,9)} = 5.75$, $P = 0.04$) (Fig. 2.6). Psocid emergence varied among hybrid treatments depending on the sampling date but the overall response was in the opposite direction from that of the fungivorous coleopterans. Captures of Psocoptera in emergence traps were 70.7% higher in the insecticide-treated plots ($F_{(1,12)} = 8.41$, $p = 0.01$) (Fig. 2.7).

Parasitic hymenopterans comprised 6.2% of all arthropods captured in emergence traps and were relatively low in their frequency of occurrence, except for scelionids and charipids (Table 2.1). Overall numbers of parasitoids averaged 25.7, 28.4, 24.9, and 19.1 per trap in the Bt, Bt treated, non-Bt control, and non-Bt treated plots, respectively; thus, indicating numerically that the lowest emergence occurred in the non-Bt treated plots (Fig. 2.8). However, differences were not statistically significant due to considerable variance in the numbers of emerging parasitoids. Predatory and phytophagous insects accounted for 2.4% and 11.9% of the total emergence but no major taxa represented in these functional groups were affected by insecticide or Bt exposure.

Litter colonization. Decomposers comprising 89.8% of the litterbag community were by far the majority of the colonizing arthropods extracted (Table 2.1). Collembola, soil mites (Oribatida), dipteran larvae, and fungivorous coleopterans were the predominate taxa in this functional group. Of the beetles, the most common families were

essentially the same as those captured in emergence traps (see listed above). Shannon-Wiener indices indicated that diversity of the colonizing community gradually decreased during the fall months as average temperatures dropped and then increased slightly in the spring (Fig. 2.9). The litter from the different hybrid treatments had no effect on community diversity.

Collembola, the most abundant colonizing group, reached the highest density of 438 per litterbag in September, gradually declined during October and November, and then dropped significantly the following spring (Fig. 2.10). Entomobryidae, Isotomidae, and Hypogasturidae accounted for 17.4, 48.1, and 34.3% of the Collembola numbers, respectively, and all three families followed the similar population trends over time. The density of springtails in litterbags placed in non-Bt plots treated with five insecticide applications averaged 700 per bag and was numerically much higher than densities in the other treatments, particularly on 11 September. However, the high variance of data precluded the possibility of a statistical significant effect. Populations of oribatid mites (Fig. 2.11) and predatory mites (Mesostigmata) (Fig. 2.12) infesting litterbags followed a similar trend over time and were not influenced by exposure to Bt or insecticide-treated litter. The combined group of fungivorous coleopterans showed the greatest response to the hybrid treatments ($F_{(3,9)} = 5.55$, $P = 0.02$), which was mainly evident as significantly fewer beetles during the 2002 sampling times in the non-Bt plots treated with insecticides (Fig. 2.13). The majority of the coleopterans captured were sap beetles (Nitidulidae), hairy fungus beetles (Mycetophagidae) and false blister beetles (Oedemeridae).

Predators were the second most abundant functional group and constituted 8% of the arthropods extracted from litterbags. With the exception of the predatory mites

(Mesostigmata), data on predators were too over-dispersed to statistically test for treatment effects. Moreover, there were no consistent, numerical trends in predator densities that might suggest a Bt or insecticide effect.

Litter decomposition. At the time of sweet corn harvest, the dry matter content in Bt plant material (68.0%) was significantly lower than the level of dry matter in non-Bt corn (76.4%). Thus, a different conversion factor was used for each corn type to estimate the initial dry weights of litterbags placed in the plots. Overall percentage dry matter loss significantly increased over time ($F_{(1,29.6)} = 10.63$, $P < 0.001$), losing incrementally 17.4, 13.6, and 10.8% of the dry weight at the September, October, and November sampling dates (Fig. 2.14). The rate of decomposition was apparently very low during the winter, as indicated by a slight overall gain in weight by mid-April. This was probably the result of inorganic materials entering the bags during the winter, since the final ash content of each bag was not determined to correct for contamination. Litterbags lost another 9.6% of the dry matter during the last month ending in mid-May. Statistically, there was no overall treatment or treatment by time interaction effects on dry matter loss. Percentage losses averaged 38.7, 34.0, 34.3, and 32.6% for litterbags containing Bt, Bt treated, non-Bt control and non-Bt treated plant material and placed in their respective plots. Significance was indicated by contrasting the highest loss in Bt plots with the lowest loss in the non-Bt treated plots ($F_{(1,6.2)} = 6.90$, $P = 0.04$). However, there was no strong evidence of any consistent trend indicating that the type of sweet corn influenced the rate of decomposition.

Discussion

Parasitism. The egg-laying behavior of chrysopids and high natural occurrence of egg parasitism provided an ideal system to examine the effects of the hybrid treatments on the rate of parasitism. An overall 39% of the chrysopid eggs were parasitized by scelionid wasps in the non-Bt plots. Egg parasitism is one of the limiting factors of *C. carnea* population, as described by Conard (1984) and Szentkiralyi (2001). Scelionid species reported attacking chrysopid eggs include *Telenomus acrobates* Giard and *T. chrysopae* Ashmead. The levels of parasitism was not significantly affected by exposure to the Bt sweet corn hybrid, thus providing evidence that these particular parasitoids did not avoid Bt plants and were equally able to find hosts compared with parasitoids in the non-Bt plots. Although egg densities were not recorded on a per plant basis, the amount of sampling effort required to locate equal numbers of eggs was similar between Bt and non-Bt plots. Furthermore, non-target studies of Bt sweet corn at the same location during the previous two years showed no differences in lacewing egg densities between Bt and non-Bt plots. Thus, it is unlikely that host density influenced the rate of parasitism in Bt and non-Bt plots. These results agree with other studies that showed no changes in parasitoid searching behavior in Bt crops (Johnson and Gould 1992, Schuler *et al.* 2003) or no mortality effects due to Bt intoxicated hosts (Chilcutt and Tabashnik 1999b). Additionally, Cry1Ab transgenic corn had no significant impact on the percentage of larvae parasitized by *Macrocentris grandii* Goidanich (Orr and Landis 1997, Venditti and Steffey 2003).

In contrast, applications of the pyrethroid insecticide significantly reduced the level of egg parasitism and permitted a greater percentage of chrysopid eggs to hatch.

This response could be partly attributed to lower numbers of host eggs in the treated plots if searching ability of the scelionid wasps was directly related to host density. Although the actual consequences of this effect would be beneficial, it does suggest that the conventional insecticide approach in sweet cornfields may have a greater non-target impact on parasitoids in general than the impact of Bt sweet corn.

Predation. Observations of sentinel egg masses of *O. nubilalis* allowed for discrimination of the amount of predation by both chewing and piercing-sucking insects active on the sweet corn plants. The majority of the eggs consumed on the Bt and non-Bt plants showed feeding signs of chewing predators. Coccinellids were the most likely cause of this predation, based on sightings of adults and larvae feeding on sentinel masses and the fact that coccinellids were consistently the most abundant chewing predator in field studies conducted at Upper Marlboro during the previous year. The smaller portion of the total predation attributed to piercing-sucking predators was probably caused by *O. insidiosis*, the most abundant plant-dwelling predator in previous field studies. All together, consumption of egg masses was not affected by their placement in Bt plots. These results confirm other field studies that found no significant effects from the Cry1Ab protein on several predators, including coccinellids and anthocorids (Orr and Landis 1997, Lozzia and Rigamonti 1998, Lozzia 1999, Lozzia *et al.* 1999, Pilcher *et al.* 1997, Zwahlen *et al.* 2000, Jasinski *et al.* 2003, Candolfi *et al.* 2003, Steffey *et al.* 2004, Sisterson *et al*. 2004).

However, exposure in plots of insecticide-treated hybrids significantly reduced the incidence of predation by more than 60%. Since the placement of egg masses occurred after the last insecticide application, this effect was probably not influenced by

density differences in the natural population of *O. nubilalis* egg masses, but more likely due to direct effects on the predators. In previous studies at the same location, anthocorids, coccinellids, and other predators were all adversely affected by the insecticide treatments, averaging 27 and 58% less overall in the Bt and non-Bt treated plots, respectively.

Recruitment. The emergence traps provided estimates of the number of arthropods emerging per unit of surface litter before, during and after the crop season. Results were biased toward those taxa that were mobile enough to find the funnel opening of the traps. Also, the initial captures during the first few days probably included adult insects that were active when the traps were placed over the litter. However, the majority of insects captured over the two-week period were newly-emerged recruits added to the litter community. Collembolans, psocids, and several families of flies and beetles with saprophagous feeding habits comprised the majority of the emerging community. Herbivores and predators accounted for an insignificant percentage of the total numbers.

Exposure in Bt plots, either via carryover transgenic plant litter or pollen that fell to the surface, had no effect on the overall community diversity and numbers of emerging arthropods. Any direct effect of Bt exposure on this community was unlikely due to the insensitivity of decomposers to the expressed Bt toxins. Furthermore, collembolans, psocids, flies, and fungivorous beetles feed primarily on fungal spores associated with degraded pollen deposits and litter organic matter. Exposure to Bt proteins via feeding on fungal spores is unlikely because studies have shown no toxin transfer to fungi growing on transgenic tissue (Donegan *et al.* 1995). Other studies using different sampling

techniques have shown that Bt plant material does not affect Collembola in the field (Sims and Martin 1997, Yu *et al*. 1997).

The diversity of arthropods emerging was highest before and after the crop season when the litter biomass was greater and moister, thus it probably provided a more favorable breeding site. Overall diversity and recruitment of all major taxa, except collembolans and psocids, declined in the insecticide-treated plots during the period when insecticides were applied. These effects were predictable based on the available literature on the non-target effects of pesticides (see reviews in Theiling and Croft 1988, Jepson 1989, Croft 1990,). Recruitment of collembolan and psocid populations was significantly enhanced in non-Bt plots requiring five insecticide applications. Collembolans and psocids are important diet items of many spiders, carabids, staphylinids, and mesostigmatid mites. Field studies conducted in the same plots during the previous year showed significant disruptions in populations of these predators following insecticide treatments. Negative effects on predators have been shown to generate a trophic cascade resulting in blooming of collembolans (Moore *et al*. 1984, Frampton *et al*. 1992, Czarnecki and Losinski 1995). Thus, it is concluded that the enhanced emergence of collembolans and psocids was due to disruption of their natural enemy populations.

Litter colonization. Changes in the colonizing densities of selected non-target arthropods in litter bags over time gave a direct bioassay assessment of the effects of exposure to plant material from the different hybrid treatments.
Collembola, oribatid mites, dipteran larvae, and fungivorous coleopterans comprised 90% of the litterbag community. The diversity of the colonizing community responded to seasonal changes in average temperatures during the nine months. Litter from the

different hybrid treatments had no effect on community diversity. Similar to the recruitment results, the density of colonizing springtails in litterbags placed in insecticide-treated non-Bt plots was numerically much higher than densities in the other treatments. This response agrees with the recruitment results and further confirmed that collembolan populations were enhanced by the pyrethroid treatments. Populations of oribatid mites, other decomposers, and predators extracted from the litter were not influenced by exposure to Bt or insecticide-treated litter. Laboratory and field studies thus far have shown no consistent adverse effects of Bt crops or Bt proteins on micro- and macro-organisms in related soil communities (Yu *et al.* 1997, Donegan *et al.* 1997, Sims and Martin 1997, Saxena and Stotzky 2001, Head *et al.* 2002, Al-deeb *et al.* 2003).

Litter decomposition. Previous studies determined that detectable amounts of Bt proteins persisted as long as the plant litter remains on the ground (Zwahlen *et al.* 2003). Thus, rates of decomposition could be affected if organisms that decompose litter are directly affected by Bt exposure or indirectly by content changes in the Bt litter itself. In this study, percentage dry matter loss significantly increased over time, losing 9.6 to 17.4% of the dry weight each month, except during the winter. However, the type of litter, or whether it came from an insecticide-treated or untreated plot, had no statistically significant effect on the rate of dry matter loss, although the highest loss occurred in Bt plots. These results disagree with the study by Flores *et al.* (2005) that showed a slower decomposition rate, lower amounts of carbon evolving as CO_2 ,and higher lignin content in Bt plant litter compared with non-Bt plant litter. Saxena and Stotzky (2001) reported evidence that Bt plant tissue may have higher levels of lignin which could result in slower decomposition.

Further research is needed to investigate whether Bt plant litter decomposes at a different rate. However, it is unclear if changes in litter decomposition rates will have positive or negative effects on the corn agroecosystem. An increase in persistence of plant litter may actually provide beneficial habitat for ground-dwelling organisms or may aid in reduction of soil erosion after harvest.

References Cited

Al-Deeb, M.A., G.E. Wilde and R.A. Higgins. 2001. No effect of Bacillus thuringiensis corn and Bacillus thuringiensis on the predator *Orius insidiosus* (Hemiptera : Anthocoridae). Environ. Entomol. 30: 625-629.

Andow, D.A. 1990. Characterization of predation on egg masses of *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 83: 482-486.

Betz, F.S., B.G. Hammond, and R.L. Fuchs. 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. Reg. Tox. Pharmacol. 32: 156-173.

Canard, M., Y. Semeria, and T. R. New. 1984. Biology of Chrysopidae. Dr. W. Junk Publishers, The Hague, The Netherlands. 294 pp.

Candolfi, M., K. Brown, and B. Reber. 2003. A faunistic approach to assess potential side-effects of genetically modified *Bt*-corn on non-target arthropods under field conditions. Environ. Biocontrol Science and Technology 14: 129-170.

Cheng W. and D.C. Coleman. 1990. Effect of living roots on soil organic matter decomposition. Soil Biol. Biochem. 22: 781-787.

Chilcut, C.F. and B.E. Tabashnik. 1999a. Simulation of integration of *Bacillus thuringiensis* and the parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae) for control of susceptible and resistant diamondback moth (Lepidoptera: Plutellidae). Environ. Entomol. 28: 505-512.

Chilcut, C.F. and B.E. Tabashnik. 1999b. Effects of *Bacillus thuringiensis* on adults of *Cotesia plutellae* (Hymenoptera: Braconidae), a parasitoid of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). Biocon. Sci. Tech. 9: 435-440.

Coll, M. and D.G. Bottrell. 1992. Mortality of European corn borer larvae by natural enemies in different corn microhabitats. Bio. Con. 2: 95-103.

Cortet, J., D. Gillon, R. Joffre, J. Ourcival and N. Poinsot-Balaguer. 2002. Effects of pesticides on organic matter recycling and microarthropods in a maize field: use and discussion of the litterbag methodology. Euro J Soil Bio. 38: 261-265.

Crecchio C. and G. Stotzky. 1998. Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subspecies *kurstaki* bound to humic acids in soil. Soil Biol. Biochem. 30: 463-470

Crossley,D.A and M.P. Hoglund. 1962. A litterbag method for the study of microarthropods inhabiting leaf litter. Ecology. 43: 571–573.

Czarnecki, A., and J. Losinski. 1995. The effect of GT seed dressing on the community of Collembola in soil under sugar beet. Pedobiologia 28: 427-31.

Donegan, K.K., C.J. Palm, V.J. Fieland, L.A. Porteous, L.M. Ganio, D.L. Schaller, L.Q Bucao, R.J. Seidler. 1995. Changes in levels, species and DNA fingerprints of soil-microorganisms associated with cotton expressing the *Bacillus thuringiensis* var *kurstaki* endotoxin. Appl. Soil Ecol. 2: 111-124.

Donegan, K.K, R.J. Seidler, V.J. Fieland, D.L. Schaller, C.J. Palm, L.M. Ganio, D.M. Cardwell, and Y. Steinberger. 1997. Decomposition of genetically engineered tobacco under field conditions: persistence of proteinase inhibitor I product and effects on soil microbial respiration and protozoa, nematode and microarthropod populations. J. Applied Ecol. 34: 767-777.

Dutton, A., H. Klein, J. Romeis and F. Bigler. 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. Ecol Entomol. 27: 441-447.

Flores, S., D. Saxena and G. Stotzky. 2005. Transgenic *Bt* plants decompose less in soil than non-*Bt* plants. Soil Biol. Biochem. In press.

Frampton, G. K., S. D. Langton, P. W. Greig-Smith, and A. R. Hardy. 1992. Changes in the soil fauna at Boxworth. In Pesticides, *Cereal Farming and the Environment: the Boxworth Project* (ed. P. Greig-Smith, G.K. Frampton, and T. Hardy), pp. 132-43. HMSO, London.

French, B.W., L.D. Chandler, M.M. Ellsbury, B.W. Fuller and M. West. 2004. Ground beetle (Coleoptera: Carabidae) assemblages in a transgenic corn-soybean cropping system. Environ. Entomol. 33: 554-563.

Griffiths B.S., Ritz K., Ebblewhite N., and G. Dobson. 1999. Soil microbial community structure: effects of substrate loading rates. Soil Biol. Biochem. 31: 145-153.

Groot, A.T. and M. Dicke. 2002. Insect-resistant transgenic plants in a mulit-trophic contect. Plant Journ. 31: 387-406.

Hagerty, A., S.G. Turnipseed and M.J. Sullivan. 2001. Impact of predaceous arthropods in cotton IPM. In Proceedings of the Beltwide Cotton Conferences, National Cotton Council, Memphis, TN, pp. 812-815.

Head, G., B. Freeman, W. Moar, J. Ruberson and S. Turnipseed. 2001. Natural enemy abundance in commercial Bollgard and conventional cotton fields. In Proceedings of the Beltwide Cotton Conferences, National Cotton Council, Memphis, TN, pp. 796-798.

Jasinski, J.R., J.B. Eisley, C.E. Young, J. Kovach and H. Willson. 2003. Select nontarget arthropod abundance in transgenic and nontransgenic field crops in Ohio. Environ. Entomol. 32: 407-413.

Jensen, L.S. and J. Soerensen. 1994. Microscale fumigation-extraction and substrate induced respiration methods for measuring microbial biomass in barley rhizosphere. Plant Soil. 162: 151-161

Jepson, P.C., B.A. Croft, and G.E. Pratt. 1994. Test systems to determine the ecological risks posed by toxin release from *Bacillus thuringiensis* genes in crop plants. Mol. Ecol. 3: 81-89.

Jepson, P.C. 1989. The temporal and spatial dynamics of pesticide side-effects on non-target invertebrates. Pesticides and non-target invertebrates. editor: Paul C. Jepson. Wimborne, Dorset, England : Intercept. pp. 95-127.

Johnson, M.T. 1992. Interaction of genetically engineered host plant resistance and natural enemies of *Heliothis virescens* (Lepidoptera: Noctuidae) in tobacco. Environ. Entomol. 21:586-597.

Johnson, M.T. 1997. Interaction of resistant plants and wasp parasitoids of tobacco budworm (Lepidoptera: Noctuidae). Environ. Entomol. 26: 207-214.

Koskella J. and J. Stotzky. 1997. Microbial utilization of free and clay-bound insecticidal toxins from *Bacillus thuringiensis* and their retention of insecticidal activity after incubation with microbes. Appl. Environ. Microbiol. 63: 3561-3568

Langrock, I., T. Hurley and K. Ostlie. 2003. Farmer Demand For Corn Rootworm Bt Corn: Do Insect Resistance Management Guidelines Matter? Annual Meeting of American Agricultural Economics Association 2003. http//agecon.lib.umn.edu/cgi-bin/pdf%5Fview.pl?paperid=9055.

Lozzia, G.C. & Rigamonti, I.E. 1998. Preliminary study on the effects of transgenic maize on non target species. IOBC Bulletin, 21, 171–180.

Lozzia, G.C. 1999. Biodiversity of ground beetle assemblages (Coleoptera: Carabidae) in Bt corn and its effects on non target insects. Bollentino di Zoologia agraria e di Bachicoltura. 31: 37-50.

MacIntosh, S.C., T.B. Stone, S.R. Sims, P.L. Hunst, J.T. Greenplate, P.G. Marrone, F.J. Perlak, D.A. Fischhoff, and R.L. Fuchs. 1990. Specificity and efficay of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invert. Path. 56: 258-266.

Manachini, B., M. Agosti and M. Rigamonti. 1999. Environmental impact of Bt-corn on non target entomofauna: synthesis of field and laboratory studies. XI Symposium Pesticide Chemistry.

Marra, M., G. Carlson, and B. Hubbell. 1998. Economic Impacts of the First Crop Biotechnologies. Electronic publication, North Carolina State University, Department of Agricultural and Resource Economics.
http://www.ag-econ.ncsu.edu/faculty/marra/FirstCrop/sld001.htm

Melin, B.E. and E.M. Cozzi. 1990. Safety to nontarget invertebrates of lepidopteran strains of *Bacillus thuringiensis* and their beta-exotoxins. In Safety of microbial insecticides.

Meharg A.A. 1994. A critical review of labeling techniques used to quantify rhizosphere carbon flow. Plant Soil. 166: 55-62.

Moore, J. C., R. J. Snider, and L. S. Robertson. 1984. Effects of different management practices on Collembola and Acari in corn production systems. I. The effects of no-tillage and atrazine. Pedobiologia 26: 143-152.

Naranjo, S.E. and P.C. Ellsworth. 2002. Arthropod communities and transgenic cotton in the western United States: implications for biological control. In Proceedings of the First International Symposium of Biological Control of Arthropods, U.S. Forest Service, Amherst, MA.

NCFAP. 2002. National Center for Food and Agricultural Policy. Leonard P. Gianessi, Cressida S. Silvers, Sujatha Sankula and Janet E. Carpenter. Plant Biotechnology: Current and Potential Impact for Improving Pest Management in U.S. Agriculture – An Analysis of 40 Case Studies.

Orr, D.B. and D.A. Landis. 1997. Oviposition of European corn borer (Lepidoptera: Pyralidae) and impact on natural enemy populations in transgenic versus isogenic corn. J. Econ. Entomol. 90: 905-909.

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. Environ. Entomol. 26: 446-454.

Russek-Cohen, E. and L.W. Douglas. 1999. Mixed models data analysis: using PROC MIXED for biological research. Mixed model shortcourse manual. P. 89-103. Jan. 11-14, 1999. Beltsville, MD.

SAS Institute. 1997. SAS user=s guide. SAS Institute, Raleigh, NC. 494 pp.

Saxena, D. and G. Stotzky. 2001. *Bt* corn has a higher lignin content. Am. J. Bot. 88: 1704-1706.

Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D.R. Zeigler, and D.H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Molec. Biol. Rev. 62: 775-806.

Schuler, T.H. , G.M. Poppy, B.R. Kerry, and I. Denholm. 1999. Potential side effects of insectresistant transgenic plants on arthropod natural enemies. Trends in Biotech. 17: 210-216.

Schuler, T.H., R.P.J. Potting, I. Denholm, S.J. Clark, A.J. Clark, C.N. Stewart and G.M. Poppy. 2003. 12: 351-361. Tritrophic choice experiments with Bt plants, the diamondback month (*Plutella xylostella*) and the parasitoid *Cotesia plutellae*. Transgen Res. 12: 351-361.

Shannon, C. E. and W. Weaver. 1949. The mathematical theory of communication. Urbana IL: University of Illinois Press.

Siedentop, S. 1995. A litterbag test for the assessment of side effects of pesticides on soil. mesofauna, Acta Zool. Fennica. 196: 357–360.

Sims, S.R. 1995. *Bacillus thuringiensis* var. *kurstaki* [CryIA (C)] protein expressed in transgenic cotton: effects on beneficial and other nontarget insects. Southwestern Entomol. 20: 493-500.

Sims, S.R. 1997. Host activity spectrum of the CryIIA *Bacillus thuringiensis* subsp. *kurstaki* protein. Effects on Lepidoptera, Diptera, and nontarget arthropods. Southwest. Entomol. 22: 395404

Sims, S.R. and J.W. Martin. 1997. Effect of the *Bacillus thuringiensis* insecticidal proteins on CryIA(b), CryIA(c), CryIIA, and CryIIIA on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola). Pediobiologia. 41: 412-416.

Sisterson, M.S., R.W. Biggs, C.O. Olson, Y. Carriere, T.J. Dennehy and B.E. Tabashnik. 2004. Arthopod abundance and diversity in Bt and non-Bt cotton fields. Environ. Entomol. 33: 921-929.

Steffey, K. L., M. Venditti, B. Ria Barrido, and A. S. Felsot. 2004. Effect of *Bacillus thuringiensis* corn on natural enemies of the European corn borer. Agricultural biotechnology: challenges and prospects: 139-150. American Chemical Society, Washington D. C.

Stotzky, G. 2000. Persistence and biological activity in soil of insecticidal proteins from Bacillus thuringiensis and of bacterial DNA bound on clays and humic acids. J. Environ. Qual. 29: 691-705.

Szentkiralyi, F. 2001. Ecology and habitat relationships. In: P. McEwen, T. New and A. E. Whittington (eds.), Lacewings in the Crop Environment, Cambridge University Press, Cambridge, UK.

Tapp, H. and G. Stotzky. 1998. Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. Soil Biol. Biochem. 30: 471-476.

Theiling, K.M. and B.A. Croft. 1988. Pesticide side effects on arthropod natural enemies. Agric. Ecosys. Environ. 21: 191-218.

Venditti, M.E. and K.L. Steffey. 2002. Field effects of Bt corn on the impact of parasitoids and pathogens on European corn borer in Illinois. In Proceedings of the First International Symposium of Biological Control of Arthropods, U.S. Forest Service, Amherst, MA.

Wilson and Fitt. 2002. Presentation at the National Biological Control Conference.

Wold, S.J., E.C. Burkness, W.D. Hutchison and R.C. Venette. 2001. In-field monitoring of beneficial insect populations in transgenic corn expressing *Bacillus thuringiensis* toxin. J. Entomol. Sci. 36: 177-187.

Yu, L., R.E. Berry, and B.A. Croft. 1997. Effects of *Bacillus thuringiensis* toxins in transgenic cotton and potato on *Folsomia candida* (Collembola: Isotomidae) and *Oppia nitens* (Acari: Orbatidae). J. Econ. Entomol. 90: 113-118.

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

Zwahlen, C., A. Hilbeck, P. Gugerli and W. Nentwig. 2003. Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. Mol. Ecol. 12: 765-775.

Table 2.1 Frequency of occurrence and abundance of arthropods emerging from the surface litter and colonizing litterbags after harvest at Upper Marlboro, MD. Taxa are listed by ecological function in order of overall abundance. Means were computed by pooling data over all sampling dates and treatments.

		Emergence traps		Litterbags	
Common name	Taxonomic group	Mean density per 0.4 m^2	Percentage occurrence	Mean density per 0.4 m^2	Percentage occurrence
		Decomposers			
Springtails	Collembola	530.75	95.3	2099.23	44.7
Mites	Oribatida	-	-	256.83	43.8
Fly larvae	Diptera	-	-	66.20	38.8
Fungus beetle larvae	Coleoptera	-	-	46.90	37.5
Humpbacked flies	Phoridae	37.95	97.8	-	-
Darkwinged fungus flies	Sciaridae	29.60	99.4	-	-
Frit flies	Chloropidae	23.73	99.4	3.90	10.9
Fungus beetles	Coleoptera	5.72	82.2	23.94	42.5
Picture winged flies	Otitidae	18.98	91.3	-	-
Psocids	Psocoptera	11.52	80.9	3.80	23.1
Minute scavenger flies	Scatopsidae	9.45	89.1	-	-
Gall gnats	Cecidomyiidae	0.17	2.5	-	-
Sap beetles	Nitidulidae	5.31	80.6	*	*
Fungus gnats	Mycetophilidae	0.74	21.6	-	-
Small dung fly	Sphaeroceridae	0.89	35.0	-	-
Sowbugs	Isopoda	-	-	0.33	13.8
Midges	Chironomidae	0.20	13.1	-	-
Frit flies	Chloropidae	0.14	8.4	-	-
Crickets	Gryllidae	0.03	2.8	0.13	3.1
		Herbivores			
Blotch leaf miners	Agromyiidae	1.88	8.1	-	-
Thrips	Thripidae	17.69	83.8	0.19	10.9
Plant bugs	Miridae	3.02	25.3	0.98	7.8
Flea beetles	Chrysomelidae	5.72	71.9	0.02	1.9
Hoppers	Orthoptera	1.55	45.6	0.02	1.9
Caterpillars	Lepidoptera	0.80	25.3	0.84	8.1
Aphids	Aphididae	0.22	8.8	0.20	9.4
Cucumber beetles	Chrysomelidae	0.35	19.4	-	-
Click beetles	Elateridae	0.22	14.7	0.17	9.1
		Parasitoids			
Scelionid wasps	Scelionidae	15.46	93.1	-	-
Parasitic wasps	Hymenoptera	-	-	6.61	35.9
Encyrtid wasps	Encyrtidae	0.82	11.3	-	-
Chalcidoid wasps	Chalcidoidea	0.53	11.3	-	-
Fairyflies	Mymaridae	2.28	48.8	-	-

Charipid wasps	Charipidae	2.75	69.1	-	-
Braconid wasps	Braconidae	0.45	13.4	-	-
Diapriid wasps	Diapriidae	0.72	21.6	-	-
Ceraphronid wasps	Ceraphronidae	0.54	25.0	-	-
Eulophid wasps	Eulophidae	0.09	5.0	-	-
Trichogrammatid wasps	Trichogrammatidae	0.27	15.9	-	-
Pteromalid wasps	Pteromalidae	0.09	5.9	-	-
Tachinid flies	Tachinidae	0.01	0.9	-	-
Spider wasps	Pompilidae	0.14	12.8	-	-
Ichneumonid wasps	Ichneumonidae	0.02	1.9	-	-
		Predators			
Predaceous mites	Mesostigmata	-	-	150.12	40.3
Spiders	Aranea	2.77	80.0	9.62	42.8
Rove beetles	Staphylinidae	0.77	30.3	4.92	38.4
Ground beetles	Carabidae	-	-	3.76	26.9
Ants	Formicidae	0.61	36.9	7.12	28.1
Predatory bugs	Hemiptera	0.49	29.1	2.62	23.4
Thrips	Phlaeothripidae	1.14	21.9	0.02	1.3
Long legged flies	Dolichopodidae	0.31	17.2	-	-
Lady beetles	Coccinellidae	0.11	7.5	0.18	3.4
Soldier beetles	Cantharidae	-	-	0.12	4.7
Hister beetles	Histeridae	-	-	0.03	2.8

Percentage occurrence is based on a sampling unit of four emergence traps and two litterbags per plot.

- Not recorded.

* Nitidulids were combined with other fungivorous beetles in litter bag data.

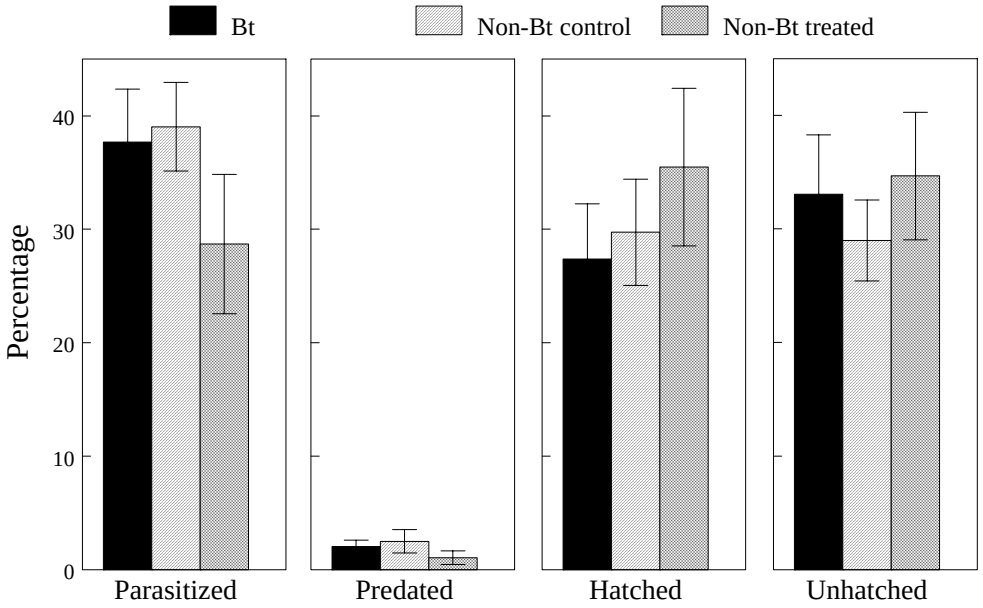

Figure 2.1. The fate of chrysopid eggs expressed as the percentage parasitized, predated, hatched and unhatched. Data were pooled over two sampling periods approximately one week after peak anthesis in 2002 and 2003. Upper Marlboro, MD.

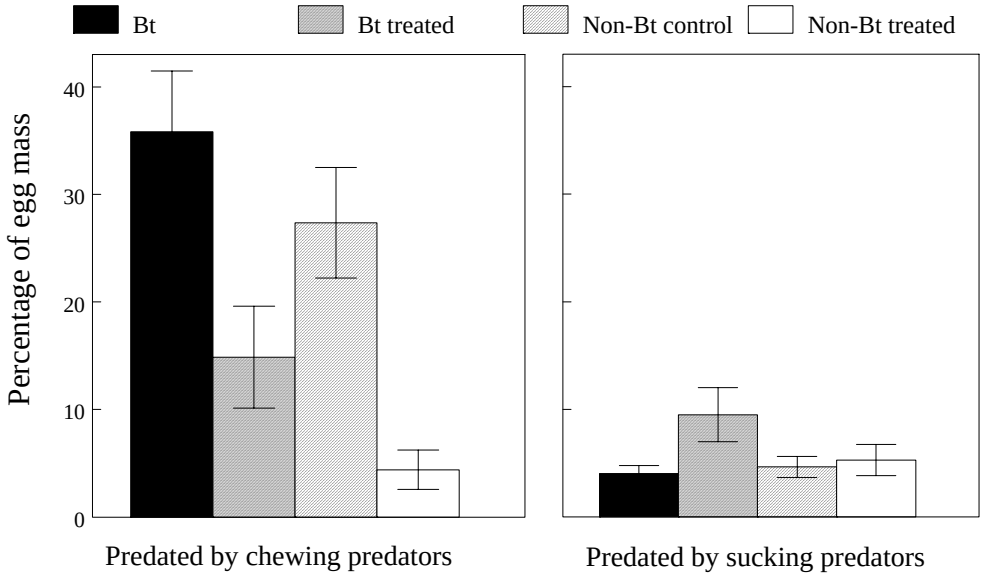

Figure 2.2 Percentage portion of *O. nubilalis* egg masses consumed by chewing and piercing-sucking predators in Bt, non-Bt insecticide-treated, and non-Bt control plots in sweet corn. Data were averaged over six sampling dates in 2002 and 2003. Upper Marlboro, MD.

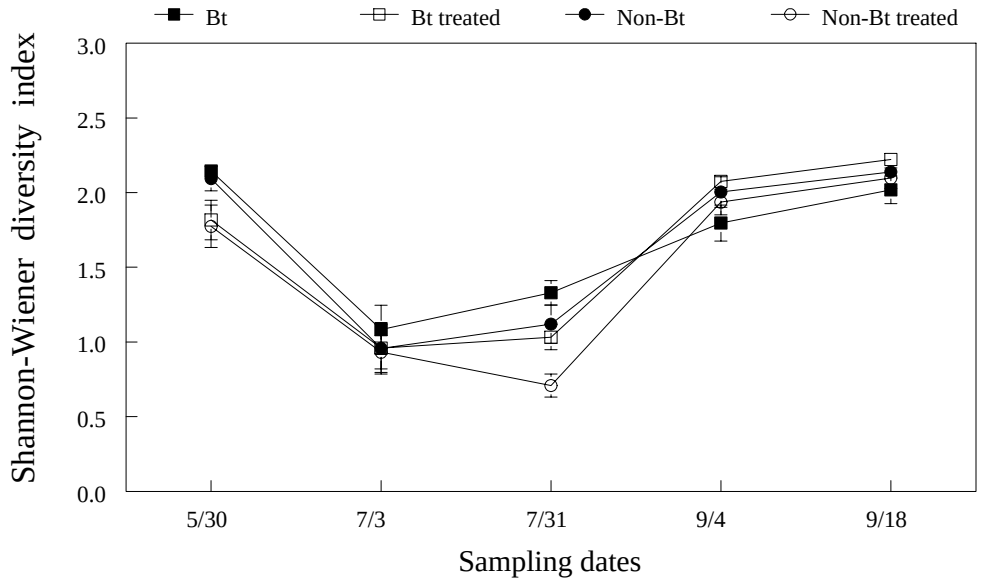

Figure 2.3. Mean Shannon-Wiener diversity index of the arthropod community emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002.

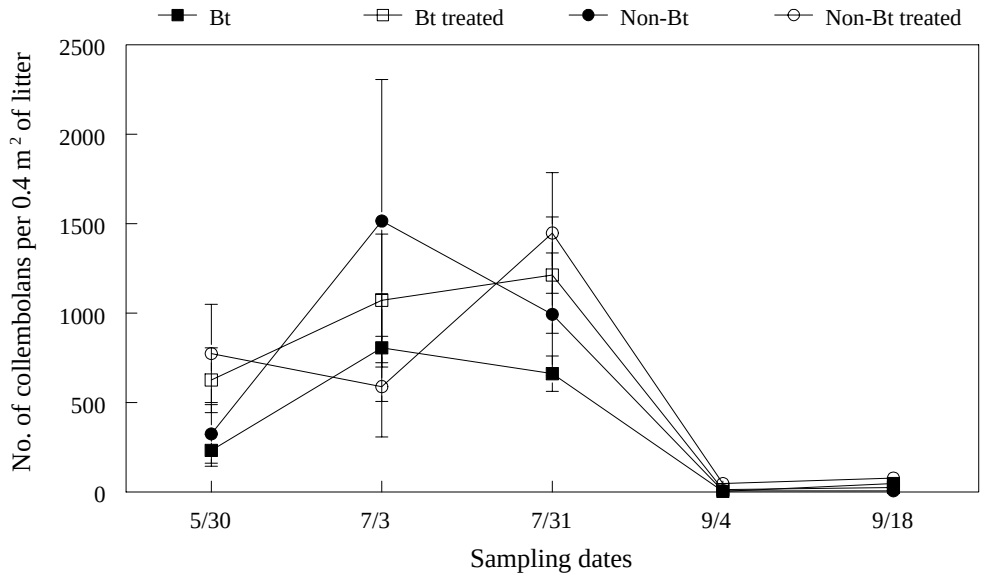

Figure 2.4. Mean number of Collembola emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002.

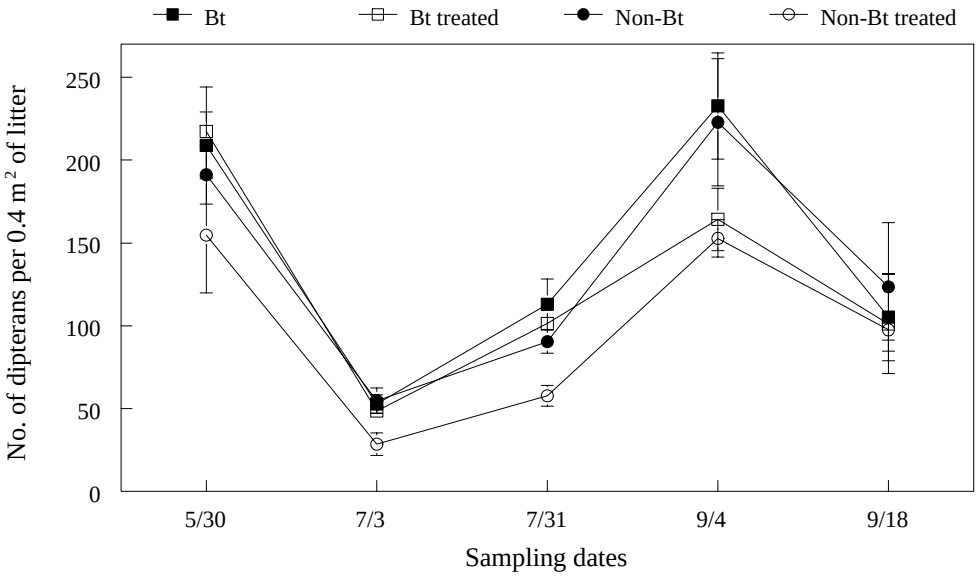

Figure 2.5. Mean number of Diptera emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002.

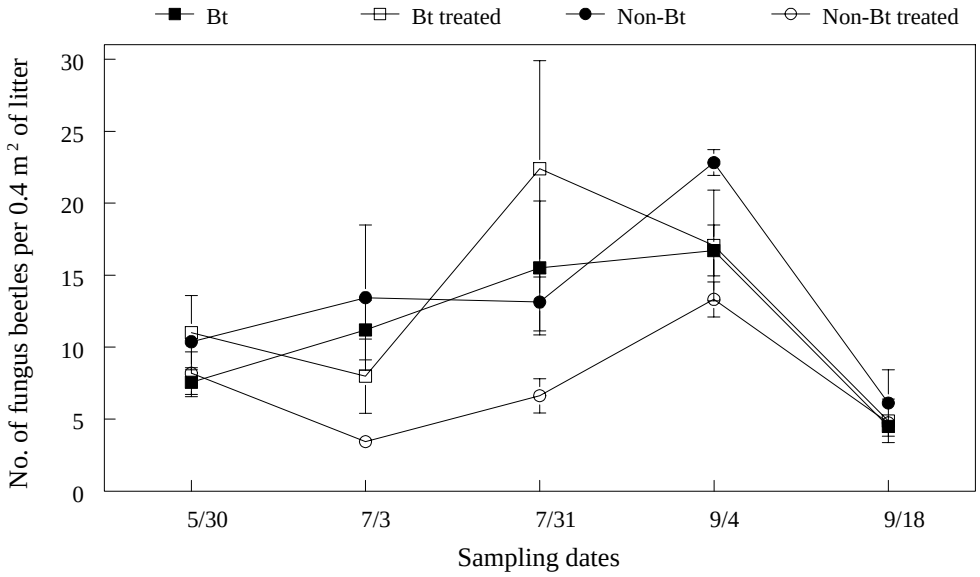

Figure 2.6. Mean number of fungivorous Coleoptera emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002.

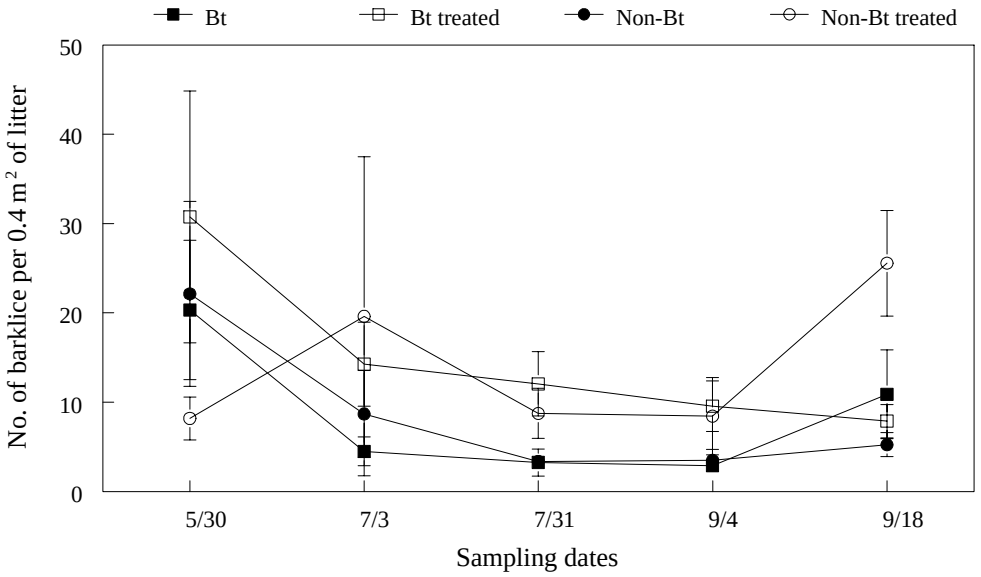

Figure 2.7. Mean number of Psocoptera emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002.

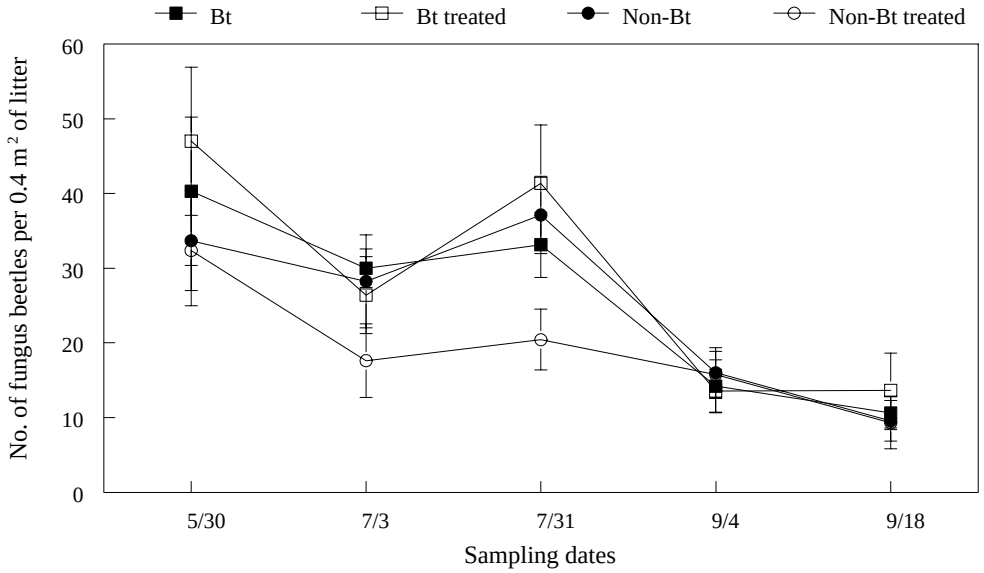

Figure 2.8. Mean number of parasitic Hymenoptera emerging from surface litter prior to, during, and after the crop season in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. 2002.

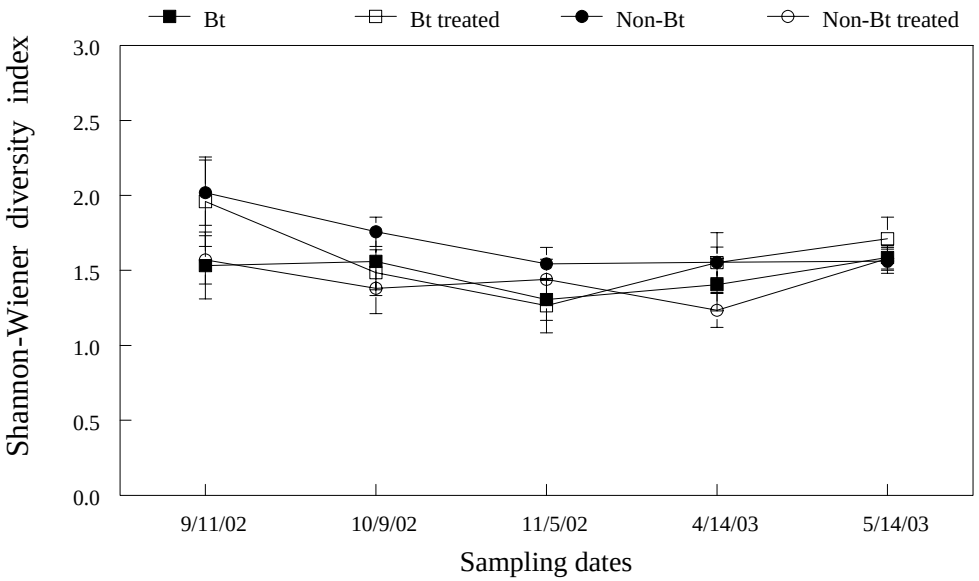

Figure 2.9. Mean Shannon-Wiener diversity index of the arthropod community colonizing litterbags at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002.

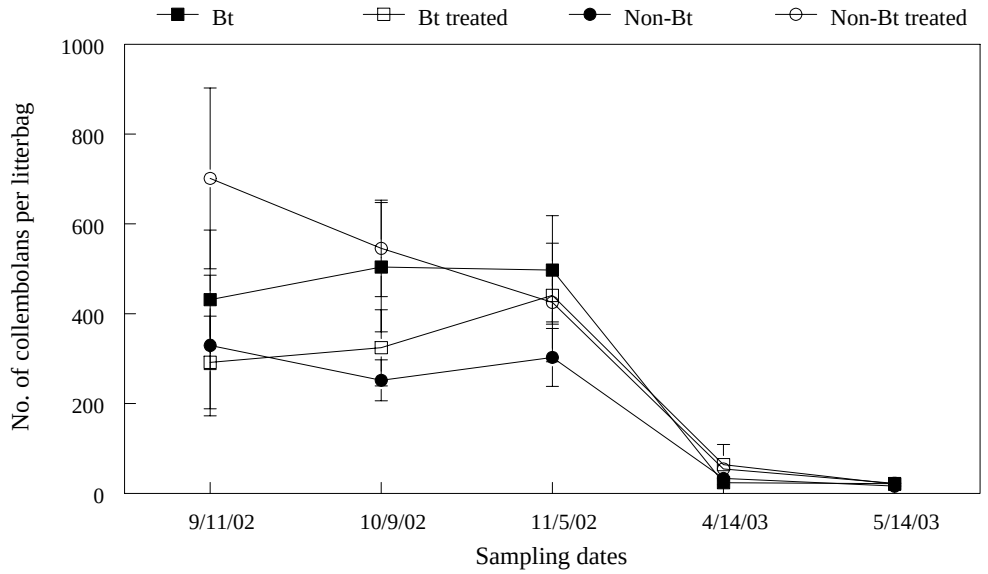

Figure 2.10. Mean number of Collembola colonizing litterbags at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002.

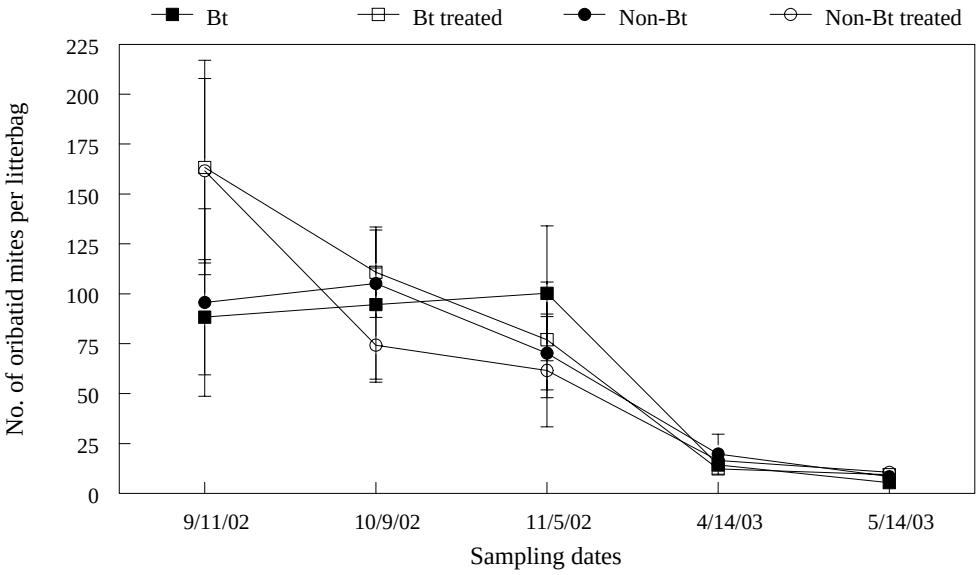

Figure 2.11. Mean number of Oribatid mites colonizing litterbags at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002.

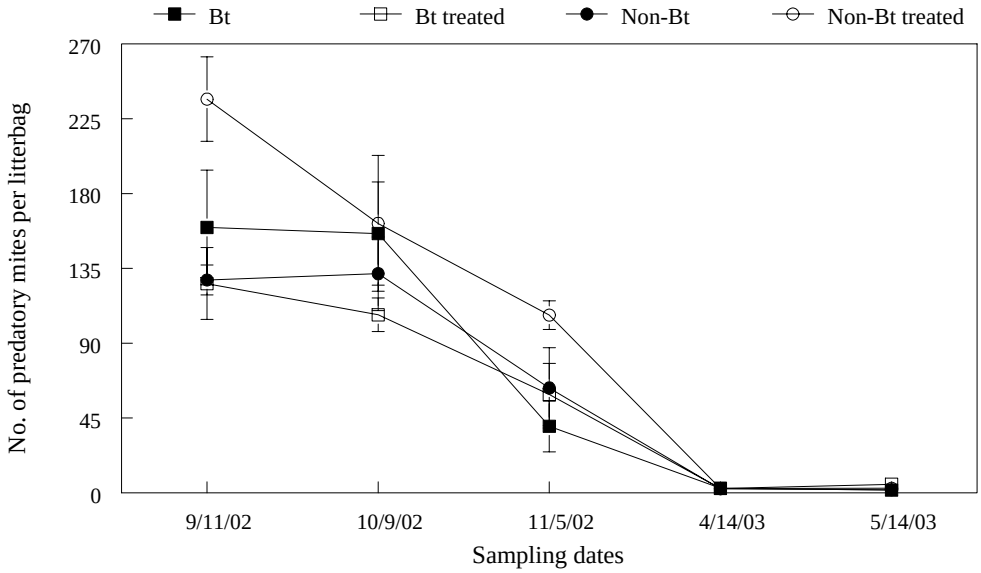

Figure 2.12. Mean number of predatory mites colonizing litterbags at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002.

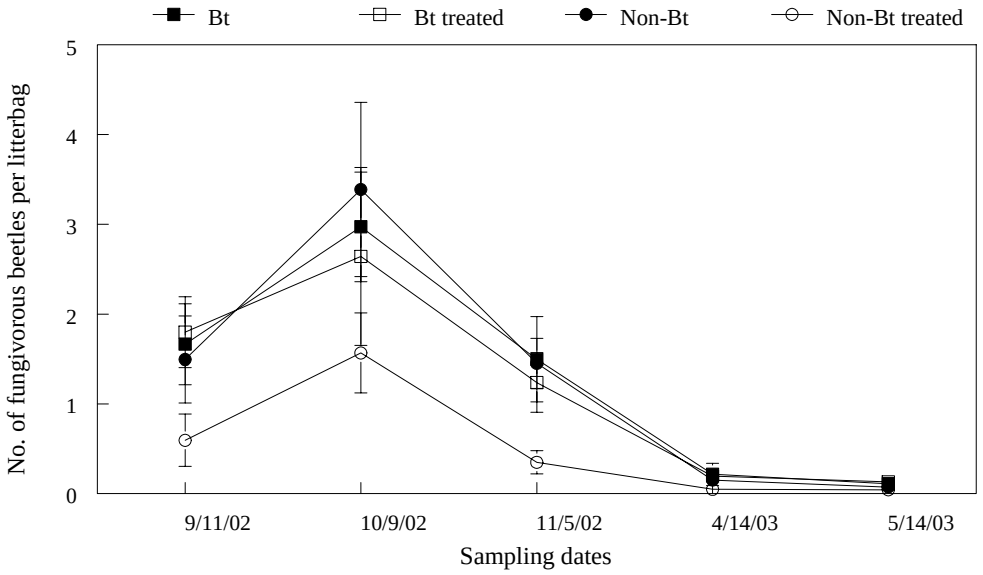

Figure 2.13. Mean number of fungivorous beetles colonizing litterbags at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002.

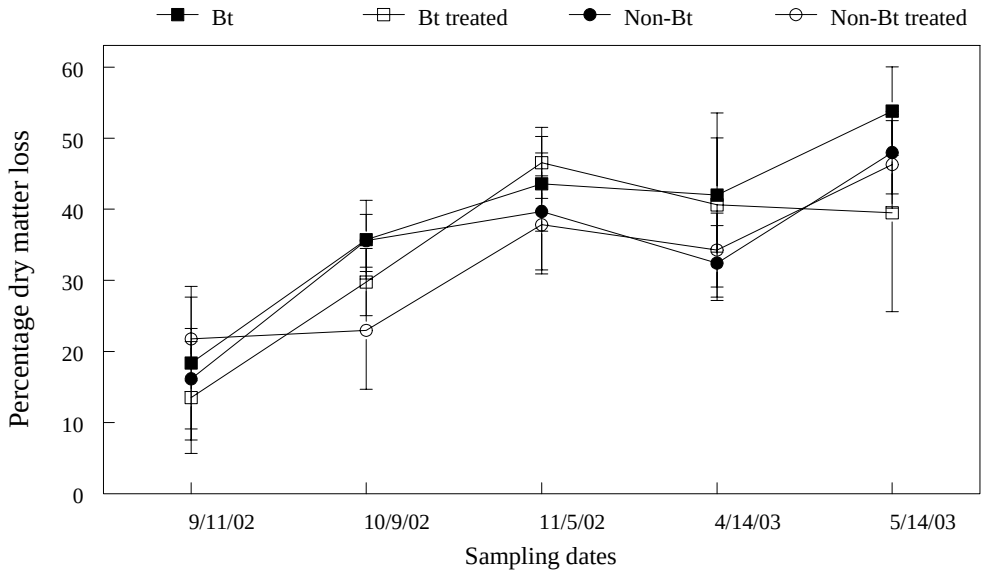

Figure 2.14. Percentage dry matter loss of Bt and non-Bt sweet corn plant material in litter bags removed at various times after placement in Bt, insecticide-treated, and non-Bt control plots at Upper Marlboro, Maryland. Bags were placed flat on the ground surface on 16 August 2002.

CHAPTER III

Effects of Bt Corn Pollen on Honey Bees with Emphasis on Protocol Development

Abstract

Corn hybrids containing genes from the soil bacterium *Bacillus thuringiensis* (Bt) have been developed to express insecticidal proteins for insect control purposes. Exposure to pollen produced by these plants may have direct and indirect effects on honey bees. Laboratory and field studies were conducted to assess the effects of Bt pollen on worker survival, brood development and foraging behavior of bees feeding on Bt corn pollen. Results were used to develop test protocols and levels of replication required to detect a 50% effect size with 80% statistical power. Laboratory results showed that survival and head weight gain of newly-emerged bees (less than 24 hrs old) were not significantly affected when fed Bt pollen for 21 days. These endpoints were the most appropriate for laboratory toxicity testing, and the latter can be used as an indirect measure of hypopharyngeal gland development.

Field studies to assess effects of Bt pollen on honey bees involved small hives placed in replicated Bt and non-Bt sweet corn plots at several locations and repeated over two years. Bees were allowed to forage for 28 days during pollen shed, during which hives were provisioned with pollen cakes to increase exposure to the Bt proteins. During the exposure period, colonies in the Bt, non-Bt, and positive control plots consumed an average of 7.9 g of pollen per day, which amounted to approximately 44% of the daily pollen requirements of a small hive. Positive control plots with bee colonies exposed to insecticides were also included. Results from both years showed no significant effects on

bee foraging behavior, survival, weight or hive performance. Foraging bee weight, number of foragers returning with pollen loads, pollen load weight and brood size were the most appropriate endpoints to indicate effects on the general fitness of colonies. Number of bees returning with pollen loads was a more reliable indicator than the total number of returning foragers. Based on the replication used in this study, a non-target effect equal to a 40% reduction in the foraging parameters can be detectable with 80% power. Since most variation in foraging activity was due to between plot differences rather than hives within plots, increasing the number of replicate plots achieved more statistical power than adding more hives per plot. This study identified several experimental design elements, sensitive endpoints, and factors to consider in planning short-term field studies with open, functional hives to evaluate the non-target effects of transgenic pollen.

Introduction

Many insecticides used in agriculture have significant adverse effects on honey bees, *Apis mellifera* L. (Johansen 1977, Johansen and Mayer 1990). For this reason, toxicity testing to assess the potential hazards to honey bees is a routine part of the pesticide regulatory process by the U.S. Environmental Protection Agency (40 CFR §158.74(d) 2003). The Cry1Ab endotoxin from the soil bacterium *Bacillus thuringiensis* (Bt) formulated in microbial insecticides or expressed in Bt transgenic crops meets EPA's reduced risk status, partly due to the lack of acute toxicity to honey bees. Numerous laboratory tests of microbial insecticides containing Bt spores and crystals have shown that the endotoxins pose no risk to honey bees (Flexner *et al.* 1986, EPA 1998).

Studies to measure acute mortality effects have typically involved feeding or exposing the toxin directly to adult or larval bees (EPA 1996a, EPA 1996b). For microbial insecticides, experimental protocols that simulate exposure of foliar-applied endotoxin residues to potentially vulnerable life stages may be sufficient to characterize toxicity and potential hazards to honey bees. However, the Cry1Ab endotoxin expressed in Bt corn is delivered constitutively within the tissues of the plant; thus, the only potential exposure to honey bees is by pollen ingestion. Laboratory studies to assess potential non-target effects of Bt corn have involved feeding Bt pollen or purified endotoxin mixed with honey or sugar syrup directly to the larvae (Arpaia 1996, Malone *et al* 1999, Hanley *et al.* 2003). These studies and others have indicated no adverse effects, however, the method of endotoxin delivery may be inappropriate because pollen is processed and fed to honey bee larvae in a different way (Hilbeck *et al.* 2000). Specifically, newly-emerged bees (less than 24 hrs old) consume pollen during the first two weeks of their life and then switch to honey as the main food (Haydak 1970). Pollen is digested by nurse bees, processed with secretions from the hypopharyngeal glands, and then fed as royal jelly or brood food to larvae during early stages of their development (Sammataro and Avitabile 1986, Crailsheim 1992). Older larvae are fed a mixture of royal jelly and honey, with increasing amounts of pollen.

Exposure to Bt pollen could have both direct and indirect non-target effects on brood development in a honey bee colony. Direct ingestion by bee larvae is the primary route of exposure (Babendreier *et al.* 2004) but laboratory studies to support registration of Bt corn have not tested the processed pollen in brood food. For adult bees, the presence of Cry1Ab proteins in ingested pollen may also affect hypopharyngeal gland

development and thus the ability of nurse bees to make brood food and function later as worker bees. Orientation, foraging, and communication are all important behavioral activities of worker bees in finding and relocating food sources (Winston 1987). These potential indirect effects on nurse bee development and on subsequent worker foraging behavior have not been addressed in current laboratory protocols. Reliable scientific methods that evaluate the risks to honey bees posed by plant-incorporated protectants (PIPs) are needed as part of the regulatory process (Stark *et al.* 1995, Hilbeck *et al.* 2000). Thus, reported here are results of both laboratory and field studies to develop appropriate test procedures and to assess the effects of Bt pollen on honey bee survival, brood development and foraging activity. Sweet corn was chosen to represent worst-case exposure conditions because it produces greater amounts of pollen and is more attractive to honey bees than field corn (Schur *et al.* 2000, Somerville 2001).

Materials and methods

Laboratory studies No-choice laboratory studies were conducted in 2001 and 2002 to measure effects of corn pollen on development and survival of adult honey bees. In both years, cohorts of newly-emerged bees were fed a diet of Bt pollen from sweet corn hybrid Attribute GSS0966 (Syngenta Seeds, Golden Valley, MN; event Bt11) and non-Bt pollen from an isoline (Syngenta Seeds; Prime Plus). Corn pollen was collected from 0.4 ha plots of Bt and non-Bt sweet corn grown at the Central Maryland Research and Education facilities at Beltsville and Upper Marlboro, Maryland. Pollen was collected by shaking plant tassels over large plastic trays at peak anthesis. Collections were made during early morning after the dew dried but before pollen shed was complete. Pollen was sieved to remove anthers and stored at -80° C until initiation of the

study. In 2001, bees were also fed mixed pollen that was naturally harvested by bee colonies in Beltsville, MD. Pollen traps were used to collect mixed pollen during the spring of 2001 prior to corn anthesis.

In both years, small wooden cages (11 cm x 9 cm x 7 cm) with a sliding glass front and wire mesh bottom were used to house bees during the study. In 2001, three cohorts of 60 newly emerged bees that were less than 24 h old were randomly selected from brood combs removed from each of six source colonies and held overnight in an incubator at 34°C. A cohort was introduced into each cage on day 0. Each pollen diet (Bt, non-Bt, mixed) was fed to six replicate cages representing one of each colony source. Two grams of pollen in an artificial plastic comb were placed in each cage on days 0, 5 and 10. Bees were provided continual access to a sugar syrup and water source. On days 5 and 10, a section of brood comb containing 10 eggs from the same source colony was introduced into each cage in 2001. Small plastic lures with queen pheromone (Bee Boost, Pherotech Inc, Delta B.C., Canada) were placed in cages with brood to simulate the presence of a queen and help promote brood care. The cages were kept in an incubator at 34°C, 50% RH, and 24 h of dark in both years. The screened floor of each cage allowed counting and removal of dead bees to monitor mortality daily. Combs containing eggs added to cages were removed on days 25 and 35 and examined for brood development. The amount of pollen consumed was determined on days 5, 10 and 26 for each treatment cage by weighing pollen before and after its introduction.

A measurement of bee head weight was used as an indicator of hypopharyngeal gland development, according to the method by Hrassnig and Crailsheim (1998). Samples of ten bees were randomly collected from each treatment cage on day 10, placed

in vials, and stored in a freezer until weighed. To serve as controls, ten newly emerged bees were randomly collected from each source colony on day 0 and frozen for later processing. At the same time, cohorts of greater than 30 newly emerged bees were marked with Testors 7 enamel paint and returned to each respective colony. Ten marked bees and ten unmarked bees of mixed ages were randomly collected and stored on day 10 from each source colony. All bees were thawed later and their heads removed and weighed individually.

The no-choice study was repeated in 2002 with pollen types mixed with honey and provisioned as pollen cakes to increase pollen consumption of exposed bees. The rearing procedures were the same as those described in the 2001 study, except that each cage contained a cohort of 50 newly emerged honey bees. Bees were evenly allocated from ten source colonies to establish three sets of 10 replicate cages. The pollen diets assigned to each set included Bt pollen, non-Bt pollen and non-Bt pollen treated with 2 ppm of imidacloprid (Admire 2F, Bayer CropSciences, Research Triangle, NC). The insecticide-treated pollen served as a positive control to determine whether differences in the measured endpoint could be detected. Pollen cakes of 4 parts pollen and 1 part honey by weight were prepared. One 5 g-cake was provided to each cage on days 0, 7 and 14, which allowed *ad libitum* access to food for bees. Pollen was removed on day 21 and bees were allowed to continue development. Mortality was monitored daily by counting the number of dead bees. The amount of food consumed was determined on days 7, 14 and 21 for each test cage by weighing pollen cakes before and after introduction.

Field studies Field-plot experiments were conducted in 2001 and 2002 to compare survival, brood development and foraging behavior in honey bee colonies

exposed to Bt and non-Bt sweet corn. In both years, four replicate plots of Bt hybrid Attribute GSS0966 (Syngenta Seeds) and its non-Bt isoline Prime Plus were planted at three Maryland Research and Education facilities. One replicate pair was located at each of the Upper Marlboro and Salisbury facilities, while two replicates were located at the Beltsville facility. Each plot measured 65 m x 65 m (0.4 ha) and was situated at least 300 m apart from each other. In addition, two positive control plots (one at each of the Upper Marlboro and Salibury facilities) included non-Bt sweet corn with bee colonies exposed to insecticides.

In both years, colonies were established in the spring from new queens and workers in nucleus hives, each containing five frames. Packages containing 900 g of bees (approximately 10,000) and a queen were obtained from a commercial supplier (Wilbanks Apiaries; Claxton, GA, USA). All queens originated from the same breeding line to ensure uniform genetic makeup of bees among the treatment groups. Colonies were maintained at an isolated location at the Salisbury facility for 5 - 6 wks before they were placed into field plots. Depending on available pollen sources and weather conditions, combs stocked with food were removed and new foundation frames added to prevent colonies from swarming before hives were transferred to treatment plots. Two weeks prior to anthesis, hives were opened to visually assess the brood size, food stores, and bee strength. If necessary, frames were removed from one hive and exchanged with another to equalize the bee and brood densities. After equalizing, each hive was labeled with a unique number and the sides of each comb were numbered and permanently marked 1-10 on the wood frame in full view.

Approximately 7 days prior to anthesis, three colonies were randomly selected and placed in a cleared area at the center of each plot. In 2001, each plot included a blank hive with the queen, drones and worker bees but brood frames were removed and replaced with foundation frames. In 2002, blank hives were not included. Each hive was placed in the field with one foundation frame to allow for brood expansion. Hives were positioned on wooded pallets over a 3 m x 4 m tarp, which provided a clear uniform surface for counting dead bees. Hives were placed separately on individual tarps in 2001, but placed side by side on one tarp in 2000. The entrance of the middle hive faced the opposite direction from the end hives. During the evening of the same day, each hive was opened and frames were individually photographed on both sides to obtain images of bees covering the comb. Bees were then dislodged into the hive box and each frame photographed a second time to obtain a close-up digital image of brood, pollen, and honey cells. After pre-exposure images were taken, frames were returned to the original hives.

In both years, colonies were allowed to forage freely within and outside each plot for at least 4 weeks, which overlapped the period of anthesis. In 2001, positive control plots were treated with carbaryl (Sevin® XLR Plus, Bayer CropSciences) at the rate of 0.56 kg per ha (1/4 the normal AI rate). An airblast sprayer delivered 470 liters of diluted spray per ha three times during the exposure period. In 2002, colonies were provisioned with cakes of pollen collected from plants within the plot to increase the level of exposure. Pollen was collected and processed as described above for the laboratory studies. Pollen cakes consisting of 2 parts pollen, 2 parts soy flour, 2 parts honey, and 1 part sugar were weighed and divided into portions of 50 g each. Four cakes were placed

directly on the top of the frames in each hive once or twice weekly to allow *ad libitum* access for bees. Records were kept on the amount of pollen cakes consumed by each hive. As an alternative to foliar-applied insecticide to stress bees, positive control hives in 2002 were provisioned with pollen cakes containing either 10, 100 or 1,000 ppb of imidacloprid (Admire 2F, Bayer CropSciences). Each concentration was assigned to one of the three hives in each control plot. Concentrations were based on studies conducted to assess the impact of imidacloprid on honey bee behavior in France (Schmuck 1999).

Bee mortality was estimated three times weekly during the exposure period by counting and removing the number of dead bees found on the tarp surrounding hives. Observations of each hive were made on the same days to record the number of foraging bees returning to the hive. Counts of bees with and without pollen pellets entering the hive entrance were tallied over a 5-minute period in the morning between the hrs of 0900 and 1100. Each week, weights of foraging bees and their pollen loads were measured from a sample of 10 bees returning to each hive. At the end of the exposure period, combs of each hive were photographed as described above to obtain post-exposure measurements of bee strength, brood development, and stores of food.

Pre- and post-exposure images were displayed on a computer monitor with a grid overlay to measure the percentage of each frame covered with bees, capped brood, pollen and honey cells. The strength of the colony was derived by visually estimating the percentage of combs covered with bees to the nearest 10%. A representative set of grid sections ranging from 10 to 100% covered with bees was displayed at higher resolution to count the actual number of bees. This data set was used to compute a linear regression function to estimate the total number of bees per hive from the average percentage of

combs covered with bees. Food stores were expressed as percentages of honey and pollen cells on each comb. Brood development was expressed as percentage of capped brood cells on each comb.

Statistical Analysis All data sets were tested before analysis for normality and homogeneity of variances using Spearman Rank Correlation and Shapiro-Wilk tests. For data not meeting the assumptions of ANOVA, an appropriate transformation was used or variances were grouped prior to analysis (Russek-Cohen and Douglas 1999). Field data were analyzed either as individual observations or as an average of weekly observations. The PROC MIXED procedure of ANOVA (SAS Institute 1996) was used to test for main and interaction effects for each variable. In 2001, the two hives that began with brood in each replicate plot were averaged as an experimental unit, while data on hives starting with foundation frames were analyzed separately. The mean of the three hives per replicate plot in 2002 were analyzed as the experimental unit, including the hives exposed to different doses of imidacloprid. The repeated measures option was used for time series data to correct for intercorrelation between sampling dates. Means were separated following a significant F test by using the Tukey's multiple comparison adjustment ($P < 0.05$). Contrast tests were also performed to compare specific pollen diets individually or grouped.

A variance component analysis (PROC MIXED option CL) was conducted on specific endpoints to estimate the variance at each level of the experimental design. A retroactive power analysis using PASS 2000 (Hintze 2001) was then performed using variance data to determine the number of replicates and subsamples (bees per cage, hives per plot) required to detect a range of effect sizes at 80% statistical power. An 80%

probability of rejecting a false null hypothesis (Type II error) was chosen as the accepted level of power for non-target risk assessment studies (Candofi *et al.* 2000).

Results

2001 Laboratory study Overall survival of bees after feeding for 35 days averaged 47.2% and was not significantly affected by the pollen diets (Fig. 3.1). Of the treatment groups, only three of the six mixed-pollen cages showed evidence of rearing brood. However, sixteen brood cells of 120 possible were capped but only a few adults emerged due to chalk brood (*Ascosphaera apis*), a common fungal pathogen of bees. Furthermore, the number of larvae maintained by the tending bees was too small to analyze for treatment effects. The interaction effect for the amount of pollen consumed per bee was significant ($F = 5.17$; df = 4, 30; $p = 0.003$), indicating that differences among pollen diets were not the same for each time period (Fig. 3.1). Adult bees consumed significantly more mixed pollen than corn pollen but this preference declined significantly as the bees aged, probably because the older bees utilized less pollen in their diet (Crailsheim *et al.* 1992). Moreover, the consumption of Bt pollen was significantly higher than the consumption of non-Bt pollen during the first five days ($p < 0.038$) (Fig. 1).

The body weight of bees was significantly affected by age and pollen source ($F = 5.09$; df = 5, 25; $p = .002$). Bees fed non-Bt corn pollen in the laboratory for 10 days ate less pollen and were significantly smaller than bees fed mixed pollen. Also, bees fed for 10 days on either Bt corn or mixed pollen were significantly larger than newly emerged bees collected on day 0. An increase in body weight indicated that some pollen was ingested during exposure. Bee head weights were also significantly different indicating

that the age of bees or the food that they consumed had an effect on hypopharyngeal glands ($F = 9.36$; df = 5, 25; $p < .0001$) (Fig. 3.2). Heads of bees fed for 10 days on mixed pollen either in treatment cages or source colonies were significantly heavier than the heads of newly emerged bees on day 0, which should have no glandular development. Head weights of bees fed mixed pollen after 10 days in cages were statistically the same as bees reared in the source colonies, indicating that the mixed pollen diet and laboratory conditions in cages were sufficient to sustain bees comparable to those reared in hives. Results of an analysis including only data from the treatment cages showed significant differences among pollen diets ($F = 10.87$; df = 1, 10; $p = .003$). Bees fed Bt corn pollen resulted in heavier heads than bees fed non-Bt pollen but were not different from those fed mixed pollen.

2002 Laboratory study Survivorship curves were different among pollen diets as indicated by a significant treatment by time effect ($F = 1.92$; df = 40, 460; $p < .001$) and overall treatment effect ($F = 4.67$; df = 2, 23; $p < .02$). Survival of bees fed imidacloprid-treated pollen cakes was significantly reduced, particularly during the first five days, but there was no difference in the number of bees surviving in the Bt and non-Bt pollen groups (Fig. 3.3). When cake consumption was adjusted for the proportion of pollen, the overall amount of pollen ingested per bee was higher than the amount consumed by bees in the 2001 study, indicating that the addition of honey significantly enhanced exposure under the test conditions. Differences in pollen consumption among treatments were consistent over time and statistically significant among pollen types ($F = 10.43$, df = 2, 15.9; $p = .001$). More non-Bt than Bt pollen was consumed per surviving bee but there was no difference in consumption between the non-Bt and imidacloprid-

exposed bees (Fig. 3.2). This result differed from the 2001 study that indicated greater Bt than non-Bt pollen consumption.

Foraging activity Overall mean numbers of foragers returning to hives exposed in Bt, non-Bt, and non-Bt insecticide treated plots were 146.4, 167.1, and 114.7 bees per 5 min, respectively. Main and interaction effects between non-Bt and Bt treatments for foraging activity and the proportion of bees carrying pollen loads were not significant. The main effect for time was significant ($F = 5.42$; $df = 5, 39$; $p < .001$), indicating higher foraging activity at earlier sampling times. Contrast tests revealed that significantly fewer bees were foraging in the positive control plots with hives exposed to carbaryl ($F = 4.15$; $df = 1, 39$; $p = .048$). This was particularly evident in hives initially stocked with only foundation frames, where a greater number of workers were probably tending the queen and rearing brood (Fig 3.4).

In 2002, exposure of bees to pollen within the sweet corn plots and directly to pollen cakes had no significant effect on foraging activity and the percentage of bees loaded with pollen (Fig 3.5). Noteworthy is the fact that bees from the imidacloprid-exposed hives were foraging at about the same level of activity but were numerically less efficient in bringing back pollen. Although sample size was smaller for the positive control hives, a separate analysis of foraging activity among hives exposed to the three insecticide doses showed that more bees were foraging but less were returning with pollen in hives exposed to 1000 ppm of imidacloprid compared to the lower doses. However, this response was significant only at a probability level of 0.065.

Bee mortality In 2001, hives were located on separate tarps, so dead bee counts were associated with each hive, whereas counts in 2002 consisted of dead bees from all three hives positioned on a single tarp. For consistency in data interpretation, the number of dead bees per hive was averaged per plot in both years. These average counts probably underestimated the actual mortality because workers carried some dead bees beyond the tarp area. Main and interaction effects were not significant in both years. Bee mortality varied widely among treatments over time ranging from 0 to 352 dead bees per hive, with no consistent trends that would indicate exposure effects by the positive control (Fig. 3.6). In 2002, noticeable peaks in dead bees occurred during the second and third weeks of exposure but overall differences were not statistically significant. In general, more dead bees were recorded in 2001 and this was attributed to the presence of the hives initially exposed with only foundation frames. These hives were stressed by the lack of food stores and experienced heavy bee losses and the death of several queens.

Bee weights and pollen loads In 2001, foraging bee weights averaged 77.0, 77.1, and 76.5 mg and pollen loads averaged 14.1, 16.7, and 12.5 mg for hives exposed in Bt, non-Bt, and non-Bt insecticide treated plots, respectively. Exposure had no significant effect on bee weights or pollen loads at days 7 and 14 (Fig. 3.7). There were also no statistical differences in body weight between bees from hives initially stocked with brood frames and those with only foundation frames. Similarly, there were no statistical differences in the weight of bees returning to hives or their pollen loads between treatments in 2002 (Fig. 3.8). However, the overall weight of pollen loads significantly increased in size over time ($F = 3.93$; df = 2, 12; $p = 0.049$).

Hive performance In 2001, hives with only foundation frames experienced difficulty in establishing brood and maintaining the size of the colony. At least 20% of these hives became queenless during the exposure period resulting in death of the colony and many hives resulted in significant colony decline. Thus, data collected on these colonies were not included in the evaluation of hive performance. Another problem in the 2001 study was the pre-exposure differences among treatments in the number of bees in the hives. Colony size of hives placed in the Bt, non-Bt, and positive control plots averaged 6,066, 7,485, and 7,110 bees, respectively. These inequalities made it difficult to evaluate changes in absolute levels of each hive measurement due to treatment exposure. Thus, pre- and post-measurements of bee and brood strength, and stores of honey and pollen within hives were analyzed both as absolute values and as relative differences before and after exposure. In 2001, the number of bees, percent of capped brood cells, and stores of honey declined during exposure but were not significantly affected by the treatments (Fig. 3.9). The amount of stored pollen varied widely among hives and was not significantly affected by the treatments.

In 2002, more consideration was given to equalizing hives before they were allocated to treatment plots. Colony size of hives placed in the Bt, non-Bt, and positive control plots averaged 4,160, 4,747, and 5,170 bees, respectively. The pre-exposure levels of capped brood cells were statistically the same among treatments, although hives placed in the positive control plots had a numerically greater percentage of brood cells. During the exposure period, colonies in all hives consumed an average of 7.9 g of pollen per day by feeding on the pollen cakes. In all plots, bees were also observed foraging on the sweet corn tassels during anthesis. The pollen cake consumption alone represented

approximately 44% of the expected daily pollen requirements of a small hive of 5,000 bees (Seeley 1995, Crailsheim *et al.* 1992, J. Pettis, personal communication), indicating that colonies were exposed to a substantial dosage of Bt pollen. Exposure to the pollen treatments had no significant effect on the size of the colony, as indicated by the non-significant exposure by treatment interaction (Fig. 3.10). Colony size increased by an overall 33% ($F = 28.71$; $df = 1, 12.3$; $p < .001$) and the increment change was statistically the same for each treatment. Stores of honey and pollen dropped about 20% during exposure and neither absolute levels nor relative changes were statistically affected by treatments. The relative change in the amount of capped brood was the only hive measurement significantly affected by the treatments ($F = 12.52$; $df = 2,4$; $p < .019$) (Fig. 3.10). The percentage change in brood increased by 1.5 and 31.7% in the Bt and non-Bt plots, respectively, but decreased by 18.9% in the positive control plots. The difference was significant for contrasts between non-Bt and the positive control ($F = 24.35$; $df = 1,4$; $p < .001$) but not between non-Bt and Bt treatments.

Discussion

Laboratory studies Current Tier 1 dietary toxicity tests in support of a registration of Bt proteins with EPA involve dosing larval honey bees with microbially-derived endotoxins or Bt pollen. Another route of exposure is through consumption of pollen by young nurse bees, particularly during the first 10 days after emergence. In this study, newly emerged bees were fed different pollen types for up to 35 days to assess the effects on feeding, survival, and weight gain. Bees consumed significantly more mixed (non-corn) pollen than sweet corn pollen and more corn pollen when it was mixed with honey. Inconsistent differences in pollen consumption were observed between the non-Bt

and Bt cohorts of bees but the Bt pollen did not significantly affect survival in both years. These results agree with studies by Hanley *et al*. (2003) and others that indicated no adverse effects when Cry1Ab field corn pollen was fed directly to honey bee larvae in brood cells. In 2002, the average survival at 21 days was 71.4% with a standard deviation of 20.6. Because the variance was relatively low, the experimental design of ten cohorts of 50 bees per treatment used in the 2002 study had 80% power to detect a 35% reduction in survival with a significance level of 0.05 using a one-tailed t-test. In this case, the one-tailed test is appropriate because only reductions in survival relative to the control indicate a potential risk or the need for further studies. Figure 3.11 shows the number of replicate cohorts of 50 bees required to detect a range of effect sizes at 80% power for both one- and two-tailed tests. It is clear that considerable improvement in power or the ability to detect smaller differences is achieved if only reductions in survival are assumed.

In the 2001 study, measurements of head weights were recorded on a subset of bees exposed for 10 days to each pollen type for the purpose of evaluating effects on hypopharyngeal glandular development. Newly emerged bees initially function as nurse bees and consume pollen during the first two weeks of their life. Nurse bees have highly developed hypopharyngeal glands to process pollen into brood food; thus adverse effects to this gland may diminish the bee's ability to produce food for the colony. Fresh weight of honey bee heads has been directly correlated to the development and size of hypopharyngeal glands (Hrassnig and Crailsheim 1998). Thus, differences observed in the head weight of young bees can be an indirect measure of the effects on hypopharyngeal glandular development when evaluating risk of transgenic pollen to

honey bees. In the 2001 study, the head weight of young bees fed mixed pollen for 10 days either in treatment cages or source colonies gained an average of 1.66 mg, which agrees closely with the weight added by a fully developed hypopharyngeal gland (Hrassnig and Crailsheim 1998). Bt proteins are not expected to accumulate in honey bee hypopharyngeal glands (Malone *et al.* 2004). Interestingly, bees fed Bt corn pollen gained more head weight than bees fed non-Bt pollen but were not different from those fed mixed pollen. This is likely due to the consumption of significantly more pollen in the Bt cohorts compared to non-Bt pollen-fed bees. The standard deviation in head weight at 10 days was 1.24 mg (75% of the mean), so it follows that a high level of replication would be needed to detect small differences. By measuring head weights of 10 bees from each of six replicate cohorts per treatment, the 2001 study had 80% power to detect a 70% reduction in head weight gain with a significance level of 0.05 using a one-tailed t-test. Smaller differences of less than 50% of the control response would require studies with subsamples of 10 or more bees drawn from 12 replicate cohorts (Figure 3.12). Furthermore, little efficiency in detecting significant differences is gained by sampling more than ten bees per cohort.

In both studies, newly emerged bees were drawn from different colonies and allocated as replicates within treatments. This was done to partition the between hive variability from the residual term and also to make inferences about the effects on honey bees in general. For the head weight data, differences among sources of bees accounted for 30% of the total variance. Greater statistical power may be achieved by establishing replicate cohorts from a more homogenous pool of bees collected from one representative colony. Another source of variability in the survival of cohorts fed imidacloprid-treated

pollen was apparently due to uneven distribution of imidacloprid within the cakes. The 2 ppm dose of imidacloprid was more than adequate for stressing cohorts to demonstrate an adverse effect. However, a less variable response may be achieved by thoroughly mixing a lower dose of imidacloprid with the honey prior to adding pollen to ensure an even distribution of the insecticide.

Based on the laboratory studies, the following guidelines will aid in developing a Tier 1 protocol for assessing the effects of Bt corn pollen on the development and survival of newly emerged honey bees. To ensure maximum consumption and exposure to the expressed protein, fresh pollen should be mixed with honey and fed to cohorts of adults for at least 21 days. Mortality should be recorded at weekly intervals, although survival data collected at 21 days is a reliable endpoint alone for detecting differences. As described above, sample sizes of ten cohorts of 50 bees per treatment will detect a 35% reduction in survival with 80% power, while only six cohorts of 50 bees is needed to detect a 50% reduction. If the endpoint is head weight as an indirect measure of glandular development, a larger sample size is required to detect a 50% change with 80% power. Since glandular development is influenced by the presence of brood (Hrassnigg and Crailsheim 1998), a section of comb containing eggs should be included in the rearing cages and proper steps taken to avoid the problems of chalk brood. A test environment that includes a queen attached to the cages or queen pheromone also provides a more natural environment for caged bees. A positive control, such as a highly toxic gut-active protein or chemical, should be included to confirm that the test organisms are exposed to the test protein.

Field studies Hive studies in both years showed no significant effects of exposure to Bt pollen on bee foraging behavior, mortality, body weight and brood development. These results agree with other laboratory and field studies that reported no effects of Bt pollen or Cry proteins on honey bees (Malone and Pham-Delegue 2001). The exposure regime used in this study was different from previous non-target work on honey bees because it represents the first attempt to expose functional colonies of honey bees to Bt corn pollen under open field conditions. A related study by Schur *et al.* (2000) reported no effects on similar endpoints of honey bee performance in caged hives exposed to Bt sweet corn. Non-target studies on other transgenic crops such as canola have been conducted under confinement, semifield or open field conditions. Studies to evaluate honey bee foraging behavior on canola were conducted in an environmentally controlled flight chamber and an outdoor cage intended to represent field conditions (Grallien *et al.* 1995, Picard-Nizou *et al.* 1995). A two year study conducted by Huang *et al*. (2004) exposed honey bees to transgenic glyphosate-tolerant canola under open field and semifield conditions to evaluate larval survival, adult recovery, pupal weight and hemolymph protein concentrations. In all of these studies, there were no effects detected due to direct exposure to the pollen or protein produced by the transgenic crop. It is clear that non-target studies so far have provided abundant evidence that honey bees are insensitive to the Cry1 proteins expressed in transgenic corn pollen. However, transgenes expressing other novel proteins in crops are being developed (Malone and Pham-Delegue 2001) and may have subtle effects on honey bee colonies. Thus, currently applied protocols of field studies must be improved to provide data with sufficient statistical

power on the most sensitive endpoints of hive performance under realistic routes of exposure.

Experimental procedures to ensure exposure to Bt pollen should be taken into consideration when assessing the risk of transgenic crops to honey bees in an open field situation. In the first year, honey bees were allowed to forage freely for nectar and pollen from hives placed in the middle of sweet corn plots. Since the foraging radius of honey bees from the colony ranges from a few hundred meters to 10 km in agricultural areas (Winston 1987, Seeley 1995), it is uncertain whether foraging bees actually collected sweet corn pollen within the plots. Several methods could be used to verify the presence of sweet corn pollen in the hives. Samples of pollen collected from returning bees or from stored pollen added to combs during the exposure period could be processed and counted for corn pollen content according to the methods described by Jones and Coppedge (2000). Enzyme-linked immunosorbent assays (ELISA) have been used in other studies (Hansen and Obrycki 2000, Wraight *et al.* 2000, Chilcutt and Tabashnik 2004) to confirm that corn pollen contains the Cry1Ab protein. However, the level of expression in sweet corn (event Bt11) pollen may be too low to accurately detect its presence (D. Vlachos, Syngenta, personal communication). Even if an ELISA approach is feasible, it still cannot quantify the exposure level of active protein or confirm that pollen from foragers or stored pollen came from sweet corn plants within the plots. Honey bees are known to ingest corn pollen but their guts will only hold 1.52-2.04 mg of pollen per larva until defecation (Babendreier *et al.* 2004). A more direct route of exposure was used in the second year by providing pollen cakes to hives and measuring the amount consumed during the exposure period. Since pollen cake consumption alone represented about half

of the daily pollen requirements of a nuclear colony and stores of pollen dropped about 20% during exposure, it can be assumed that nurse bees were consuming pollen and utilizing it to make brood food. Other studies have exposed bees to pollen in similar ways. Huang *et al.* (2004) fed cakes of transgenic canola pollen mixed with 50% sugar syrup to honey bees to ensure ingestion prior to returning them to hives in a non-target field study. Other studies have also fed transgenic pollen or the Cry protein mixed with sugar syrup directly to adult bees outside the hive or fed directly in hives (Arpaia 1996, Picard-Nizou *et al.* 1997, Malone *et al.*2001).

A positive control was included in this study to verify that the bees and brood in the hive were exposed to the treatments. This is particularly critical in hive studies due to the various ways that naturally collected or artificially provisioned pollen is processed. For instance, if pollen is stored and not used immediately to make brood food, then a potential non-target effect on brood development may be delayed beyond the experimental duration. The aim of a positive control is to expose bees to a dose of known toxicant that mimics an expected non-target effect. In the first year, a reduced dose of the insecticide carbaryl was applied to plots to create a positive control effect. This rather indirect approach was successful in reducing the foraging activity of bees in the treated plots but had no detectable effect on the endpoints of hive performance. Moreover, foliar applications of insecticides are inherently problematic for establishing positive controls because it is difficult to deliver an appropriate dose over a sufficient duration to achieve the desired effect. In the second year of the field study, 10, 100 and 1000 ppb doses of imidacloprid were incorporated into pollen cakes to expose bees directly to a known toxicant. Many insecticides and other stomach poisons would be likely candidates for

positive controls, however, imidacloprid was selected because it is relatively safe to humans and much information is known about its effects on honey bees (Schmuck 1999). This imidacloprid-spiked cake approach resulted in a significant 20% reduction in the number of brood cells in the imidacloprid-treated plots, confirming that consumption of the pollen cakes were either adversely affecting the worker bees tending to brood and/or indirectly affecting larvae via brood food. Unfortunately, since a single hive was exposed to each dose in two replicate plots, only the combined effect of all doses was tested. Thus, the minimum dose of imidacloprid to add to pollen cakes in future studies could not be determined. Generally, results of chronic feeding studies with worker bees report that no adverse effects are expected under field conditions for imidacloprid residue levels of 20 ppb or less and that no loss of foraging bees occurs at concentrations of <100 ppb (Maus *et al*. 2003). Therefore, a concentration of around 100 ppb in pollen cakes may be a reasonable exposure level for a positive control.

Another important consideration in a non-target field study of honey bee colonies is the duration of the exposure. In this study, functional hives were exposed to Bt pollen for at least 4 weeks in all plots after which various components of hive performance were measured and compared with pre-exposure levels. Because the generation period of honey bees is 21 days from egg to adult, all the immature stages and development of new nurse bees were exposed through at least one generation, ending the third week of August. The exposure period could not be extended any longer in this study because of confounding effects due to physiological changes in the bees in preparation for overwintering. However, longer experimental durations may be more desirable to detect possible delayed effects if the exposure period starts earlier in the summer.

A common criticism of field studies is that the level of replication does not allow for enough statistical power to detect differences between the Bt treatment and controls if they exist. A generally accepted aim in ecotoxicological studies is to detect a 50% difference relative to the control with 80% statistical power (Candolfi *et al*. 2000). Using this as a standard, retrospective power analyses were conducted on each endpoint measured in the field study to determine the number of plots and hives within plots needed to detect a range of effect sizes. The power of testing is dependent partly on the selection of relevant endpoints that are the most sensitive and consistent in response to non-target effects. Based on coefficients of variability of data recorded from the untreated control hives, the number of foragers returning, counts of dead bees, bee population size, and measurements of honey and pollen stores were more variable and thus less reliable to detect relative fitness over an entire generation. Given the level of replication in this study, only differences in stores of pollen and honey greater than 75% from the control could be detected with 80% power (Figs. 3.13 and 3.14). If 50% differences are important to detect in future hive studies, at least four hives exposed in each of six replicate plots are required to achieve 80% power. Counts of dead bees on the tarp around Bt and non-Bt hives were highly variable and showed no significant trends or differences among treatments. As mentioned above, these data underestimated bee mortality because dead bees are often carried beyond the tarp and the number of dead bees lost or dying during foraging flights is not known. An efficient trap designed to capture the majority of dead bees removed from nuclear hives could greatly improve the reliability of bee mortality estimates in future studies. Power curves for dead bee counts are not included because the variance estimate in this study was considered inappropriate. Changes in the bee

population were also difficult to detect because the size of hives was not completely standardized at the beginning of the study. To reduce the variance due to pre-exposure differences in bee strength, data expressed as a relative change within each hive were analyzed rather than using the absolute values. Based on the variance of the relative data, at least four hives placed in five replicate plots would be needed to detect a 50% change in the number of bees with 80% power (Fig. 3.15). Foraging activity expressed as the total number of bees returning to the hive per 5 minutes was highly variable from day to day and not sensitive enough to detect significant changes that were less than 65% of the control. Increasing the observation time may improve the consistency of the foraging data but could create logistical problems in monitoring multiple hives of all treatments of a replicate within the required time frame. Tests designed to evaluate overall foraging activity will require six plots per treatment and six hives per plot to detect a 50% effect with 80% power (Fig. 3.16).

The most consistent endpoints were foraging bee weight, number of foragers returning with pollen loads, pollen load weight, and brood size. Together the former three endpoints indicate the general fitness of foraging bees to locate sources of pollen and return to the hive. Based on the level of replication in this study, a non-target effect equal to a 40% reduction in these foraging fitness parameters should be detectable with 80% power (Figs. 3.17, 3.18 and 3.19). Surprisingly, more statistical power is possible by focusing on the number of bees returning with pollen loads rather than counting all returning foragers, which is the common indicator used in most studies of foraging activity. The majority of variation in foraging activity was due to hives between replicate plots rather than hives within plots. Thus, significantly less gain in statistical power is

achieved by increasing the number of hives beyond three per plot. The one endpoint of hive performance that did differ significantly among treatments was the relative change in the percentage of capped brood cells. This parameter was the best indicator of queen health and brood development, although it was too variable to detect a 50% effect size with 80% power. Given the variation in brood development in control hives over the 4 week period, future studies to evaluate changes in brood strength should include four hives in each of six replicate plots to detect a 50% difference from the control (Fig. 3.18).

The EPA Scientific Advisory Panel held in 1999 (EPA 2000) recommended that field studies be conducted as the most direct way to assess potential non-target invertebrate impacts of planting Bt corn on a commercial scale. This is particularly appropriate for honey bee studies because more relevant information on sub-lethal effects can be obtained under field conditions where the bees encounter their natural environment within the social context of their colony. However, the impact of transgenic plants on honey bees needs to be considered on a case-by-case basis (Malone and Pham-Delegue 2001). This study has identified several experimental design elements, sensitive endpoints, and factors to consider in planning short-term field studies with open, functional hives to evaluate the non-target effects of transgenic pollen. Although there is no evidence thus far of any sub-lethal effects of the Cry proteins, insecticidal products expressed by other transgenes may need more extended field testing to assess the longer term consequences of sub-lethal changes in colonies, such as over-wintering survival and subtle modifications in bee behavior.

References

40 Code of Federal Regulations (CFR) Parts 150 to 189 (2003) Published by the Office of the Federal Register National Archives and Records Administration,Washington, DC.

Arpaia, S. (1996) Ecological impact of Bt-transgenic plants: Assessing possible effects of CryIIIB toxin on honey bees (*Apis mellifera* L.) colonies, J. Genet. Breed. 50, 315-319.

Babendreier, D, N. Kalberer, J. Romeis, P. Fluri and F. Bigler. (2004) Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants, Apidologie 35, 293-300.

Candolfi, M.P., S. Bluemel, R. Forester, F.M. Bakker, C. Grimm, S.A. Hassan, U. Heimbach, M.A. Mead-Briggs, B. Reber, R. Schmuck and H. Vogt (2000) Guidelines to evaluate side-effects of plant protection products to non-target arthropods: IOBC, BART and EPPO joint initiative, 158 pp.

Chilcutt C.F. and B.E. Tabashnik (2004) Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic corn, PNAS 101: 7526-7529.

Crailsheim, K., L.H.W. Schneider, N. Hrassnigg, G. Buhlmann, U. Brosch, R. Gmeinbauer and B. Schoffmann (1992) Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): dependence on individual age and function, J. Insect Physiol. 38, 409-419.

Doanne Marketing Research, Inc (1998) Multi Client Studies.

Environmental Protection Agency (1996a) Honey bee testing, tier I, OPPTS 85.4380, EPA 712-C-96-337, www.epa.gov/pesticides/biopesticides/regtools/guidelines/oppts_885_4380.htm.

Environmental Protection Agency (1996b) Honey bee acute contact toxicity, OPPTS 850.3020, EPA 712-C-96-147, www.epa.gov/opptsfirs/oppts_harmonized/850_ecological_effects_test_guidelines/drafts/850-3020.pdf.

Environmental Protection Agency (1998) Registration Eligibility Decision (RED) *Bacillus thuringiensis*, EPA738-R-98-004.

Environmental Prtoection Agency (2000) Characterization and non-target organism data requirements for protein plant-pesticides, SAP report no. 99-06A for FIFIRA Scientific Advisory Panel Meeting

Felton, J.C., P.A. Oomen, and J.H. Stevenson (1986) Toxicity and hazard of pesticides to honeybees: harmonization of test methods, Bee World 67, 114-124.

Flexner, J.L., B. Lighthart, B.A. Croft (1986) The effect of microbial pesticides on non-target, beneficial arthropods, Ag. Eco. Environ. 15, 203-254.

Grallien, E., R. Marilleau, M.H. Pham-Delegue, A.L. Picard-Nizou, L. Jouanin and F. Marion-Poll (1995) Impact of pest insect resistant oilseed rape on honeybees, Proc. 9th Int. Rapeseed Congr. "Rapeseed today and tomorrow", Cambridge, United Kingdom, 4-7 July 1995, pp. 784-786.

Hanley, A.V., Z.Y. Huang and W.L. Pett (2003) Effects of dietary transgenic Bt corn pollen on larvae of *Apis mellifera* and *Galleria mellonella*, J. Apic. Res. 42, 77-81.

Haydak, M.H. (1970) Honey Bee Nutrition, Ann. Rev. of Entomol. 15, 143-156.

Hilbeck, A., M.S. Meier, and A. Raps (2000) Review on Non-Target Organisms and Bt-Plants, EcoStrat GmbH (Ecological Technology Assessment & Environmental Consulting), Report to Greenpeace International, Amsterdam, 77pp.
Hintze, J. (2001) NCSS and PASS, Number cruncher statistical systems, Kaysville, Utah, www.ncss.com.

Hrassnigg, N. and K. Crailsheim (1998) Adaptation of hypopharyngeal gland development to the brood status of honeybee (*Apis mellifera* L.) colonies, J. Insect Physiol. 44, 929-939.

Huang, Z.Y., A.V. Hanley, W.L. Pett, M. Langenberger, J.J. Duan (2004) Field and semifield evaluation of impacts of transgenic canola pollen on survival and development of worker honey bees, J. Econ. Entomol, 97, 1517-1523.

Jesse, L.C.H. and J.J. Obrycki (2000) Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly, Oecologia, 125 241-248.

Johansen, C.A. (1977) Pesticides and pollinators, Ann. Rev. Entomol, 22, 177-192.

Johansen, C.A., and D.F. Mayer (1990) Pollinator protection: a bee & pesticide handbook, Wicwas, Cheshire, CT, 212 pp.

Jones, G. D. & Coppedge J. R. (2000) Foraging resources of adult Mexican corn rootworm (Coleoptera: Chrysomelidae) in Bell County, Texas, J. Econ. Entomol. 93**,** 636-643.

Knecht, D. and H.H. Kaatz (1990) Patterns of larval food production by hypopharyngeal glands in adult worker honey bees, Apidologie. 21, 457-468.

Malone, L.A., E. P.J. Burgess, D. Stefanovic (1999) Effects of a *Bacillus thuringiensis* toxin, two *Bacillus thuringiensis* biopesticide formulations, and a soybean trypsin

inhibitor on honey bee (*Apis mellifera* L.) survival and food consumption, Apidologie 30, 465-473.

Malone, L.A. and M. Pham-Delegue (2001) Effects of transgenic products on honey bees (*Apis mellifera*) and bumblebees (*Bombus* sp.), Apidologie 32, 287-304.

Malone, L.A., E.P.J. Burgess, H.S. Gatehouse, C.R. Voisey, E.L. Tregidga and B.A. Philip (2001) Effects of ingestion of a *Bacillus thuringiensis* toxin and a trypsin inhibitor on honey bee flight activity and longevity, Apidologie 32, 57-68.

Malone, L.A., J.H. Todd, E.P.J. Burgess and J.T. Christeller (2004) Development of hypopharyngeal glands in adult honey bees fed with a Bt toxin, a biotin-binding protein and a protease inhibitor, Apidologie 35, 655-664.

Maus, C., G. Curé, and R. Schmuck (2003) Safety of imidacloprid seed dressings to honey bees: a comprehensive overview and compilation of the current state of knowledge, Bull. of Insectology 56, 51-57. Moffett, J.O., R. H. Macdonald, and M.D. Levin (1970) Toxicity of carbaryl-contaminated pollen to adult honey bees, J. Econ. Entomol. 63, 475-476.

Picard-Nizou, A.L., M.H. Pham-Delegue, V. Kerguelen, P. Douault, R. Marilleau, L. Olsen, A. Toppan and C. Masson (1995) Foraging behavior of honey bees (*Apis mellifera* L. *var. oleifera*), Trans. Res. 4, 270-276.

Picard-Nizou, A.L., R. Grison, L. Olsen, C. Pioche, G. Arnold and M.H. Pham-Delegue (1997) Impact of proteins used in plant genetic engineering: toxicity and behavioral study in the honeybee, J. Econ. Entomol, 90, 1710-1716.

Russek-Cohen, E. and L.W. Douglas. 1999. Mixed models data analysis: using PROC MIXED for biological research. Mixed model shortcourse manual. P. 89-103. Jan. 11-14, 1999. Beltsville, MD.

Sammataro, D. and A. Avitabile (1986) The Beekeeper's Handbook Macmillan Publishing Company, New York, 148 pp.

SAS Institute. 1997. SAS user=s guide. SAS Institute, Raleigh, NC. 494 pp.

Schmuck, R. (1999) No causal relationship between Gaucho® seed dressing in sunflowers and the French bee malady, Pflanzenschutz-Nachrichten Bayer 52, 257– 299

Schur, A., I. Tornier, and C. Neumann (2000) Association of Institutes for Bee Research Report of the 47th seminar in Blaubeuren near Ulm Heinrich-Fabri-Institut of the Eberhard-Karis-University Tubingen 3-5 April 2000, Apidologie 31, 557-668.

Seeley, T.D (1985) Honeybee Ecology a Study of Adaptation in Social Life, Princeton University Press, Princeton, NJ, pp. 80-106.

Seeley, T.D. (1995) The Wisdom of the Hive The Social Physiology of Honey Bee Colonies, Harvard University Press, Cambridge Massachusetts, 295 pp.

Sommerville, D.C. (2001) Nutritional value of bee collected pollens, A report for the Rural Industries Research and Development. Corporation #01/047, NSW Agriculture Goulburn, NSW Kustry, 166pp.

Stark, J.D., P.C. Jepson, and D.F. Mayer (1995) Limitations to use of topical toxicity data for predictions of pesticide side effects in the field, J. Econ. Entomol. 88, 1081-1088.

Winston, M.L. (1987) The Biology of the Honey Bee, Harvard University Press, Cambridge, Massachusettes, 281 pp.

Wraight, C.L., A.R. Zangerl, M.J. Carroll, M.R. Berenbaum (2000) Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions, PNAS 97, 7700-7703.

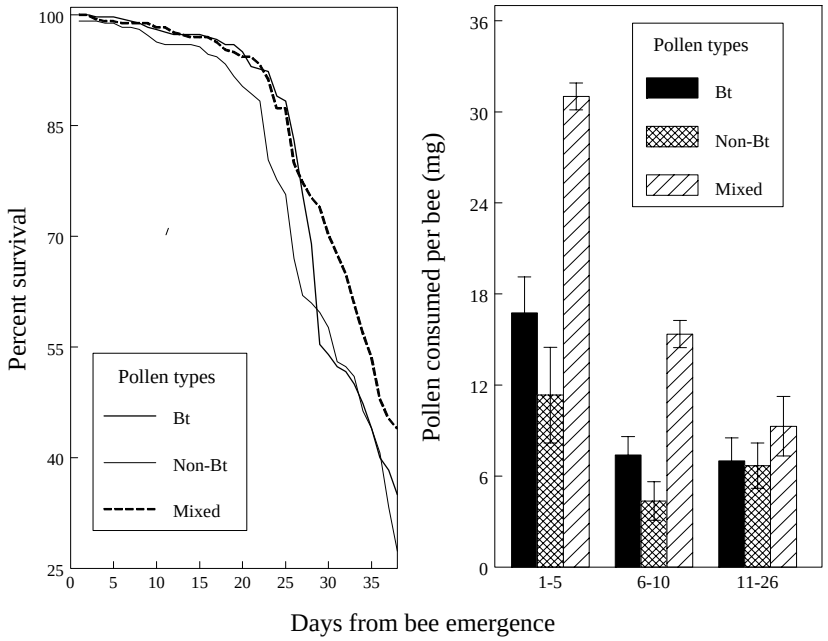

Figure 3.1. Percentage survival and pollen consumption of honey bees fed Bt and non-Bt sweet corn pollen and mixed pollen from natural sources for 35 days in a no-choice laboratory study conducted in 2001.

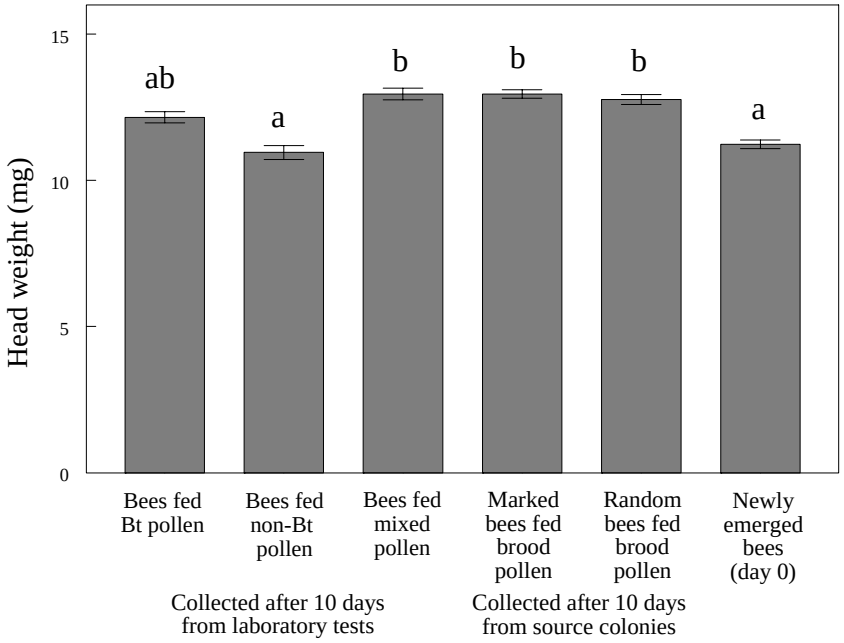

Figure 3.2. Average head weight of bees collected from colonies at test initiation (day 0) and removed at 10 days from treatment cages in 2001. Marked colony bees were the same age as laboratory exposed bees, whereas random bees were of mixed ages representative of the colonies. Newly-emerged bees collected day 0 were exposed only to hive pollen as larvae. Mean bars with the same letter are not significantly different at the 5% probability level.

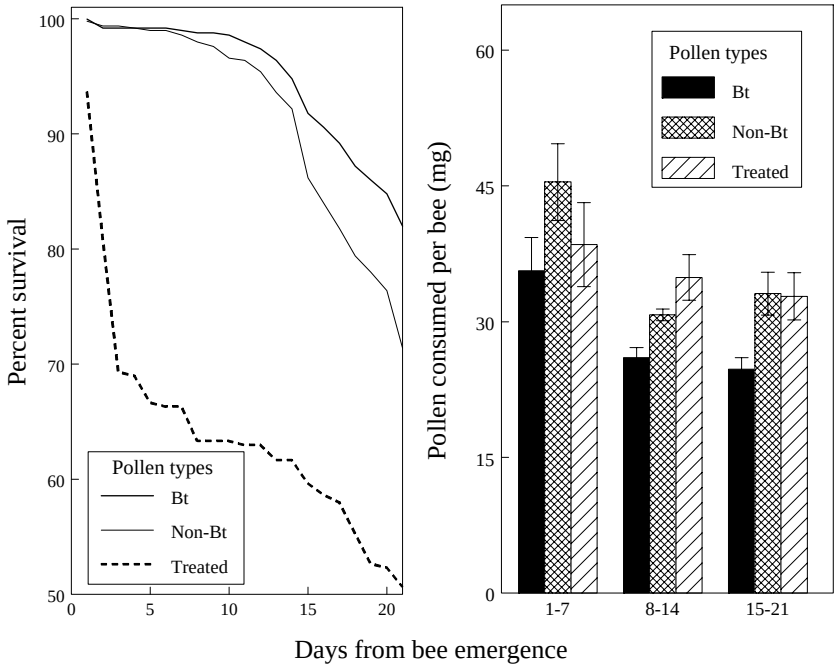

Figure 3.3 Percentage survival and pollen consumption of bees fed Bt, non-Bt and treated sweet corn pollen for 20 days in a no-choice laboratory study conducted in 2002. The treated pollen contained a 2 ppm concentration of imidacloprid.

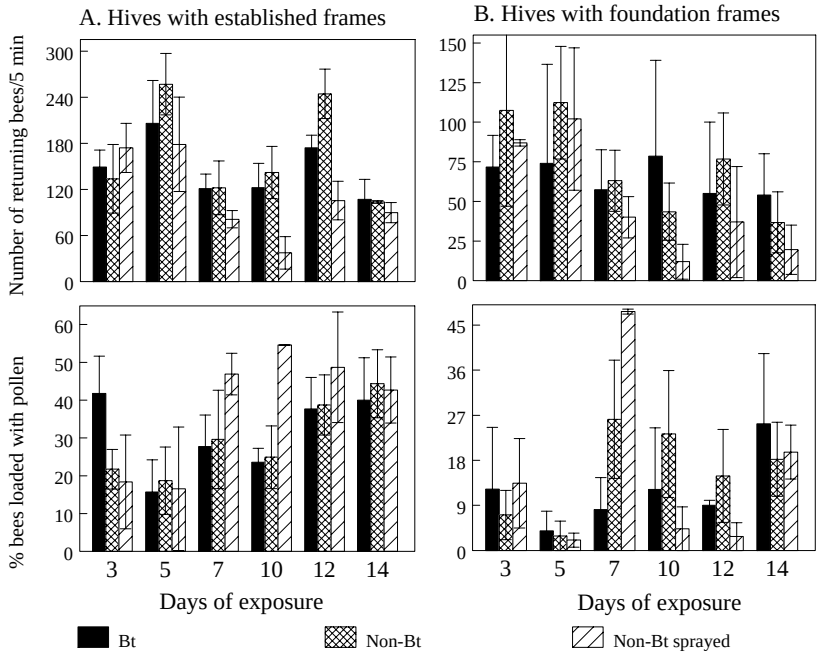

Figure 3.4. Average number of foraging bees and the proportion carrying pollen loads returning to A) hives with established brood and food frames and B) hives that began with blank foundation frames. Data are shown for hives exposed during anthesis in plots of Bt and non-Bt sweet corn, and in control plots of non-Bt sweet corn treated with carbaryl. 2001.

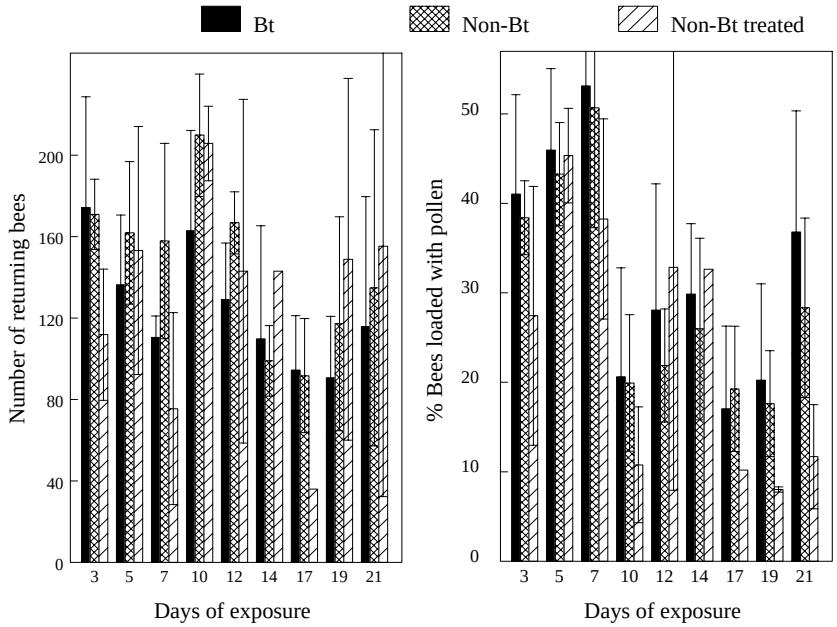

Figure 3.5. Average number of foraging bees and the proportion carrying pollen loads returning to hives exposed during anthesis in plots of Bt and non-Bt sweet corn. Data are shown for hives fed Bt, non-Bt, and non-Bt pollen treated with imidacloprid in the respective plots. 2002.

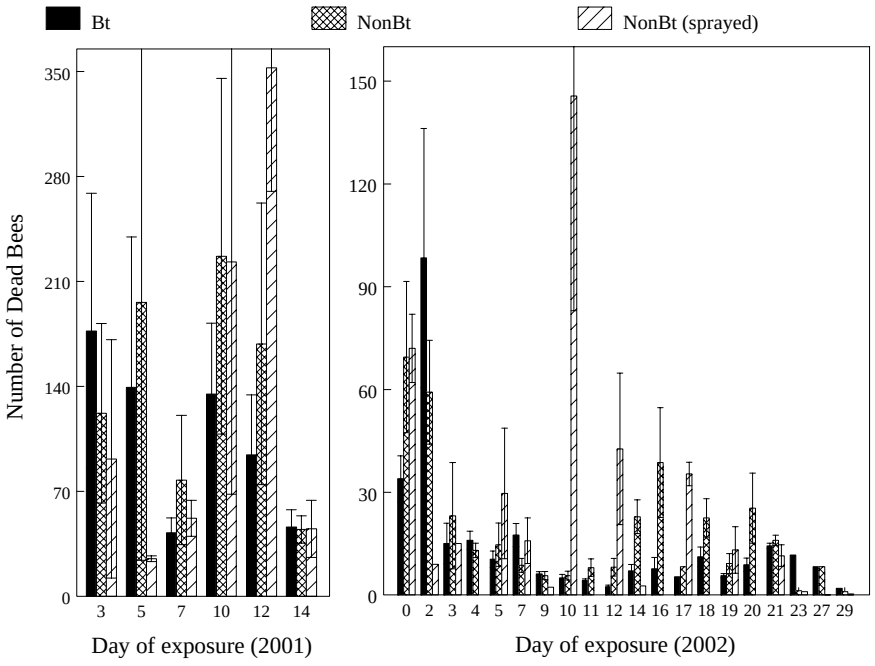

Figure 3.6. The number of dead bees found on a 8' x 10' tarp around the hives located in the middle of Bt and nonBt (treated and untreated) cornfields during pollination in the 2001and 2002 growing seasons. Non-Bt treated plots were sprayed with Sevin7 in 2001 and pollen cakes were mixed with imidacloprid in 2002.

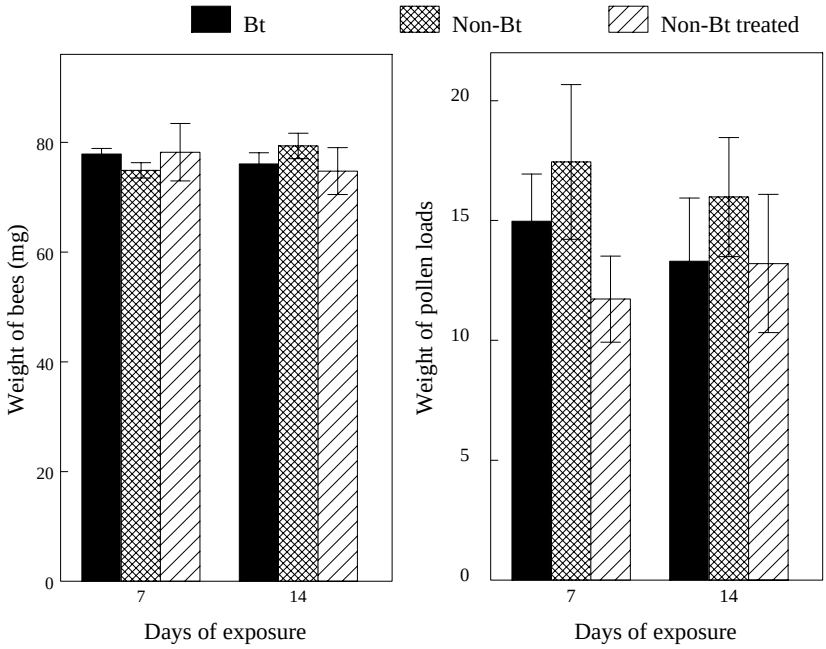

Figure 3.7. Average weight of foraging bees carry pollen loads to hives and the weight of their pollen loads for A) hives with established brood and food frames and B) hives that began with blank foundation frames. Data are shown for hives exposed during anthesis in plots of Bt and non-Bt sweet corn, and in control plots of non-Bt sweet corn treated with carbaryl. 2001.

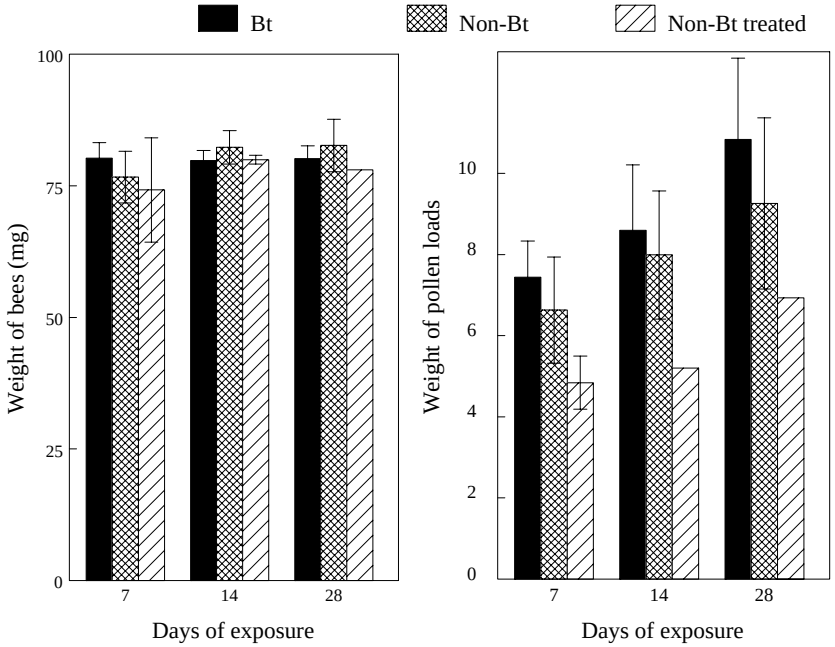

Figure 3.8. Average weight of foraging bees carry pollen loads to hives and the weight of their pollen loads. Data are shown for hives exposed during anthesis in plots of Bt and non-Bt sweet corn, and in control plots of non-Bt sweet corn exposed to imidacloprid treated pollen cakes. 2002.

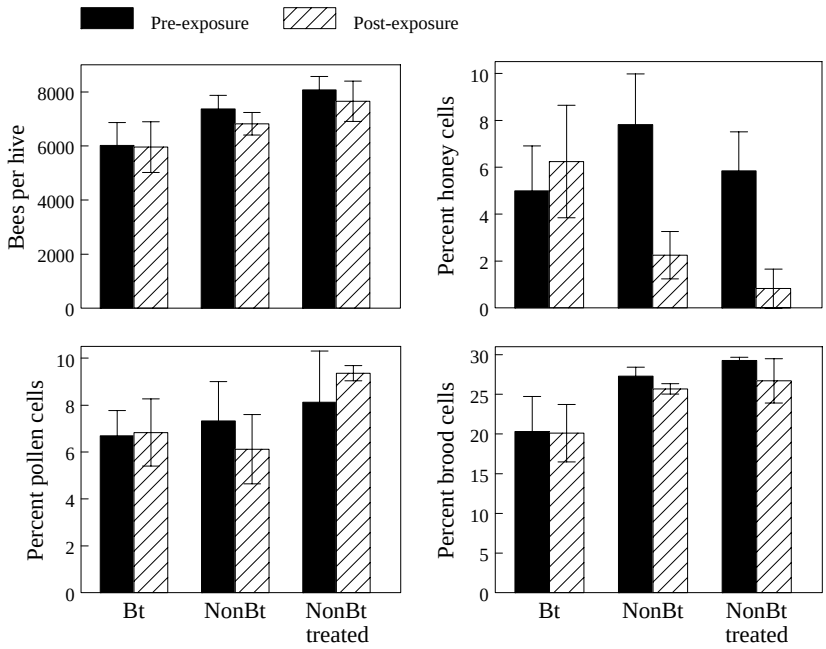

Figure 3.9. Pre- and post-exposure bee strength, percent honey, percent pollen and percent capped brood. Data are shown for hives exposed during anthesis in plots of Bt and non-Bt sweet corn, and in control plots of non-Bt sweet corn treated with carbaryl. Number of bees and standard error for number of bees graphed are 100-fold less than actual numbers. 2001.

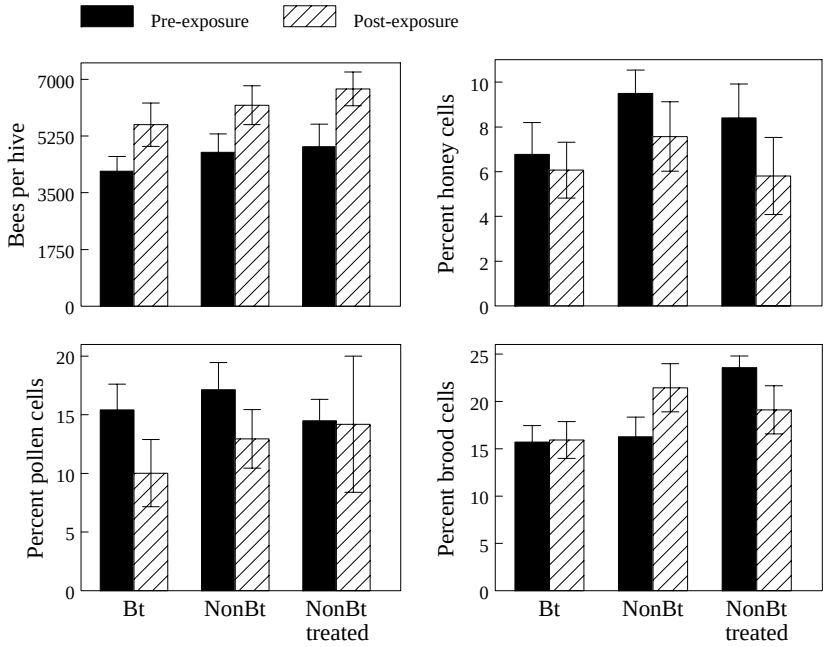

Figure 3.10. Pre- and post-exposure bee strength, percent honey, percent pollen and percent capped brood. Data are shown for hives exposed during anthesis in plots of Bt and non-Bt sweet corn, and in control plots of non-Bt sweet corn pollen treated with imidacloprid. Number of bees and standard error for number of bees graphed are 100-fold less than actual numbers. 2002.

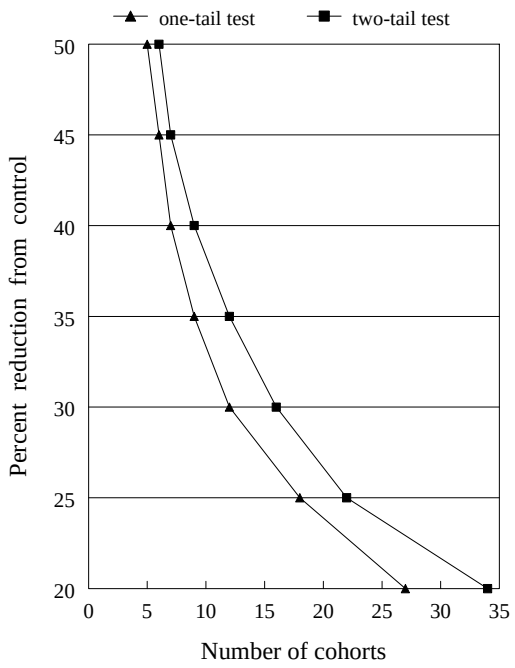

Figure 3.11. Number of replicate cohorts of 50 bees required in laboratory tests to detect a range of relative differences in percent bee survival from the control at 80% statistical power.

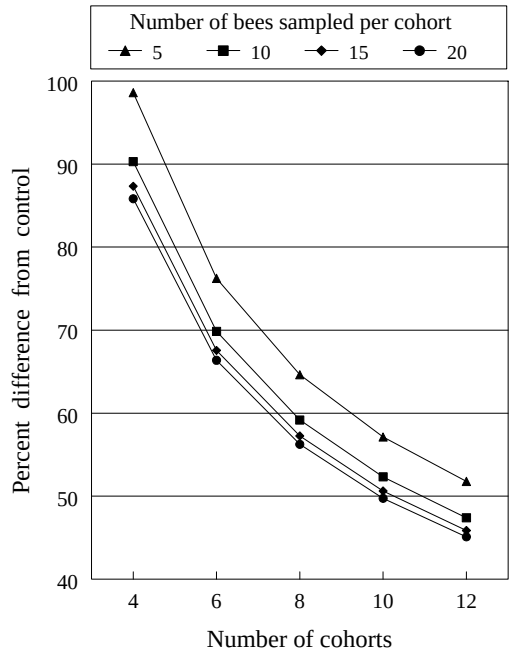

Figure 3.12. Combinations of replicate cohorts and number of bees per cohort required in laboratory tests to detect a range of differences in bee head weight at 80% statistical power.

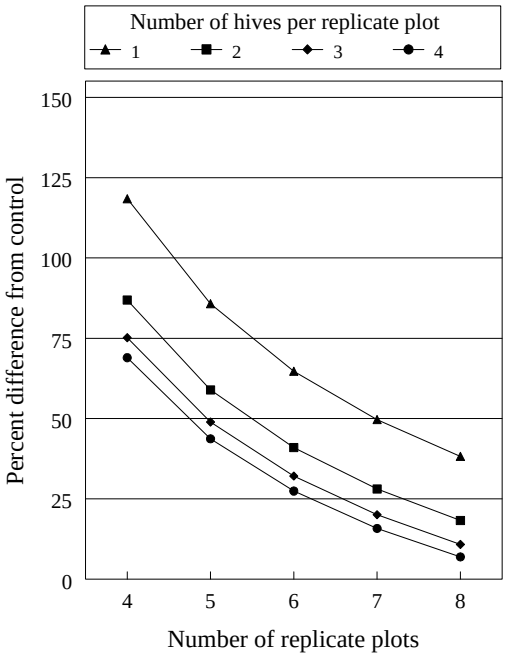

Figure 3.13. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in pollen stores from the control at 80% statistical power.

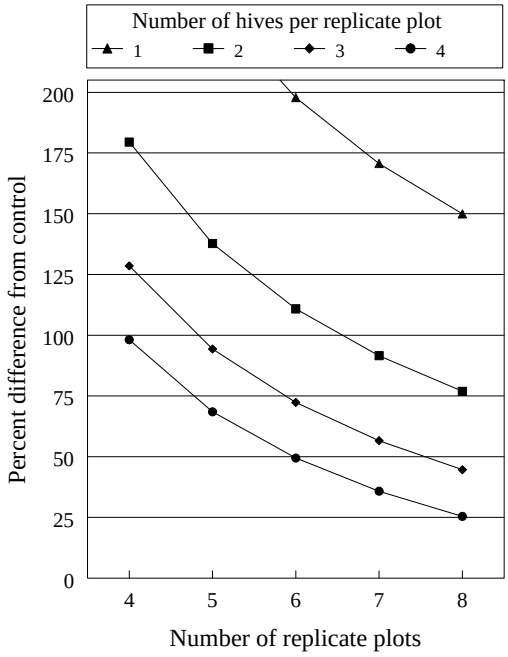

Figure 3.14. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in honey stores from the control at 80% statistical power.

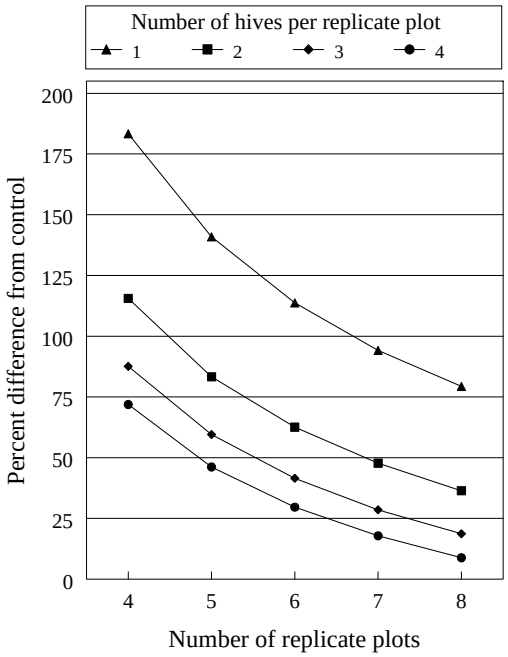

Figure 3.15. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in the bee population from the control at 80% statistical power.

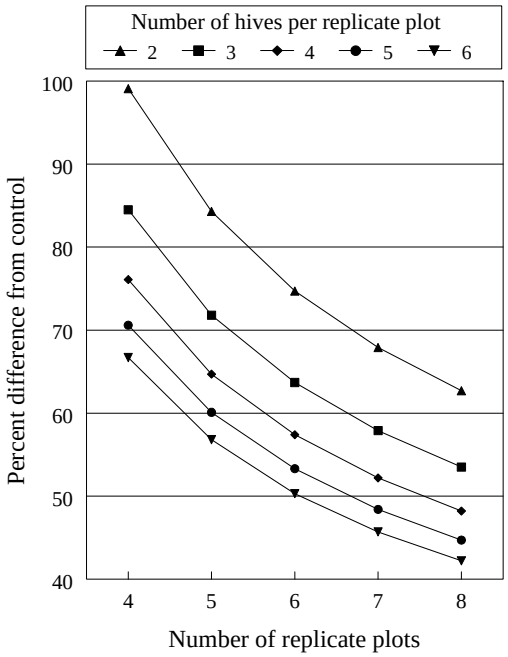

Figure 3.16. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in the number of bees returning to hives from the control at 80% statistical power. Variance and mean values for power calculations are based on counts per 5 minutes at the entrance of the hive.

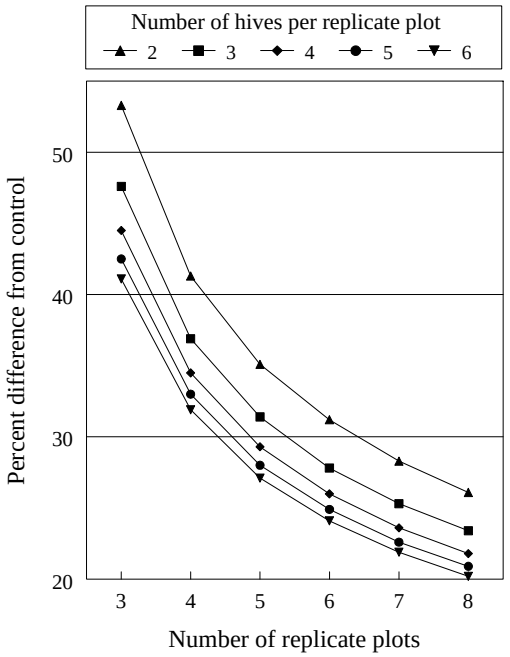

Figure 3.17. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in the number of foraging bees returning to hives with pollen loads from the control at 80% statistical power. Variance and mean values for power calculations are based on counts per 5 minutes at the entrance of the hive.

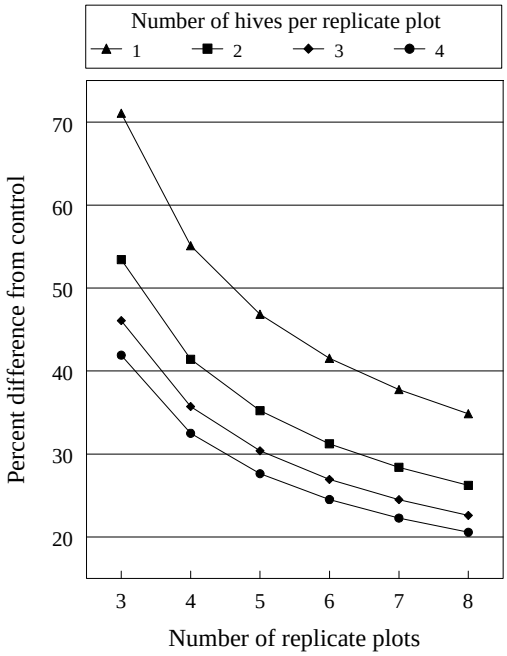

Figure 3.18. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in the weight of pollen loads carried back to hives by foraging bees relative to the control at 80% statistical power. Variance and mean values for power calculations are based on average weights of ten pollen loads combined per replicate.

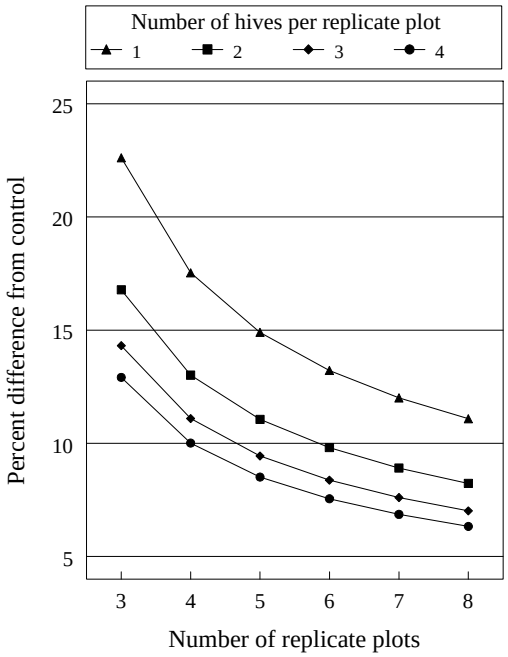

Figure 3.19. Number of replicate plots and number of hives per plot required in field studies to detect a range of differences in the weight of foraging bees from the control at 80% statistical power. Variance and mean values for power calculations are based on average weights of cohorts of ten bees.

REFERENCES CITED

40 Code of Federal Regulations (CFR) Parts 150 to 189 (2003) Published by the Office of the Federal Register National Archives and Records Administration,Washington, DC.

Al-Deeb, M.A., G.E. Wilde and R.A. Higgins. 2001. No effect of Bacillus thuringiensis corn and Bacillus thuringiensis on the predator *Orius insidiosus* (Hemiptera : Anthocoridae). Environ. Entomol. 30: 625-629.

Andow, D.A. 1990. Characterization of predation on egg masses of *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 83: 482-486.

Arpaia, S. 1996. Ecological impact of Bt-transgenic plants: Assessing possible effects of CryIIIB toxin on honey bees (*Apis mellifera* L.) colonies, J. Genet. Breed. 50, 315-319.

Babendreier, D, N. Kalberer, J. Romeis, P. Fluri and F. Bigler. 2004. Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants, Apidologie 35, 293-300.

Betz, F.S., B.G. Hammond, and R.L. Fuchs. 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. Reg. Tox. Pharmacol. 32: 156-173.

Canard, M., Y. Semeria, and T. R. New. 1984. Biology of Chrysopidae. Dr. W. Junk Publishers, The Hague, The Netherlands. 294 pp.

Candolfi M, Bigler F, Campbell P, Heimbach U, Schmuck R, Angeli G, Bakker F, Brown K, Carli G, Dinter A, Forti D, Forster R, Gathmann A, Hassan S, MeadBriggs M, Melandri M, Neumann P, Pasqualini E, Powell W, Reboulet JN, Romijn K, Sechser B, Thieme T, Ufer A, Vergnet C, Vogt H. 2000. Principles for regulatory testing and interpretation of semifield and field studies with nontarget arthropods. J. Pest Sci. 73: 141-147.

Candolfi, M.P., S. Bluemel, R. Forester, F.M. Bakker, C. Grimm, S.A. Hassan, U. Heimbach, M.A. Mead-Briggs, B. Reber, R. Schmuck and H. Vogt. 2000. Guidelines to evaluate side-effects of plant protection products to non-target arthropods: IOBC, BART and EPPO joint initiative, 158 pp.

Candolfi, M., K. Brown, and B. Reber. 2003. A faunistic approach to assess potential side-effects of genetically modified *Bt*-corn on non-target arthropods under field conditions. Environ. Biocontrol Science and Technology 14: 129-170.

Cheng W. and D.C. Coleman. 1990. Effect of living roots on soil organic matter decomposition. Soil Biol. Biochem. 22: 781-787.

Chilcut, C.F. and B.E. Tabashnik. 1999a. Simulation of integration of *Bacillus thuringiensis* and the parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae) for control of susceptible and resistant diamondback moth (Lepidoptera: Plutellidae). Environ. Entomol. 28: 505-512.

Chilcut, C.F. and B.E. Tabashnik. 1999b. Effects of *Bacillus thuringiensis* on adults of *Cotesia plutellae* (Hymenoptera: Braconidae), a parasitoid of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). Biocon. Sci. Tech. 9: 435-440.

Chilcutt C.F. and B.E. Tabashnik. 2004. Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic corn, PNAS 101: 7526-7529.

Coll, M. and D.G. Bottrell. 1991. Microhabitat and resource selection of the European corn borer (Lepidotpera: Pyralidae) and its natural enemies in Maryland field corn. Environ. Entomol. 20: 526-533.

Coll, M. and D.G. Bottrell. 1992 Mortality of European corn borer larvae by natural enemies in different corn microhabitats. Bio. Con. 2: 95-103.

Cortet, J., D. Gillon, R. Joffre, J. Ourcival and N. Poinsot-Balaguer. 2002. Effects of pesticides on organic matter recycling and microarthropods in a maize field: use and discussion of the litterbag methodology. Euro J Soil Bio. 38: 261-265.

Crailsheim, K., L.H.W. Schneider, N. Hrassnigg, G. Buhlmann, U. Brosch, R. Gmeinbauer and B. Schoffmann. 1992. Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): dependence on individual age and function, J. Insect Physiol. 38, 409-419.

Crecchio C. and G. Stotzky. 1998. Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subspecies *kurstaki* bound to humic acids in soil. Soil Biol. Biochem. 30: 463-470

Crossley,D.A and M.P. Hoglund. 1962. A litterbag method for the study of microarthropods inhabiting leaf litter. Ecology. 43: 571–573.

Czarnecki, A., and J. Losinski. 1995. The effect of GT seed dressing on the community of Collembola in soil under sugar beet. Pedobiologia 28: 427-31.

Doanne Marketing Research, Inc. 1998. Multi Client Studies.

Dogan, E.B. R.E. Berry, G.L. Reed, and P.A. Rossignol. 1996. Biological parameters of convergent lady beetle (Coleoptera: Coccinellidae) feeding on aphids (Homoptera: Aphididae) on transgenic potato. J. Econ. Entomol. 89: 1105-1108.

Donegan, K.K., D.L. Schaller, J.K Stone, L.M. Ganio, G. Reed, P.B. Hamm, and R.J. Seidler. 1996. Microbial populations, fungal species diversity and plant pathogen levels in field plots of potato plants expressing the *Bacillus thuringiensis* var. *tenebrionis* endotoxin. Transgenic Research. 5:25-35.

Dutton, A., H. Klein, J. Romeis and F. Bigler. 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. Ecol. Entomol. 27:441-447.

Environmental Protection Agency. 1996a. Honey bee testing, tier I, OPPTS 85.4380, EPA 712-C-96-337, www.epa.gov/pesticides/biopesticides/regtools/guidelines/oppts_885_4380.htm.

Environmental Protection Agency. 1996b. Honey bee acute contact toxicity, OPPTS 850.3020, EPA 712-C-96-147, www.epa.gov/opptsfirs/oppts_harmonized/850_ecological_effects_test_guidelines/drafts/850-3020.pdf.

Environmental Protection Agency. 1998. Registration Eligibility Decision (RED) *Bacillus thuringiensis*, EPA738-R-98-004.

Environmental Protection Agency. Scientific Advisory Panel. 2000. Characterization and non-target organism data requirements for protein plant-pesticides. SAP Report No. 99-06A dated February 4, 2000, of December 8, 1999, FIFRA SAP meeting, Arlington, VA.

Environmental Protection Agency. Scientific Advisory Panel. 2002. Corn rootworm plant-incorporated protectant insect resistance management and non-target insect issues. Transmittal of meeting minutes of the FIFRA Scientific Advisory Panel Meeting held August 27-29 at the Marriott Crystal City Hotel, Arlington, VA.

Felton, J.C., P.A. Oomen, and J.H. Stevenson. 1986. Toxicity and hazard of pesticides to honeybees: harmonization of test methods, Bee World 67, 114-124.

Fitt, G. and L. Wilson. 2003. Non-Target Effects of Bt Cotton: A Case Study from Australia. In R. J. Akhurst, C. E. Beard and P. Hughes (eds.). *Biotechnology of Bacillus thuringiensis and its environmental impact : Proceedings of the 4th Pacific Rim conference*, Canberra, AU, CSIRO Entomology.

Flexner, J.L., B. Lighthart, B.A. Croft. 1986. The effect of microbial pesticides on non-target, beneficial arthropods, Ag. Eco. Environ. 15, 203-254.

Flores, S., D. Saxena and G. Stotzky. 2005. Transgenic *Bt* plants decompose less in soil than non-*Bt* plants. Soil Biol. Biochem. In press.

Frampton, G. K., S. D. Langton, P. W. Greig-Smith, and A. R. Hardy. 1992. Changes in the soil fauna at Boxworth. In Pesticides, *Cereal Farming and the Environment: the Boxworth Project* (ed. P. Greig-Smith, G.K. Frampton, and T. Hardy), pp. 132-43. HMSO, London.

French, B.W., L.D. Chandler, M.M. Ellsbury, B.W. Fuller and M. West. 2004. Ground beetle (Coleoptera: Carabidae) assemblages in a transgenic corn-soybean cropping system. Environ Entomol. 33: 554-563.

Gill, S.S., E.A. Cowles, and P.V. Pietrantonio. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Ann. Rev. Entomol. 37: 615-636.

Grallien, E., R. Marilleau, M.H. Pham-Delegue, A.L. Picard-Nizou, L. Jouanin and F. Marion-Poll. 1995. Impact of pest insect resistant oilseed rape on honeybees, Proc. 9th Int. Rapeseed Congr. "Rapeseed today and tomorrow", Cambridge, United Kingdom, 4-7 July 1995, pp. 784-786.

Griffiths B.S., Ritz K., Ebblewhite N., and G. Dobson. 1999. Soil microbial community structure: effects of substrate loading rates. Soil Biol. Biochem. 31: 145-153.

Groot, A.T. and M. Dicke. 2002. Insect-resistant transgenic plants in a mulit-trophic contect. Plant Journ. 31: 387-406.

Hagerty, A., S.G. Turnipseed and M.J. Sullivan. 2001. Impact of predaceous arthropods in cotton IPM. In Proceedings of the Beltwide Cotton Conferences, National Cotton Council, Memphis, TN, pp. 812-815.

Hanley, A.V., Z.Y. Huang and W.L. Pettis. 2003. Effects of dietary transgenic Bt corn pollen on larvae of *Apis mellifera* and *Galleria mellonella*, J. Apic. Res. 42, 77-81.

Haydak, M.H. (1970) Honey Bee Nutrition, Ann. Rev. of Entomol. 15, 143-156.

Head, G., C.R. Brown, M.E. Groth and J.J. Duan. 2001a. Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. Entomol. Exp. Applic. 99: 37-45.

Head, G., B. Freeman, W. Moar, J. Ruberson and S. Turnipseed. 2001b. Natural enemy abundance in commercial Bollgard and conventional cotton fields. In Proceedings of the Beltwide Cotton Conferences, National Cotton Council, Memphis, TN, pp. 796-798.

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

Hilbeck, A., W.J. Moar, M. Pusztai-Carey, A. Filippini, and F. Bigler. 1998b. Toxicity of *Bacillus thuringiensis* CryIAb toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 27:1255-1263.

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*. Entomol. Exper. Appl. 91: 305-316.

Hilbeck, A., M.S. Meier, and A. Raps. 2000. Review on Non-Target Organisms and Bt-Plants, EcoStrat GmbH (Ecological Technology Assessment & Environmental Consulting), Report to Greenpeace International, Amsterdam, 77pp.
Hintze, J. 2001. NCSS and PASS, Number cruncher statistical systems, Kaysville, Utah, www.ncss.com.

Hrassnigg, N. and K. Crailsheim. 1998. Adaptation of hypopharyngeal gland development to the brood status of honeybee (*Apis mellifera* L.) colonies, J. Insect Physiol. 44, 929-939.

Huang, Z.Y., A.V. Hanley, W.L. Pett, M. Langenberger, J.J. Duan. 2004. Field and semifield evaluation of impacts of transgenic canola pollen on survival and development of worker honey bees, J. Econ. Entomol, 97, 1517-1523.

Jasinski, J.R., J.B. Eisley, C.E. Young, J. Kovach and H. Willson. 2003. Select nontarget arthropod abundance in transgenic and nontransgenic field crops in Ohio. Environ. Entomol. 32: 407-413.

Jensen, L.S. and J. Soerensen. 1994. Microscale fumigation-extraction and substrate induced respiration methods for measuring microbial biomass in barley rhizosphere. Plant Soil. 162: 151-161

Jepson, P.C. 1989. The temporal and spatial dynamics of pesticide side-effects on non-target invertebrates. Pesticides and non-target invertebrates. editor: Paul C. Jepson. Wimborne, Dorset, England : Intercept. pp. 95-127.

Jepson, P.C., B.A. Croft, and G.E. Pratt. 1994. Test systems to determine the ecological risks posed by toxin release from *Bacillus thuringiensis* genes in crop plants. Mol.Ecol. 3: 81-89.

Jesse, L.C.H. and J.J. Obrycki (2000) Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly, Oecologia, 125 241-248.

Johansen, C.A. 1977. Pesticides and pollinators, Ann. Rev. Entomol, 22, 177-192.

Johansen, C.A., and D.F. Mayer. 1990. Pollinator protection: a bee & pesticide handbook, Wicwas, Cheshire, CT, 212 pp.

Johnson, M.T. and F. Gould. 1992. Interaction of genetically engineered host plant resistance and natural enemies of *H. virescens* (Lepidoptera: Noctuidae) in tobacco. Environ. Entomol. 21: 586-597.

Johnson, M.T. 1997. Interaction of resistant plants and wasp parasitoids of tobacco budworm (Lepidoptera: Noctuidae). Environ. Entomol. 26: 207-214.

Johnson, M.T., F. Gould, and G.C. Kennedy. 1997. Effects of natural enemies on relative fitness of *Heliothis virescens* genotypes adapted and not adapted to resistant host plants. Entomiol. Exper. Appl. 82: 219-230.

Jones, G. D. & Coppedge J. R. 2000. Foraging resources of adult Mexican corn rootworm (Coleoptera: Chrysomelidae) in Bell County, Texas, J. Econ. Entomol. 93**,** 636-643.

Knecht, D. and H.H. Kaatz. 1990. Patterns of larval food production by hypopharyngeal glands in adult worker honey bees, Apidologie. 21, 457-468.

Koskella J. and J. Stotzky. 1997. Microbial utilization of free and clay-bound insecticidal toxins from *Bacillus thuringiensis* and their retention of insecticidal activity after incubation with microbes. Appl. Environ. Microbiol. 63: 3561-3568.

Langrock, I., T. Hurley and K. Ostlie. 2003. Farmer Demand For Corn Rootworm Bt Corn: Do Insect Resistance Management Guidelines Matter? Annual Meeting of American Agricultural Economics Association 2003. http//agecon.lib.umn.edu/cgi-bin/pdf%5Fview.pl?paperid=9055.

Lozzia, G.C. & Rigamonti, I.E. 1998. Preliminary study on the effects of transgenic maize on non target species. IOBC Bulletin, 21, 171–180.

Lozzia, G.C. 1999. Biodiversity of ground beetle assemblages (Coleoptera: Carabidae) in Bt corn and its effects on non target insects. Bollentino di Zoologia agraria e di Bachicoltura. 31: 37-50.

MacIntosh, S.C., T.B. Stone, S.R. Sims, P.L. Hunst, J.T. Greenplate, P.G. Marrone, F.J. Perlak, D.A. Fischhoff, and R.L. Fuchs. 1990. Specificity and efficay of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invert. Path. 56: 258-266.

Malone, L.A., E. P.J. Burgess, D. Stefanovic. 1999. Effects of a *Bacillus thuringiensis* toxin, two *Bacillus thuringiensis* biopesticide formulations, and a soybean trypsin inhibitor on honey bee (*Apis mellifera* L.) survival and food consumption, Apidologie 30, 465-473.

Malone, L.A. and M. Pham-Delegue. 2001. Effects of transgenic products on honey bees (*Apis mellifera*) and bumblebees (*Bombus* sp.), Apidologie 32, 287-304.

Malone, L.A., E.P.J. Burgess, H.S. Gatehouse, C.R. Voisey, E.L. Tregidga and B.A. Philip. 2001. Effects of ingestion of a *Bacillus thuringiensis* toxin and a trypsin inhibitor on honey bee flight activity and longevity, Apidologie 32, 57-68.

Malone, L.A., J.H. Todd, E.P.J. Burgess and J.T. Christeller. 2004. Development of hypopharyngeal glands in adult honey bees fed with a Bt toxin, a biotin-binding protein and a protease inhibitor, Apidologie 35, 655-664.

Manachini, B., M. Agosti and M. Rigamonti. 1999. Environmental impact of Bt-corn on non target entomofauna: synthesis of field and laboratory studies. XI Symposium Pesticide Chemistry.

Marra, M., G. Carlson, and B. Hubbell. 1998. Economic Impacts of the First Crop Biotechnologies. Electronic publication, North Carolina State University, Department of Agricultural and Resource Economics.
http://www.ag-econ.ncsu.edu/faculty/marra/FirstCrop/sld001.htm

Maus, C., G. Curé, and R. Schmuck. 2003. Safety of imidacloprid seed dressings to honey bees: a comprehensive overview and compilation of the current state of knowledge, Bull. of Insectology 56, 51-57. Moffett, J.O., R. H. Macdonald, and M.D. Levin (1970) Toxicity of carbaryl-contaminated pollen to adult honey bees, J. Econ. Entomol. 63, 475-476.

Melin, B.E. and E.M. Cozzi. 1990. Safety to nontarget invertebrates of lepidopteran strains of *Bacillus thuringiensis* and their beta-exotoxins. In Safety of microbial insecticides.

Meharg A.A. 1994. A critical review of labeling techniques used to quantify rhizosphere carbon flow. Plant Soil. 166: 55-62.

Men, X., F. Ge, C.A. Edwards and E.N. Yardim. 2004. Influence of pesticides applications on pest and predatory arthropods associated with trangenic *Bt* cotton and nontransgenic cotton plants. Phytoparasitica. 32: 246-254.

Moore, J. C., R. J. Snider, and L. S. Robertson. 1984. Effects of different management practices on Collembola and Acari in corn production systems. I. The effects of no-tillage and atrazine. Pedobiologia 26: 143-152.

Naranjo, S.E. and P.C. Ellsworth. 2002. Arthropod communities and transgenic cotton in the western United States: implications for biological control. In Proceedings of the First International Symposium of Biological Control of Arthropods, U.S. Forest Service, Amherst, MA.

NCFAP. 2002. National Center for Food and Agricultural Policy. Leonard P. Gianessi, Cressida S. Silvers, Sujatha Sankula and Janet E. Carpenter. Plant Biotechnology: Current and Potential Impact for Improving Pest Management in U.S. Agriculture – An Analysis of 40 Case Studies.

Picard-Nizou, A.L., M.H. Pham-Delegue, V. Kerguelen, P. Douault, R. Marilleau, L. Olsen, A. Toppan and C. Masson. 1995. Foraging behavior of honey bees (*Apis mellifera* L. *var. oleifera*), Trans. Res. 4, 270-276.

Picard-Nizou, A.L., R. Grison, L. Olsen, C. Pioche, G. Arnold and M.H. Pham-Delegue. 1997. Impact of proteins used in plant genetic engineering: toxicity and behavioral study in the honeybee, J. Econ. Entomol, 90, 1710-1716.

Orr, D.B. and D.A. Landis. 1997. Oviposition of European corn borer (Lepidoptera: Pyralidae) and impact on natural enemy populations in transgenic versus isogenic corn. J. Econ. Entomol. 90: 905-909.

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. Environ. Entomol. 26: 446-454.

Raps, A., J. Kehr, P. Gugerli, W.J. Moar, F. Bigler and A. Hilbeck. 2001. Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. Molec. Ecol. 10: 525-533.

Reed, G. L., A. S. Jensen, J. Riebe, G. Head, and J.J. Duan. 2001. Transgenic Bt potato and conventional insecticides for Colorado potato beetle management: Comparative efficacy and non-target impacts. Entomologia Experimentalis et Applicata 100: 89-100.

Riddick, E.W. and P. Barbosa. 1998. Impact of Cry3Aintoxicated *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and pollen on consumption, development, and fecundity of *Coleomegilla maculata* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 91: 303-307.

Riddick, E.W., G. Dively and P. Barbosa. 1998. Effect of a seedmix deployment of Cry3A transgenic and nontransgenic potato on the abundance of *Lebia grandis* (Coleoptera: Carabidae) and *Coleomegilla maculata* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 91: 647-653.

Romeis, J., A. Dutton and F. Bigler. 2004. *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). J Insect Physiol. 50: 175-183.

Russek-Cohen, E. and L.W. Douglass. 1999. Mixed models data analysis: using PROC MIXED for biological research. Mixed model shortcourse manual. P. 89-103. Jan. 11-14, 1999. Beltsville, MD.

SAS Institute. 1997. SAS user=s guide. SAS Institute, Raleigh, NC. 494 pp.

Saxena, D. and G. Stotzky. 2001. *Bt* corn has a higher lignin content. Am. J. Bot. 88: 1704-1706.

Sammataro, D. and A. Avitabile. 1986. The Beekeeper's Handbook Macmillan Publishing Company, New York, 148 pp.

Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D.R. Zeigler, and D.H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Molec. Biol. Rev. 62: 775-806.

Schmuck, R. 1999. No causal relationship between Gaucho® seed dressing in sunflowers and the French bee malady, Pflanzenschutz-Nachrichten Bayer 52, 257– 299

Schuler, T.H. , G.M. Poppy, B.R. Kerry, and I. Denholm. 1999. Potential side effects of insect-resistant transgenic plants on arthropod natural enemies. Trends in Biotechnology 17: 210-216.

Schuler, T.H., R.P.J. Potting, I. Denholm, S.J. Clark, A.J. Clark, C.N. Stewart and G.M. Poppy. 2003. 12: 351-361. Tritrophic choice experiments with Bt plants, the diamondback month (*Plutella xylostella*) and the parasitoid *Cotesia plutellae*. Transgen Res. 12: 351-361.

Schur, A., I. Tornier, and C. Neumann. 2000. Association of Institutes for Bee Research Report of the 47th seminar in Blaubeuren near Ulm Heinrich-Fabri-Institut of the Eberhard-Karis-University Tubingen 3-5 April 2000, Apidologie 31, 557-668.

Seeley, T.D. 1985. Honeybee Ecology a Study of Adaptation in Social Life, Princeton University Press, Princeton, NJ, pp. 80-106.

Seeley, T.D. 1995. The Wisdom of the Hive The Social Physiology of Honey Bee Colonies, Harvard University Press, Cambridge Massachusetts, 295 pp.

Shannon, C. E. and W. Weaver. 1949. The mathematical theory of communication. Urbana IL: University of Illinois Press.

Sims, S.R. 1997. Host activity spectrum of the CryIIA *Bacillus thuringiensis* subsp. *kurstaki* protein. Effects on Lepidoptera, Diptera, and nontarget arthropods. Southwest. Entomol. 22: 395-404.

Siedentop, S. 1995. A litterbag test for the assessment of side effects of pesticides on soil. mesofauna, Acta Zool. Fennica. 196: 357–360.

Sims, S.R. 1995. *Bacillus thuringiensis* var. *kurstaki* [CryIA (C)] protein expressed in transgenic cotton: effects on beneficial and other nontarget insects. Southwestern Entomol. 20: 493-500.

Sims, S.R. and J.W. Martin. 1996. Effect of the *Bacillus thuringiensis* insecticidal proteins on CryIA(b), CryIA(c), CryIIA, and CryIIIA on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola). Pediobiologia. 41: 412-416.

Sims, S.R. 1997. Host activity spectrum of the CryIIA *Bacillus thuringiensis* subsp. *kurstaki* protein. Effects on Lepidoptera, Diptera, and nontarget arthropods. Southwest. Entomol. 22: 395404

Sisterson, M.S., R.W. Biggs, C.O. Olson, Y. Carriere, T.J. Dennehy and B.E. Tabashnik. 2004. Arthopod abundance and diversity in Bt and non-Bt cotton fields. Environ. Entomol. 33: 921-929.

Sommerville, D.C. 2001. Nutritional value of bee collected pollens, A report for the Rural Industries Research and Development. Corporation #01/047, NSW Agriculture Goulburn, NSW Kustry, 166pp.

Sparks, A.N., H.G. Chiang, C.C. Burkhardt, M.L. Fairchild, and G.T. Weekman. 1966. Evaluation of the influence of predation on corn borer populations. J. Econ. Entomol. 59(1): 104-107.

Stark, J.D., P.C. Jepson, and D.F. Mayer. 1995. Limitations to use of topical toxicity data for predictions of pesticide side effects in the field, J. Econ. Entomol. 88, 1081-1088.

Steffey, K. L., M. Venditti, B. Ria Barrido, and A. S. Felsot. 2004. Effect of *Bacillus thuringiensis* corn on natural enemies of the European corn borer. Agricultural biotechnology: challenges and prospects: 139-150. American Chemical Society, Washington D. C.

Stotzky, G. 2000. Persistence and biological activity in soil of insecticidal proteins from Bacillus thuringiensis and of bacterial DNA bound on clays and humic acids. J. Environ. Qual. 29: 691-705.

Szentkiralyi, F. 2001. Ecology and habitat relationships. In: P. McEwen, T. New and A. E. Whittington (eds.), Lacewings in the Crop Environment, Cambridge University Press, Cambridge, UK.

Tapp, H. and G. Stotzky. 1998. Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. Soil Biol. Biochem. 30: 471-476.

Theiling, K.M. and B.A. Croft. 1988. Pesticide side effects on arthropod natural enemies. Agric. Ecosys. Environ. 21: 191-218.

Van den Brink, P.J. and C.J.F Ter Braak. 1999. Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. Environ. Tox. Chem. 18: 138-148.

Venditti, M.E. and K.L. Steffey. 2002. Field effects of Bt corn on the impact of parasitoids and pathogens on European corn borer in Illinois. In Proceedings of the First International Symposium of Biological Control of Arthropods, U.S. Forest Service, Amherst, MA.

Wilson and Flint. 2002. Presentation at the National Biological Control Conference.

Winston, M.L. 1987. The Biology of the Honey Bee, Harvard University Press, Cambridge, Massachusettes, 281 pp.

Wold, S.J., E.C. Burkness, W.D. Hutchison and R.C. Venette. 2001. In-field monitoring of beneficial insect populations in transgenic corn expressing a *Bacillus thuringiensis* toxin. J. Entomol. Sci. 36: 177-187.

Wraight, C.L., A.R. Zangerl, M.J. Carroll, M.R. Berenbaum (2000) Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions, PNAS 97, 7700-7703.

Xia. J.Y., J.J. Cui, L.H. Ma, S.X. Dong and X.F. Cu. 1999. The role of transgenic Bt cotton in integrated insect pest management. Acta Gossypii Sinica. 11: 57-64.

Yu, L., R.E. Berry and B.A. Croft. 1997. Effects of *Bacillus thuringiensis* toxins in transgenic cotton and potato on *Folsomia candida* (Collembola: Isotomidae) and *Oppia nitens* (Acari: Orbatidae). J Econ Entomol. 90: 113-118.

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

Zwahlen, C., A. Hilbeck, P. Gugerli and W. Nentwig. 2003. Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. Mol. Ecol. 12: 765-775.