IMPACT OF *Bacillus thuringiensis* TRANSGENIC CORN ON NON-TARGET AND PHYTOPHAGOUS ARTHROPOD POPULATIONS

by

TIMOTHY DALY

(Under the direction of G. David Buntin)

ABSTRACT

Transgenic field corn has been genetically engineered with the gene from the *Bacillus thuringiensis* var. *kurstaki* (Bt), which secretes a proteinaceous endotoxins that are toxic to certain species of lepidopteran pests. The effects of transgenic Bt corn containing the MON 810 event on non-target arthropods were compared to those in conventional, near isogenic corn at two locations in Georgia. The study was conducted in 2001 and 2002 using visual counts, pitfall traps, and corn ear insect evaluations. The only insect whose numbers and mortality was consistently affected by the Bt corn was the corn earworm, *Helicoverpa zea* (Boddie), a target insect. There were no consistent effects of the Bt toxins on non-target phytophagous and predaceous arthropods observed in the visual counts, nor on those non-target arthropods recovered from the pitfall traps and the corn ear dissection. From these results, transgenic Bt field corn containing the MON 810 event does not have an adverse effect on non-target phytophagous or predaceous arthropods.

INDEX WORDS: *Bacillus thuringiensis*, non-target arthropods, Cry1Ab toxins, soil dwelling arthropods, maize.

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DEDICATION

This thesis is dedicated to my parents, Thomas and Gloria Daly of Stone Mountain Georgia, to my Grandmother, Mrs. Flora Graham, of Decatur GA, Georgia.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Recent advances in genetic engineering have permitted the development of certain crop plants expressing the insecticidal toxins produced by the bacterium, *Bacillus thuringiensis* Berliner (Bt). This allows for the control of certain pestiferous insects with reduced use of synthetic insecticides. In 1995, the United States Environmental Protection Agency (EPA) approved the first registration of Bt corn, cotton, and potatoes. Presently the United States has the highest number and acreage of Bt crops grown in the world. More than 20 million acres of Bt transgenic crops were planted in 1999 and 2001. The percentage of Bt corn has grown from 1% (0.16 million hectares) in 1996 to 26% (8 million hectares) in 2001 (Shelton et al. 2002, Zhao et al. 2002).

Many researchers believe these insecticidal transgenic crops are the most important advancement in insect pest management since the development of synthetic chemical insecticides. Presently, at least 18 transgenic crops have been field tested, and three, corn, cotton, and potatoes, are widely grown in the United States. With the growing commercial use of Bt crops concern has arisen on how to manage and assess the risks associated with them. The widespread planting of millions of hectares of crops expressing the Bt insecticidal toxins increases the risk of pests developing resistance to the Bt toxins, and possible detrimental effects on non-target arthropods (Obrycki et al. 2001).

The overall impact of the Bt toxin on the environment is still poorly understood. The effect of transgenic crops may not be localized as initially believed, but could be spread in the wind and persist in the soil. Additionally, the effects of the toxin could migrate through the food chain and cause harm to other arthropod species that are distantly associated with the transgenic crops (Marvier 2001).

Many entomologists regard Bt crops as benign pesticides requiring careful management and evaluation. Ecological and evolutionary responses to these crops are quite probable given that they are used on such a large scale (Snow and Palma 1997). Recent studies indicate the impact may be subtle and complex, and potentially could be overlooked in the registration process for governmental approval of transgenic crops (Obrycki et al. 2001).

Bacillus thuringiensis **and its mode of action**

Bacillus thuringiensis is a gram-positive bacterium that normally exists in the soil. It was first isolated and described by Berliner in 1915. The bacterium secretes a proteinaceous crystalline endotoxin (Cry toxin) detrimental to certain insect species. Various strains of Bt secrete different toxins that are toxic to different groups of insects (Shelton et al. 2002). The Bt strains are classified based on the flagella (H) antigens and are increasing in number as more are isolated from different parts of the world (Cannon 2000). Most of the toxins target lepidopterans, like *B*. *thuringiensis* var. *kurstaki*. Others, like *B*. *thuringiensis* var. *israeliesis* target dipteran larvae, and *B*. *thuringiensis* var. *tenebrionis* targets coleopterans (see Table 1). Susceptibility is determined by the presence of specific receptors in the membranes lining the midgut epithelial cells (Snow and Palma 1997).

Susceptible insects have a high gut pH. Upon ingesting the Cry toxins, the protoxins are broken down enzymatically by midgut proteases. The toxins bind to certain high affinity receptors on the epithelial cells lining the midgut and insert themselves into

Bt STRAIN OR SUBSPECIES	**INSECT HOST**	**DELTA ENDOTOXINS[1]**
kurstaki HD-1	*Lepidoptera; Diptera*	Cry1Aa, Cry1Ab, Cry1Ac, Cry2A ,Cry2B
thuringiensis HD-2[2]	*Lepidoptera*	Cry1A, Cry1B
aizawai	*Lepidoptera*	CryAa, Cry1Ab, Cry1C, Cry1D, Cry1F
entomocidus	*Lepidoptera*	Cry1Aa, Cry1B, Cry1C
tenebrionis	*Coleoptera*	Cry3A
israeliensis	*Diptera*	Cry4A, Cry4b, CryC, Cry4D

[1/] All endotoxins are named by the suffix Cry which stands for crystalline protein and a number and letter system to designate affinities in toxicity and genetic specificity. Cry1 and Cry2 are specific toxins for Lepidoptera, Cry3 is specific to coleopteran, and Cry4 is specifi to Diptera, and Cry2A is also toxic to Diptera.

[2/] Bt *thuringiensis HD-2* also produces an endotoxin (extracelluarly secreted) that can be toxic to non-target insects. This strain is not used commercially in Bt products.

Table 1: A listing of endotoxins from different Bt strains and their spectrum of activity (From Carpenter et al. 2002).

transmembrane potential increasing the permeability of water, which causes the cell to the cell membranes forming ion channels (cation pores). The result is a loss of rupture (Fig 1). The insect stops feeding, becomes paralyzed, and then dies (Snow and Palma 1997, Aronson and Shai 2001).

The differing strains have different toxins, but the overall molecular structure of the toxin protein is the same. It consists of three domains. Domain I consists of seven alpha helices, which insert themselves into the cell membranes of the mid-gut epithelial cells leading to the creation of ion pores. Domain II consists of three anti-parallel beta sheets that bind to the receptors. Domain III is a beta-sheet that protects the exposed end (the C-terminus) of the active toxin from being broken down further by the midgut proteases (Aronson and Shai 2001, Li et al. 1991).

The gene in the *B. thuringiensis* bacterium has been isolated, and through either recombinant DNA tactics or by using a “gene gun,” has been inserted into the cells of

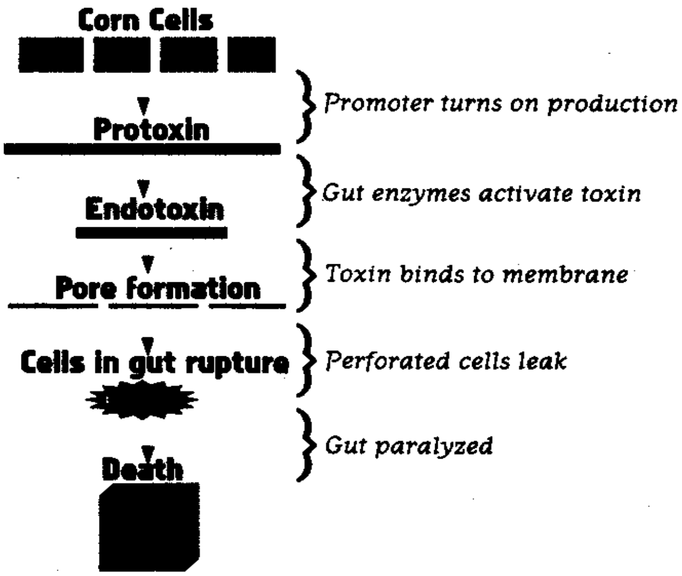

Fig. 1. Bt mode of action after being eaten by a susceptible insect (Ostlie 1997).

several different types of crop plants. There are three primary components of the genetic package placed into these plant cells: the promoter, the Bt protein gene, such as Cry1Ab, Cry1Ac, Cry3A, etc., and genetic markers. The promoter controls the amount of toxin produced and the location on the plant. Some promoters limit it to certain parts of the plant while others have whole plant expression. Genetic markers identify successful transformations and are referred to as an “event”(Ostlie et. al 1997; Tenuta et al. 2000).

Corn

Corn, *Zea mays*, is an annual grass crop seeded directly into the ground. The evolution of corn over the past millennia has brought about many different biotypes that can be cultivated in many geographic locations, from the tropics to the northern temperate regions. The majority of field corn (corn used for grain and silage production) is used for animal feed, but a substantial amount is also used for the production of ethanol and for human consumption in the form of high fructose corn syrup, starch, and corn oil. An estimated 140 million hectares of corn is grown worldwide, with the majority of it being cultivated in the United States, China, the European Union, Brazil, Mexico, Argentina, and India. In the United States, 32.2 million hectares of field corn and 227 million hectares of fresh and processed sweet corn, used for direct human consumption, were planted in the year 2000 (Carpenter et al. 2002).

Several transgenic events have been developed in corn each with a different type of endotoxin expression (Table 2). In Event 176, the toxin are expressed only in the leaves, pith, roots and pollen but is not effectively expressed in the ear or grain. The level of toxin declines as the plant ages. Two events of Bt corn on the market today; Bt11 by Syngenta and MON 810 by Monsanto. Both events contain the Cry1Ab gene and express the Bt toxins season long in both the foliage and reproductive tissue (Arches et al. 2001). Both are marketed as YieldGard.® Event CBH 351 containing the Cry9C gene and marketed as starlink Bt corn, was withdrawn because of regulatory concerns in 2001. A new event TC 1507, containing the Cry1F was deployed in 2002 as Herculex Bt corn. (Carpenter et al. 2002)

Bt target pests

Transgenic maize was originally developed for the control of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) and the Southwestern corn borer, *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae). Additionally, the Cry1Ab toxins suppress populations of the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera:

Noctuidae), and fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae).

The European corn borer (ECB) is the most destructive pest of corn in North America. The damage resulting from this insect pest exceeds $1 billion annually. An outbreak in Minnesota in 1995 alone caused losses of $285 million. Despite these losses, farmers are often reluctant to utilize current integrated pest management (IPM) strategies for controlling this pest. This is because the damage is hidden, the outbreak of heavy infestations in unpredictable, and the cost, health, and environmental concerns of using conventional insecticides (Ostlie et al.1997). ECB was first introduced into North America in the early 1900s (Steffey et al. 1999). It has expanded its distribution throughout much of North America and has the ability to adapt to a variety of environmental conditions. It also can attack other crops, such as snap beans, tomatoes, soybeans, and cotton (Tollesfon and Calvin 1994).

ECB larvae feed on all parts of the corn except the roots. Larvae hatching before the corn tassel stage feed primarily on the whorl causing minimal damage. After the corn begins to tassel, the larvae bore into stalks, and ears. This injury interferes with the plants' ability to translocate water and essential nutrients, thereby reducing plant vigor and ultimately yield. ECB over winters as late stage larvae within corn stubble and other corn debris left in the field after harvesting. Eggs are laid in masses on the underside of corn leaves. After hatching, small larvae feed on the leaves and then bore into the stalk as third instar larvae to pupate. ECB has one generation in the north, two generations per year in the Midwest, and three to four generations in the south. The first generation of adults emerge, mate, and lay eggs on the leaves near the ears. Second-generation larvae tunnel into the stalks and corn ears (Steffey et al. 1999, Carpenter 2002). ECB occurs in eastern Georgia and in some areas of the northern part of the state (Buntin 2003).

The southwestern corn borer (SWCB) is a native to Mexico and is distributed throughout the southern United States. SWCB occurs only in northwest and mountain regions of the state of Georgia (Buntin 2003). Its northern distribution is limited by temperature, soil type, and moisture levels, which impact the over wintering of the larvae. SWCB has two to three generations annually. First generation larvae seldom cause economic damage, whereas second and third generation larvae can cause severe economic damage. The larvae over winter in the root crown of the corn plant. A significant proportion of the SWCB population that over winters is killed by a variety of factors (extreme cold, rainfall, disease and predators), but only two percent of the population needs to survive to restore populations to normal levels by the next growing season. They pupate in the spring, and adults emerge from the corn stubble to feed on many of the plant tissues, depending on the growth stage of the plant. At the third instar, they tunnel into the stalk, and can cause damage to the growing point resulting in twisted, stunted plants. The damage can lead to lodging interferes with the harvest (Steffey et al.1999, Boyd and Bailey 2001).

The corn earworm (CEW) is native to the Americas and is found throughout the United States. It is a major pest of corn in Georgia. In addition to corn, it also attacks many other hosts including cotton, tomato, and soybean. The females deposit eggs on the leaves and corn silk. The larvae crawl on the plant until they find a suitable feeding site. The larvae attack the corn leaves, tassels, silk, with the most significant damage in the kernels in the corn ear. Penetration of the corn ear by this pest provides a point of entry for secondary pests and microbes. Several larvae initially infest the corn ear, but only one or two remain because of cannibalism. Mature larvae leave the feeding site, and drop to the ground, where they pupate several cm below the surface for 14 days. The adults emerge after 14 days and are mostly nocturnal. Oviposition occurs three days after emergence. There are several generations during the growing season (Steffey et al.1999).

Fall armyworm, *Spodoptera frugiperda* is found throughout the United States east of the Rocky Mountains, but it is not cold tolerant and the pupae cannot survive the winter in areas where the ground freezes. Fall armyworms over winter as larvae in later instars in southern Florida and the Caribbean. In the spring they pupate and emerge as adults that migrate northward. They deposit their egg masses on corn leaves and other vegetation like certain forage and turf grass plants (Steffey et al. 1999). When the eggs hatch the larvae begin feeding on the whorls, tassels, or ears, causing significant damage. The fall armyworm does its most significant damage by feeding on the ears even though its feeding on the leaves is the most noticeable. When larvae mature, they pupate in the soil near the base of the plant. Within 14 days, the adults emerge. Fall armyworm completes one to three generations in the northern states, more than five generations in the southern states (Dill and Handley 2001, Steffey et al. 1999).

There are several other arthropod pests of corn that are not susceptible to the Bt toxins including the chinch bug, *Blissus leucopterus leucopterus* (Say) (Hemiptera: Lygaeidae), dusky sap beetle, *Carcophilus lugribus* (Murray) (Coleoptera: Nitidulidae), and the corn rootworm complex, *Diabrotica* spp. (Coleoptera: Chysomelidae).

The control of these non-target pests of corn by Bt toxins is expected to be minimal to none. However, the reduction of caterpillar pests may indirectly reduce the

Table 2.Types of Bt for lepidopteran control in corn.

Event	Gene	Trade Name	Expressions
E-176 (1)	Cry1Ab	Knockout/Maximizer NatureGuard	Green tissue, Pollen
MON 810 (2)	Cry1Ab	YieldGard®	Whole plant
Bt-11 (2)	Cry1Ab	YieldGard®	Whole plant
DBT 418 (2)	Cry1Ac	Bt Extra	Whole plant
CBH 351 (2)	Cry9C	Starlink	Whole plant
TC 1507 (2)	Cry1F	Herculex I	Whole plant

(1) Maize pollen and phenol carboxylase promoters
(2) Cauliflower mosaic virus 35

infestation by some of these non-target pests, for example, the dusky sap beetle, *Carcinophilus dimidiatus* (Coleoptera: Nitidulidae) may be reduced because it is attracted to ear damage caused by lepidopteran larval feeding (Dowd 2000, Jepson 1994). Sustainable crop management strategies, loss prevention, and maintaining soil health are essential in maintaining the biological productivity of agricultural ecosystems (Olfert et al. 2002).

It is not expected that Bt-transgenic corn would affect sucking insects like aphids and thrips. Aphids consuming unsuitable, lower quality corn have a reduced body size, developmental delays, increased mortality, and reduced fecundity. There are no data on whether the Cry1Ab insecticidal toxins are in the phloem, which is the plant tissue that sucking insects, like aphids, feed on. By measuring parameters on both pestiferous and beneficial arthropods could help in the determination if there are indirect any tritrophic effects of Bt crops on natural enemies, like the green lacewing *Chrysoperla carnea* Stephens.

Another sap sucking arthropod that is a pest, is the two spotted spider mite, *Tetranynchus urticae* Kock. *Tetranynchus urticae* feeds by sucking the cell contents directly, as well as taking up part of the cytoplasm and the cellular bodies inside the cell. It is almost certain that *T. urticae* ingests the Cry1Ab protein when feeding on corn. Lozzia et al. (2000) examined the impact of *T. urticae* feeding directly on transgenic corn, and how it affects the life cycle of this mite. No significant interactions occurred between the life history of *T. urticae* feeding on Bt corn vs. non-Bt corn on the various parameters considered. There were no significant differences in the embryo, post embryo, pre-imaginal, imaginal or total development of *T. urticae*. These data suggest that *T. urticae* most likely is not adversely impacted by the Cry1Ab present in the green tissues of the corn.

Use of Bt toxins in agroecosystems

As agricultural ecosystems increase in size, natural habitats are decreasing. Natural enemies that are well adapted to agricultural ecosystems will play a larger role in controlling pest populations than those species of beneficial arthropods associated with natural ecosystems (Olfert et al. 2002). Plants engineered to express the Bt Cry toxins may have both direct and indirect effects on non-target species. The Cry1Ab and Cry1Ac toxins primarily target lepidopterans and the Cry3A toxins target coleopterans with Cry3A. Effects of non-pest organisms in these groups potentially could vary widely due to differing sensitivity among species and differing concentration of these toxins produced by different tissue or transgenic lines (Wolfenbarger and Phifer 2000).

Preservation of natural enemies is one of the most important aspects of integrated pest management. Natural enemies regulate and suppress many types of insect pests in a multitude of crops. Their activity is not limited to individual crops, because natural enemy populations can move among crops between fields and habitats. Any detrimental impact Bt crops have on naturally occurring biological control through natural enemies are not limited to any particular crop, location, or season. Site specific variability of the field populations of these beneficial arthropods in one field may be unable to provide control of pestiferous insects in neighboring fields or future crops. The level of the impact on natural enemy population will be proportional to the acreage planted with Bt-expressing plants (Bernal et al. 2002).

The overall understanding of the impact of the Cry1Ab toxins on these non-targets is still quite limited, because these organisms have received less attention than the targeted pests. Do these toxins disrupt with the growth, development, and distribution of these arthropods relative to conventional, isogenic corn crops? Short-term risks to natural enemies will be a function of the susceptibility of the organism to the toxin and the level of the exposure. Additionally, sublethal effects and the impact of the exposure on multiple generations could be one of the most sensitive indicators in risk assessment. The widespread use of different types of transgenic crops increases the likelihood for natural enemies to encounter hosts having varying levels of susceptibility to the Cry1Ab toxins. To assure that deployed Bt crops are sustainable and environmentally benign, long-term evaluations of these pest management strategies with naturally occurring biocontrol organisms is essential (Canon 2000).

In the agricultural ecosystem, concern exists those populations of beneficial arthropods may decrease if pests are completely controlled. In a broader context, the ecological consequences of using Bt plants in a cropping system are likely to be greater than simply abstaining from a pesticide intensive management system, but not as serious as the impact of the use of conventional pesticides. Preservation of predatory arthropods associated with crop plants is one of the most important tactics of modern pest management (Canon 2000). By studying the interactions of organisms susceptible to Bt transgenic crops and their possible impact on natural enemies under both laboratory and field conditions, more effective pest management strategies can be developed (Pilcher et al. 1997).

If the transgenic corn hybrid can affect insect pest populations, then it also has the potential to interfere with the behavior and population dynamics of non-target natural enemies of the pests. Beneficial predators and parasitoids can be adversely impacted by the depletion of prey/host, changes in predator/parasite behavior, or ingestion of the toxins that have accumulated in the host/prey that have consumed the transgenic corn material. The potential adverse effects will be greater for specialist natural enemies feeding exclusively on one type of host on a crop plant, than for generalists that feed on a wide variety of prey on a range of plants. Additionally non-target organisms could be adversely affected by feeding directly on the Bt plants, or soil insects may be impacted by soil exudates from the plants (Schuler 1999). Some studies indicate no effects on the survivorship or reproduction of predatory arthropods that consume prey that have been feeding on Bt plants. Other studies indicate that the bioaccmulation of these toxins could be detrimental (Wolfenberger and Phifer 2000).

Most of the research conducted on possible detrimental impacts of Bt has been with the application of conventional microbial sprays. There are some differences between the conventional and transgenic form of Bt. Conventional Bt applications contain numerous types of Bt toxins, whereas the transgenic Bt plants only express one of the toxins (Figure 2). Conventional Bt toxins also are in spores or modified formulations that can affect the efficacy of the toxin.

With the use of conventional sprays only chewing arthropods can ingests the Bt Proteins, whereas in the transgenic Bt plants the proteins also maybe ingested by sucking insects. Bt insecticides consist of a mixture of spores and crystalline inclusion bodies that have to undergo a complex activation process to induce the lethal effects in susceptible insects. Also, the conventional Bt insecticide degrades rapidly and requires sprays at regular intervals to maintain an effective level of control, whereas in the transgenic plant toxins are expressed continually. In transgenic plants, the Bt proteins are expressed continually as the activated toxin, thus overcoming some of the disadvantages of the conventional sprays. The rapid breakdown of the conventional microbial applications limits the temporal window of Bt exposure to herbivorous arthropods feeding on the target plant. The delta endotoxins are inactivated and the spores decrease rather rapidly. In transgenic plants, the toxins are usually expressed continually throughout the lifetime of the plant (Jepson et al.1994, Snow and Palma 1997, Cannon 2000). This could present possible hazards to non-target arthropods that are not present in treatments with conventional microbial applied Bt sprays (McEwen et al. 2001, Aronson and Shai 2001). Transgenic plants express the toxin itself instead of the inactive pro-toxin found in the conventional bacteria. Therefore, non-target insects ingesting these toxins do not need a high gut pH and the appropriate protease enzymes for solubilization and cleavage of the protoxins, thus reducing the specificity of the toxins. Non-target insects, potentially, may be susceptible to these Bt toxins as well (Tapp and Stotzky 1999).

Previous studies on the impact of conventional sprays on non-target species have yielded conflicting results. Most have shown no adverse impacts; other studies show the sprays may be of some detriment to certain non-target species of arthropods (Hilbeck et al. 1998a,1998b, 1999, Chapman and Hoy 1991, Giroux et al. 1994). Laboratory studies have show that there may be some detrimental effects of Bt on immature hymenopterans parasitoids, but the recommended application rates had little impact on the emergence of the parasitoid adults from parasitized host treated with Bt (Blumberg et al. 1997, Atwood et al. 1999).

Impact of Bt on Monarch butterflies and other non-target lepidopterans

Whaley et al. (1998) conducted research on how aerial application of Bt sprays targeting gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), in forest ecosystems impacted the populations of several non-targets lepidopterans. Aerial sprays had an effect on the non-target lepidopterans, although not all of them affected in the same way. Single applications, often used in suppression programs, probably have a negligible effect on the non-target populations. Susceptibility to single and multiple applications must be determined on a specific basis. Field and laboratory tests were conducted on the impact of aerial Bt sprays on forests in Mill Creek Canyon, Utah, and examined the impact on three non-target lepidopteran species, *Incisulia fotis* Strecker, *Callophoris sheridanii* W.H. Edwards (Lepidoptera: Lycaenidae), and *Neomis ridingsii* W.H. Edwards (Lepidoptera: Satyridae). All three have restrictive habitats, specific host

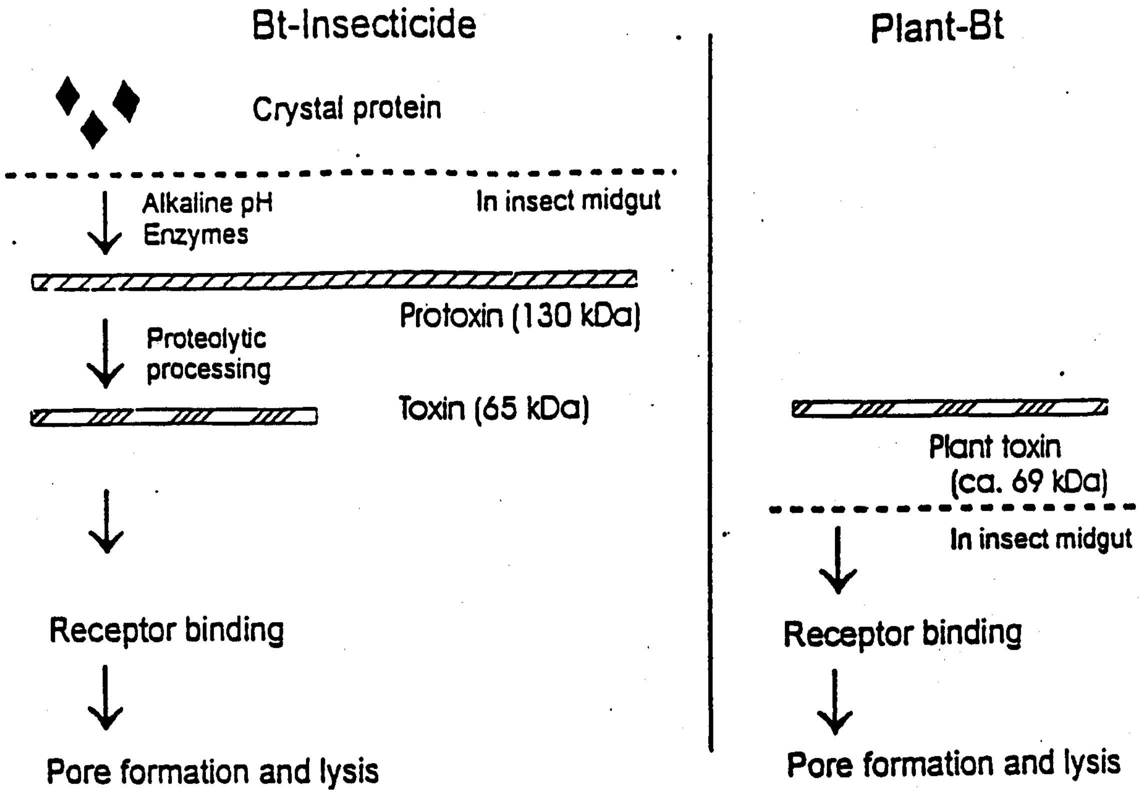

Fig. 2. Differences in Bt proteins present in most microbial formulations and expressed in transgenic plants. (From McEwen et al., 2001)

plant requirements, and their phenologies are similar to the target pest, the gypsy moth. Bioassay analyses indicate that these species had increased mortality when exposed to Bt sprays, and their populations could be significantly affected during gypsy moth eradication programs unless appropriate measures are taken. The potential impact of drift on other Bt sensitive lepidopterans could be significant even when theseorganisms are some distance from the treated areas (Whaley et al. 1998).

Recently there has been publicity about the impact of Bt corn pollen on the monarch butterfly, *Danaus plexxipus* (L.) (Lepidoptera: Danaidae). The primary food source for the monarch is the milkweed, *Asclepias* spp. (Family: Asclepiacea). This plant grows throughout North America, and is often found growing in old fields and near the edges of cornfields. Losey (1999) found, that larvae of the monarch reared on milkweed leaves dusted with Bt corn pollen consumed less, had a slower growth rate, and higher mortality rate than those dusted with pollen in laboratory bioassays from non-Bt corn plants. The average weight of surviving larvae feeding on plants dusted with Bt pollen at the end of the experiment was 0.16 ± 0.03 grams less than half the average of those feeding on leaves dusted with non-Bt pollen, which was 0.38 ± 0.02 grams.

The density of corn pollen deposited on milkweed varies. Pleasants et al. (2001) measured the densities of the pollen inside and outside cornfields in different localities. The pollen density was the highest inside the corn field at 170.6 grains/cm^2 and progressively decreased from the edge of the field outward, falling to 14.2 grains/cm^2 at 2 m from the field edge. Leaves on the upper portion of the plant, where the monarch larvae prefer to feed, had only 30-50% of the density of the pollen on the lower leaves.

Raynor et al. (1972) studied the movement of corn pollen and found around 30% as much pollen at 8 m as compared to 1m from the field edge, and corn pollen concentrations 60m were 1% of those found 1m from the source. Corn pollen can be dispersed at least 60 feet from its point of origin by the wind. Monarchs feed on milkweed growing in and near corn fields throughout their development, and the milkweed densities in agricultural environments are as high or higher than in nonagricultural environments. Most of the monarchs in the upper Midwest are likely to originate from cornfields and other agricultural habitats (Oberhauser et al. 2001).

Jesse and Obrycki (2000) showed similar results. They found that larvae feeding on *A. syriaca* plants dusted with Bt corn pollen had a much higher mortality rate after 48 hours (20 ± 0.03%) compared to larvae feeding on leaves with no pollen. Mortality at five days of monarchs exposed to 136-pollen grains/ cm^2 of Bt corn ranged from 37 to 70%. These effects may be observed at least 10 m from the borders of the transgenic corn fields based upon the quantification of the wind dispersal of the pollen beyond the field. The highest larval mortality, according to this study, is on *A. syriaca* plants within 3 m of the edge of transgenic cornfields.

The non-target impacts of transgenic Bt corn pollen on one species of oligophagous lepidoptera, the monarch butterfly, shows the insecticidal effects of transgenic crops can extend beyond the field borders and show this genetically modified crop can influence food webs that are not corn based (Jesse and Obrycki 2000). Corn plants shed pollen for eight weeks during July and August, when the monarchs are actively feeding. The majority of the monarch's summer range is in the Midwestern U.S., where most of the corn is grown in North America (Losey 1999, Wassenmar and Hobson 1998).

There is considerable evidence that refutes these findings. Critics have pointed out numerous weaknesses in the Losey study. The monarch larvae were fed high amounts of the Cry1Ab toxin that is lepidopteran specific. It is not surprising that a lepidopteran species like the monarch should be affected when force-fed plant material that expresses the Cry1Ab toxin that specifically targets lepidopterans.

These were laboratory experiments and not representative of the field conditions in which the monarch feeds. It would have been more convincing to have "choice" experiments, where, like the field, monarchs are exposed to some milkweed leaves that have been dusted with the Bt corn pollen, and some that have not. The Losey study indicates the presence of Bt or non-Bt corn pollen reduces the leaf consumption by the larvae. “Choice” experiments would have investigated the monarch larvae avoidance strategy. Ecological risks are a function of the exposure (environmental dose) and the effect (toxicological dose) on organisms. The amount of pollen dusted on the milkweed leaves were not quantified in the Losey study. Therefore we cannot establish a relationship between pollen exposure and the effect from this data. The Losey study used inappropriate controls. They used pollen from an unrelated hybrid rather than pollen from the isogenic hybrids in which N4640-Bt (used for the Bt toxin expressed by Event 176) was based. The degree of toxicity expression varies with the Bt transformation event (Hodson 1999, Hellmich 2001).

Hellmich et al. (2001) investigated the relative toxicity Cry1Ab toxin of several Bt-corn events on Monarchs: Event 176, MON 810, and Bt11. Monarch larvae were tested either by being fed the Cry toxins in an artificial diet, milkweed leaves with the Bt corn pollen applied to it, or Bt corn pollen contaminated with corn tassel material applied to milkweed leaves. Of the events tested, only event 176 consistently had detrimental impacts on the monarch larvae. Event 176 is currently less than 2% of the transgenic corn planted, and is being removed from the market. The other two events, MON 810 and Bt11, had no noticeable effects on the larvae.

Another lepidopteran that has been the subject of research and concern is the black swallowtail, *Papilio polyxenes* (Fabricius) (Lepidoptera: Papilionidae). Its range is throughout the Eastern United States and it feeds on a narrow range of apiaceous plants, such as *Daucus carota, Conium maculatum*, and these plants are often found growing around the edges of cornfields. Its larval development takes two to three weeks depending on temperature and food availability. Exposure to Bt corn is quite likely for this insect. Wraight et al. (2000), conducted field studies by placing early instars of their larvae on food plants growing along the edges of the Bt cornfields (Pioneer brand 34R07 containing the MON 810 event). Also, laboratory assays were carried out to determine the range of pollen toxicity. Only a few of the larvae in either test perished. The Bt pollen from varieties tested were shown to be unlikely to affect wild population of the swallowtails.

However, another study of Bt corn pollen on the black swallowtail (Zangerl et al. 2001) had different results. The previous study by Wraight used corn pollen from corn plants engineered with event MON 810, whereas this one used pollen from corn plants engineered with event 176. The bioassay indicates that the concentrations of event 176 pollen as low as 100 pollen grains/cm^2 caused significant mortality in the larvae. The main difference in this research when compared to that of Wraight et al. (2002), is that Bt corn from event 176 was used instead event MON 810. The pollen in event 176 has 40 times the amount of the Cry1Ab endotoxin expressed in it as compared to the pollen in event Bt 11.

Jesse and Obrycki (2002) studied the effect of Bt corn pollen on a non-target lepidopteran, the milkweed tiger moth, *Euchatia egle* (Drury) (Lepidoptera: Arctiidae). *Euchatia egle* larvae consumed *A. syriaca* that had been dusted with Bt corn pollen and anthers from two transgenic corn events, Bt11 and MON 810. There was no difference in mortality of the *E. egle* larvae following the 48-hour exposure to the transgenic corn material when compared to that of the non-transgenic corn material. The transgenic corn pollen from either event does not adversely affect lepidopteran field situations.

Effect of transgenic Bt crop on select natural enemies

Of the more than 400 species of arthropods that have been observed in the corn ecosystems during the growing season, only small percentages are pests. These pests are often attacked by natural enemies. Practical information on the effect of Bt toxins on these entomophagous arthropods is lacking, because they have been studied less than the actual pests of corn. Several species of lady beetles (Coleoptera: Coccinellidae) are common natural enemies observed in cornfields: twelvespotted lady beetle, *Coleomegilla maculata* DeGeer; the convergent lady beetle, *Hippodamia convergens* Guerin-Meneville; sevenspotted lady beetle, *Coccinella septempuncta* (L.); and the scymnus lady beetle, *Scymnus* spp. Other natural enemies that typically occur in corn include the insidious flower bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae); damsel bugs, *Nabis* spp. (Hemiptera: Nabidae); big-eyed bug, *Georcoris punctipes* (Say) (Hemiptera: Geocoridae); hooded beetles, *Notoxus* spp. (Coleoptera: Anthicidae); and numerous species of ground beetles, (Family: Carabidae); rove beetles, (Family: Staphylinidae); and green lacewings *Chrysoperla spp.*(Neuroptera: Chrysopidae), and spiders (Steffey et al. 1999).

Transgenic plants express their toxins in high doses in their tissues through most of the growing season. The toxin becomes available to natural enemies of arthropod pests by feeding on susceptible or sublethally affected herbivorous prey that consume transgenic plant material. The Bt toxins may become more widely spread in the food chain than when conventional Bt insecticides are used (Zwalen et al. 2000). Generalist predators are common in agroecosystems in North America. They play an important role in the suppression of potential crop pests and can prevent the outbreaks of secondary pests. Management practices relying solely on broad-spectrum pesticides can disrupt these populations. Because of the selective nature of transgenic plants, their use in an IPM system may help relieve the deleterious effects of traditional insecticides on these predators (Riddick et al. 2000).

The overall understanding of the possible detrimental effects, if any, of the Cry toxins on beneficial insects are still poorly understood. Previous research examining Bt toxins or their microbial produced equivalents on beneficial arthropods have resulted in conflicting results. Some of the research has shown no noticeable effects on these organisms (Dogan et al. 1996, Pilcher et al. 1997, Riddick and Barbosa 1998). Other studies have shown effects on beneficials like the green lacewing, *C. carnea* (Croft 1990; Hilbeck et al. 1998a, 1998b, 1998c). The differing results could be the result of differing methods used in these studies. This demonstrates the need of additional research in the use and development of appropriate experimental methods (Zwalen et al. 2000).

There have been several previous investigations on the Cry toxins on non-target arthropods in cotton and potatoes. Sims (1995) examined the possible effects of the Cry1Ac, the Bt toxin expressed in transgenic cotton plants, on a variety of target and non-target insects. The species include: *Anthomonis grandis* Bohemon (Coleoptera: Curculionidae), *Diabrotica undecmipunctata howardi* Barber (Coleoptera: Chrysomellidae), convergent lady beetle, the honeybee, *Apis mellifera* L. (Hymenoptera: Apidae), *C. carnea*, and the target species of the Cry1Ac toxins *H. zea*, *O. nubilalis,* and *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), and. they were tested using a diet incorporated feeding bioassays in the laboratory that exposed the insects to a high dose concentration of the Cry1Ac toxin. Of all of the species tested, only the lepidopteran species had a >50% mortality rate when fed the Cry toxin. The species of the other orders had a mortality rate that did not differ significantly from the control tests. The data from the tests indicate the Cry1Ac toxins expressed in transgenic cotton plants are specific for the targeted lepidopteran pests and its direct risks to the non-target beneficial insects are negligible.

Bacillus thuringiensis var. *tenebrionis* secretes a toxin, Cry3A that targets coleopteran pests. Transgenic potatoes have been engineered to express the Cry3A toxin with the primary target insect being the Colorado potato beetle, *Leptinotarsa decemelineata* (Say) (Coleoptera: Chrysomellidae). This beetle is a common, native phytophagous species inhabiting both managed and natural ecosystems throughout North America and is often found in abundance in potato fields.

Riddick and Barbosa (1998) examined the possible deleterious impact of the Cry3A toxin on the growth, development, and reproduction of *C. maculata* fed on eggs and larvae of *L. decemilineata* that had consumed transgenic potatoes compared to larvae consuming non-transgenic potatoes. *Coleomegilla maculata* is a common native, predatory coleopteran that inhabits both managed and natural ecosystems throughout North America and is often found in abundance in potato fields. The experimental results indicate that *C. maculata* fed eggs and larvae that had consumed transgenic potato tissue develop showed no significant differences in development and their ability to consume the prey, and showed no observable effect on the deposition of eggs than those that have consumed non-transgenic plants. Dogan et al. (1996) had similar findings for the convergent lady beetle, *H. convergens,* fed green peach aphids, *Myzus pericae* (Sulzer) (Homoptera: Aphididae), that had ingested Cry3A expressed in potatoes toxins from transgenic potatoes. No detectable effect on these lady beetles was observed. Riddick et al. (2000) examined possible impacts of the Cry3A toxins on several ground dwelling coleopterans and on dominant plant foraging lady beetles and heteropterans. Each order observed had three species. No statistically significant differences were observed in the mortality, the level of aphid consumption, development or reproduction in the beetles populations.

Pilcher et al. (1997) was one of the first to test the impact of the Cry1Ab toxins on non-target predatory insects. Three abundant predators in North American corn ecosystems were studied *O. insidiosus, C. maculata*, and *C. carnea,* all of which consume *O. nubilalis* eggs and larvae. These species were exposed to Bt corn pollen in the laboratory, and the effects on preimaginal development and survival of these insects was compared to those exposed to non-Bt corn pollen. Also, the study examined field abundance and temporal occurrence in plots of Bt and non-Bt corn plots. No significant differences were observed in the preimaginal development and survival of these three species exposed to Bt corn pollen when compared with those exposed to non-Bt corn pollen. The number of these species observed in the Bt corn plots did not differ significantly from those in non-Bt corn plots during field evaluations. Predator numbers observed before, during, and after pollen shedding suggests Bt corn pollen will not have any adverse effects on generalist natural enemy populations. However, the populations of specialist natural enemies could be affected if their prey that is susceptible to Bt decreases in number.

A common predaceous insect found in corn ecosystems is the insidious flower bug, *Orius insidiosus*, which feeds on a variety of arthropods, including thrips, mites, aphids, insect eggs, and young caterpillars (Steffey et al. 1999). Conventional insecticides often are detrimental to *O. insidiosus* populations leading to the concern that the corn plants expressing the Cry1Ab toxins could have a potential detrimental impact on the *Orius* populations.

Al-Deeb et al. (2001) assessed the Cry1Ab toxin's impact on *O. insidiosus.* They evaluated the weight, developmental time, and mortality of *O. insidiosus* after consuming Bt-fed ECB larvae, the mortality of immature *O. insidiosus* fed Bt corn silk, and visual counts of field populations of *O. insidiosus* in Bt corn. The results were compared with those from non-Bt corn. The weight, developmental time, and mortality of *O. insidiosus* nymphs fed on Bt and non-Bt corn were not significantly different. The *O. insidiosus* fed corn silk suffered 100% mortality, but the researchers attribute this to the nutritional quality of corn silk, which consists mostly of sugars and water. The insects need other supplemental nutrients in their diet to survive. The mortality was the same when fed silk from both Bt and non-Bt corn. No significant differences were detected in the abundance of field populations of *O. insidiosus* between the two types of corn.

Orius majusculus (Reuter), a close relative of *O. insidiosus,* is a common predaceous insect native to Europe. One of its major food sources is thrips. It has been commercially available to growers for years as a biological control agent of thrips and other pests. Zwalen et al. (2000) conducted tri-trophic feeding experiments to assess the rate of development and mortality of immature *O. majusculus* when reared on *Anophrothrips obscurus* (Muller) (Thysanoptera: Thripidae) that had fed on Bt corn. The main objective was to quantify the effects of Cry1Ab intercepted prey on the growth, development, and mortality of O. *majusculus.* There were no significant differences in growth, development and mortality between *O. majusculus* reared on Bt-fed prey versus non-Bt-fed prey. There are two possible reasons for this. First, the *O. majusculus* may not be sensitive to the Cry1Ab toxins because of the lack of specific receptors in their midgut epithelial cells or the lack of biochemical processing mechanisms. Second, the levels of Bt ingested by the thrips could have been too low to be effective.

Larvae of C. *carnea* are predaceous and are important natural enemies of a variety of pests in many agroecosystems. Their immature life stages consists of three instars, and it consumes many different types of pests including aphids and lepidopterans. Because of the importance of *C. carnea* for biological control, it is one of the most commonly tested species for pesticide side effects (Croft 1990). Several studies (MacIntosh et al. 1990, Sims 1995, and Pilcher et al. 1997) found no major impact of the Cry1Ab toxins on *C. carnea*. Hilbeck et al. (1998a) conducted several experiments assessing the possible impact of the Cry1Ab toxins on *C. carnea*. Two different lepidopteran prey species, *O. nubilalis*, a target of Bt, and Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval), a non-target of Bt, were reared on Bt corn and some were reared on non-Bt corn. Overall, the mortality of the immature *C. carnea* was higher when fed Bt prey (62%) as opposed to non-Bt prey (37%). The *C. carnea* larvae that consumed Bt-fed *S. littoralis* developed at the same rate as those that had consumed Bt-free *S. littoralis*. However, the larvae that consumed Bt fed *O. nubilalis* developed at a much slower rate than those that consumed Bt-free *O. nubilalis* by an average of three days. The increase in time of development for the *C. carnea* that consumed Bt fed *O. nubilalis* was more than likely due to a combined effect of being exposed to the *Bacillus thuringiensis* toxins and nutritional deficiencies caused by consuming sick prey.

There has recently been some concern amongst researchers that the current methods of assessment do not adequately account for the novel, modified activated form of the Bt toxins expressed in transgenic plants and the long duration of availability to herbivores. More precise methods are needed to be able to determine how these toxins may be impacting non-target, beneficial insects (Jepson et al.1994, Snow and Palma 1997).

The lethal and sublethal effects of Cry1Ab on *C. carnea* may be the result of the toxins on the nutritional quality of the targeted prey insect. In order to differentiate the direct effects from indirect effect, laboratory bioassays of pure Cry1Ab toxin and artificial diet are necessary. Hilbeck et al. (1998b) further tested the toxins directly against *C. carnea* by using an artificial system consisting of the Cry1Ab toxins encapsulated in paraffin spheres. Only second and third instar immature *C. carnea* can penetrate the surface of the paraffin spheres. The first instars were fed small foam cubes soaked in a liquid diet containing either the Cry1Ab toxins (100 μg/ml) or just water (control). After reaching the second instar, all larvae were fed the regular encapsulated diet. When reared only on a diet with the toxin, the mortality rate was 57% compared with the control at 29%. Also, the *C. carnea* immatures had higher mortality (29%) when they received the toxins later in their larval development compared to the control (17%). These results demonstrate that Cry1Ab is toxic to immature *C. carnea* at the rate used when fed an artificial diet. In comparison, for the corn cultivar used in previous experiments (Hillbeck et al. 1998a), the concentration of the Cry1Ab protein was never more than 4μg/g of fresh weight in leaves, and even lower in other tissues. It may have something to do with the biochemical processing of the Cry1Ab toxin in the

C. carnea gut, thereby retaining and increasing its activity. Also, *C. carnea* could possibly have some binding sites in its midgut as in the lepidopteran larvae. Neuropterans are more closely related to lepidopterans than any other order.

There is a difference in the concentration of the Cry1Ab toxin used for this study when compared with the level expressed in the transgenic corn. In Bt corn, the concentration of the Cry1Ab toxin does not exceed 4 µg per gram of fresh leaves and is lower in other tissues (Fearing et al. 1997). Most likely the amount of the toxin passed on to the immature Cry1Ab by the target prey in the field is much smaller than the 100 µg/ml by Hillbeck et al. (1998b) used in the study. Further research conducted by Hillbeck et al. (1999) sought to quantify and compare the resulting effects of *C. carnea* being fed *S. littoralis* reared on differing concentrations of activated Bt Cry1Ab endotoxin on mortality and developmental time to previous studies that have investigated prey mediated effects of transgenic Bt corn. Also, they studied the effects of feeding the Cry1Ab toxin directly to the *C. carnea*. The prey was *S. littoralis* fed three different concentrations of the Cry1Ab toxins, 25, 50, and 100 µg/g diet used. The *C. carnea* reared on the diet containing 100 µg/g of the Cry1Ab toxin had the highest mortality rate of 78% and declined with decreasing concentrations.

The research by Hilbeck et al. (1998a, 1998b, and 1999) is somewhat controversial. As mentioned earlier, other research studies (MacIntosh et al. 1990, Sims 1995, Pilcher et al. 1997, Lozzia 1998) have indicated that the Cry1Ab toxins impact on *C. carnea* is minimal to non-existent. *Chrysoperla carnea* preys on several species of arthropods, like corn aphids, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae), numerous species of thrips, and *O. nubilalis*. *Chrysoperla carnea* has a limited window of time to feed on the larvae of *O. nubilalis* due to the fact the *O. nubilalis* larvae quickly bore into the cornstalk after hatching. Thus, *O. nubilalis* is not the most abundant or preferred food choice for *C. carnea*, but in a laboratory setting, as with any generalist predator, other prey species can be a source of food, especially when nothing else is available (Lozzia et al, 1998).

Lozzia et al. (1998) examined in greater depth, the impact of Bt corn on the biology of non-target phytophagous insects and predators. They chose a tritrophic relationship of *Rhopalosiphum padi* (L.) that has consumed transgenic Bt corn and non-Bt corn as the herbivore and *C. carnea* as the predator. No significant adverse effects of the transgenic Bt corn were observed on the developmental time, longevity, and the fecundity of *R. padi*. There were no significant differences in the developmental time or mortality of *C. carnea* being reared on aphids that were fed Bt corn versus aphids that were fed non-Bt corn. These studies had different results than the ones conducted by Hilbeck et al. (1998a, 1998b, and 1999). In actual field situations, *C. carnea* is not likely to exclusively feed on *O. nubilalis*, and more than likely will prefer to feed on aphids. This study indicates that transgenic Bt corn does not affect a common sap sucking insect at least of its major predators, *C. carnea*.

The United States Environmental Protection Agency (EPA) noted that

> "The experimental evidence [conducted by Hilbeck] did not permit a distinction between direct effects due to the CryI1A(b) protein on the predator versus an indirect effect of consuming a sub-optimal diet consisting of sick or dying prey that had succumbed to the toxin."

In the second study, the authors found that high concentrations of the Cry1Ab (100 μg/ml of artificial diet) fed directly to the lacewing were toxic. The dose, according to the EPA, is at least 30 times more than the levels being expressed in most Bt corn tissues. In a field setting the lacewing larvae will have a choice of other insects to feed on, so field exposure will be intermittent rather than continuous. Furthermore, most of the *O. nubilalis* die during the first instar when consuming Bt corn tissue, and those surviving would be inside the corn plant for most of their larval life, thus unavailable for consumption by *C. carnea*. These tritrophic interactions may not be realistic relationships in the field (EPA 2000, Shelton et al. 2002).

Bt impacts on parasitoids

Extensive deployment of Bt transgenic cultivars has a greater chance of adversely impacting parasitoids than predators because they tend to have a closer association with their hosts than predators have with their prey. Parasitoids tend to have a greater ecological specialization and in some cases are specific to one organism. Ingestion of sublethal doses of Bt by the host insect may change the host's attributes that influence the foraging and oviposition of the parasitoids. Parasitoids attacking hosts consuming Bt transgenic plants or the Bt pathogen from microbial sprays may be directly or indirectly impacted by the toxins. Larvae of target lepidopterans that have ingested the Bt toxins develop at a slower rate or are much smaller than those that have not ingested the toxins (Schuler et al. 1999). This can lead to higher rates of parasitism because these larvae spend more time in susceptible stages. Conversely, larvae parasitized before contact with the Bt toxin, lack the feeding stimulus needed to ingest a lethal dose, whereas larvae free of parasitoids feed normally and have a greater chance of ingesting a lethal dose. The tactics used to suppress pest populations could potentially disrupt the activity of their natural enemies by reducing host or prey densities. The effects will be more pronounced for species, like hymenopteran parasitoids, that tend toward greater ecological specialization and are often specific to a particular pest organism or taxa (Erb et al. 2001, Bernal et al. 2002).

Similar to Bt crop cultivars, Bt spray formulations have been examined in respect to their effects on natural enemies. However, only a few of these studies have investigated the impact of these sprays on parasitoids, and the results are conflicting. Most of these previous studies used adult parasitoids that were reared on Bt toxin laced foods, or exposed by topical applications, with the larvae being more susceptible due their greater feeding activity (Bernal et. al 2002).

Salama et al. (1982) observed that the parasitoid *Microplitus demolitor* Wilkinson (Hymenoptera: Braconidae), a parasitoid of the cotton leafworm *S. littoralis*, was affected when feeding on hosts that had consumed a diet containing *B. thuringiensis.* Emergence and reproductive potential of the parasitoid were reduced. The percentage of parasitoid emergence was 92% in cocoons collected from the hosts fed a normal diet, whereas only 77% emerged in the case of larvae fed on a treated diet. The reproductive potential of the emerged parasites showed a significant variation between those reared on hosts that had consumed Bt to those who had not. On the hosts fed a normal diet, the average egg production was 84 ± 2.0 eggs/female. This decreased to 62 ± 3.0 eggs/female for those emerging from larvae that had been reared on a diet treated with Bt.

Schuler et al. (1999) investigated the tritrophic interactions of Bt canola, the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and the parasitoid *C. plutellae* (Hymenoptera: Braconidae) (Kurdjumov). The wasp is a solitary endoparasitoid and an important natural enemy of the DBM, which is the first insect to evolve resistance to microbial Bt sprays in the field. The parasitoid larvae that developed in susceptible DBM larvae died with their hosts, behavioral factors could potentially limit the impact of this effect in field conditions. Parasitoids attacking Bt-resistant DBM larvae suffered no measurable effects of Bt toxins on their behavior as adults or larval survival. Lab tests were conducted to evaluate the performance of the parasitoid in Bt susceptible larvae. The parasitoids were allowed to parasitize susceptible DBM larvae feeding on both Bt and non-Bt canola. In the hosts exposed to Bt, none of the parasitoids completed their development because all of the hosts died. Adult parasitoids did develop completely in 635 of Bt-susceptible larvae feeding on non-Bt canola.

When parasitoids infested the larvae of a highly Bt resistant lab strain of the DBM under similar conditions, there were no significant differences in the survival of the parasitoids in resistant hosts feeding on Bt or non-Bt canola (54% for hosts fed Bt, 56% for hosts fed non-Bt canola, $P = 0.8680$). Resistant host larvae having the Bt toxin had no direct effect on parasitoid survival (Schuler et al.1999)

Research on the parasitoid *C. plutellae* indicates that DBM resistance to Bt applications provided a refugium from competition with the Bt for the parasitoid, whereas in susceptible hosts, the microbial applications are generally more efficacious than the parasitoid. In susceptible DBM populations, Bt applications combined with *C. plutellae* would be effective, whereas in resistant populations, Bt would not be as effective (Canon 2000).

Johnson and Gould (1992) evaluated the effect of transgenic Bt tobacco on the target host *Heliothis virescens* (Fabr.) (Lepidoptera: Noctuidae). Its survival was measured in response to four treatments: uncaged transgenic tobacco exposed to natural enemies, caged transgenic tobacco not exposed to non-transgenic tobacco not exposed to natural enemies. They evaluated the tritrophic interactions of *H. virescens*, natural parasitoid enemies, and transgenic tobacco plants expressing low levels of the Bt toxin. The Bt toxins caused a significant decrease in first instar larvae, and the natural enemies caused a reduction in third instar larvae. The survival of uncaged first instars as a proportion of survival of caged first instars was significantly lower on the transgenic Plants than on control plants, indicating a synergism of the Bt toxins and natural enemies. The parasitoid *Completes sonorousness* (Cameron) parasitized at a much higher rate on toxic plants than on control plants, which is also another indication of synergisms. Whether the parasitized larvae are killed by the natural enemies or the transgenic plants themselves, the consequences for the population dynamics of *H. virescens* is the same: higher parasitism causes a higher mortality rate.

Bernal et al. (2002) examined the possible effects of various fitness parameters in parasitoids that develop within hosts that had been consumed Bt corn. The host was *Eoreuma loftini* Dyar, a subtropical stem borer, and the parasitoid was *Parallorhagas pyralophagus* (Marsh). The ingestion of Bt corn tissue by *E. loftini* had a negative effect on some fitness parameters of *P. pyralophagus*, whereas other parameters were not affected. Immature stage developmental mortality increased, adult longevity was a day shorter in females, and developmental times were two days longer in both sexes in hosts that had been feeding on Bt as compared with those that feeding on non-Bt. However, feeding on Bt corn tissue did not significantly affect egg loads, adult size longevity relationships, adult size of both sexes, and the sex ratio of these parasitoids. The study indicates the Bt toxin expressed in corn tissue has both lethal and sublethal effects on *P. pyralophagus* parasitoids.

Soil Insects

In transgenic plants, the Cry toxins are not only expressed in the upper parts of the plant, but sometimes in the roots as well. Concerns exist about the potential adverse effects of the exudates of the Bt toxins from the roots and dead plant material incorporated into the soil after harvest may have on non-target arthropods. The level of Bt retention in the soil varies with the type of soil, clay and mineral composition and soil pH. The amount of Bt toxins that persist in the soil environment is a function of the concentration of these toxins added to the soil and the rate of breakdown by both biotic and abiotic factors. The Cry1Ab toxin binds to the soil particles, thus reducing microbial degradation. This may lead to the possibility of these toxins accumulating in the soil and retaining their insecticidal activity (Tapp and Stotzky 1998, Head et al. 2002).

The toxins of *B. thuringiensis* var. *kurstaki* are rapidly adsorbed and tightly bound on the clay minerals montmorillonite and kaolinite. The Bt toxins would be susceptible to breakdown only for a short period of time. Previous studies have indicated that various proteins bound on clay particles are resistant to degradation. Also, the overall molecular structure of these toxins did not appear modified as a result of binding to the clay particles. Tapp and Stotzky (1995) examined the insecticidal activity of purified toxins of *B. thuringiensis* var. *kurstaki* and *B. thuringiensis* var. *tenebrionis* free in soil water and bound to the clay particles. They collected the Bt toxin extract from *B. thuringiensis* var. *kurstaki* in the laboratory, and exposed it to larvae of *Manduca sexta* (L.) (Lepidoptera: Sphingidae) to determine if it has insecticidal properties. They also collected Bt toxin from *B. thuringiensis* var. *tenebrionis* and exposed it to Colorado potato beetle, L. *decemelineata.* The insecticidal activity was retained and enhanced in some cases when the toxins from both strains were adsorbed to clay particles. These Bt toxins bound to clay were more resistant to microbial degradation. The toxins have the potential to accumulate and retain their insecticidal properties.

Saxena et al. (1999) had similar findings. *Manduca sexta* larvae were placed on a medium with Bt corn exudates and began dying two to three days later. In all, there was a 90% mortality rate, with none being observed in the non-Bt corn exudates. This indicates the Cry1Ab toxin rapidly binds to soil particles, and retains its toxic properties against targeted insects. These studies have shown that Cry1Ab toxin retains its properties when bound to soil clay particles. Crecchio and Stotzky (1998) also found the same results when analyzing the Cry1Ac toxic properties. These researchers have hypothesized that incorporation of Bt toxins in the soil that result from growing Bt plants, over the long term could exceed the rate of natural biodegradation causes the toxin to accumulate. Other research studies have contradicted these results. These was all laboratory tests, and none were under field conditions where Bt crops have been repeatedly planted and the residues incorporated into the soil.

Head et al. (2002) used insect bioassays and enzyme linked immunsorbent assays (ELISA) to examine the activity of the Cry1Ac toxins in the soil where transgenic Bt cotton had been repeatedly grown over several seasons and its residue incorporated into the soil after harvest. The test insect used was *H. virescens*. Even though many lepidopterans are susceptible to the Cry toxins, the extreme sensitivity of *H. virescens* to growth inhibition by the Cry toxins makes it an ideal species for soil degradation studies on plant tissues from transgenic crops. The ELISA tests established the level of the Cry1Ac that is extracted from the soil, and the insect bioassay determines the insect's ability to extract the Cry1Ac protein from the soil and its susceptibility to it. The tests showed that repeated use of transgenic Bt cotton in agricultural ecosystems did not result in any detectable levels of the Cry1Ac in the soil. Any accumulation of the Cry1Ac in the soil was below the level of detection of the ELISA and insect bioassays. These findings do not support the hypothesis that the large-scale use of transgenic crop plants expressing the Cry proteins brings about bioactive accumulations in the soil.

Sims and Holden (1996) had similar findings in using transgenic Bt corn expressing the Cry1Ab protein. Transgenic corn was incubated in a growth chamber for 43 days under appropriate growing conditions, both with and without soil contact. A series of dilutions was made from each incubated sample and incorporated into an artificial diet fed to neonate *H. virescens*. The growth rate of the larvae and bioactivity of the Cry1Ab proteins was determined by using regression analysis of the dose-weight response data. The Cry1Ab protein added to the soil from the transgenic corn being in contact with the soil had an estimated time that 50% of it had dissipated in the soil of 1.6 days and an estimated time that 90% of it had dissipated in the soil of 15 days. The Cry1Ab protein obtained from the tissue that did not have soil contact had an estimated 50% dissipation time of 25.6 days and an estimated 90% dissipation time of 40.7 days. The data indicate that the Cry1Ab protein in corn tissues will break down rapidly under field conditions, in both tilled and no-tilled corn ecosystems.

The Cry1F protein is secreted by *B. thuringiensis* subsp. *azawai*, the gene from this bacteria has been inserted into cotton plants to control *H. virescens* and other lepidopterans. Herman et al. (2001) conducted laboratory studies to measure the activity of the protein in the soil after short incubation periods. Based on the decrease of toxicity over time, the 50% dissipation time was estimated to be less than one day under laboratory conditions. The study shows that the Cry1F protein is unstable in soil that is representative of that in which the transgenic crops are grown. The results support previous studies that show the Cry proteins have no persistent insecticidal activity in the soil (Sims and Holden 1996, Head et al. 2002).

Although the above studies do not provide a consensus on the soil activity of the Cry proteins, it appears that in some cases the Cry proteins do retain their insecticidal activity in the soil for a certain amount of time. The data suggest that transgenic plants may have a possible impact on non-target arthropods, especially when used in agricultural practices like conservation tillage. Soil organisms, like collembolans and oribatid mites are primarily detritovores in ecosystems. They play an important role in the dissemination of fungi and the breakdown of dead plant and animal material. These organisms are bioindicators of the biological health of the soil (Wallwork 1970). Collembolans are important as decomposers of plant residues and also create humus. They fragment and digest plant debris. Because collembolans feed close to the surface, they can be impacted by any activity affecting soil conditions (Bitzer et al. 2002).
Yu et al. (1997) evaluated the possible effects of the Cry1Ab and Cry1Ac toxins expressed in cotton plants, and the Cry3A toxins expressed in potatoes on the collembolan *Folsomia candida* (Collembola: Isotomidae), and the oribatid mite *Oppia nitens* Koch (Acari: Oribatidae). They examined the possible effect of the Cry toxins on the time to oviposition, egg production, and final body weight. The Cry1Ab and

Cry1Ac did not have any noticeable affects on these three parameters when consuming transgenic plant material in comparison to those that had consumed non-transgenic plant material. *Oppia nitens* adult and nymph numbers were unaffected by consuming the leaves of transgenic plant material as well.

Sims and Martin (1997) evaluated the dietary toxicity of four Bt insecticidal proteins, Cry1Ab, Cry 1Ac, Cry 2A and Cry 3A, against two collembolan species, *Folsomia candida* and *Xenylla grisea* Axelson (Collembola: Hypogastruridae). The numbers of surviving test adults and progeny were evaluated after 21 days, and none of the Bt proteins significantly reduced collembolan survival or reproduction. The results of both of these studies indicate that relatively low concentrations of the Cry proteins present in the transgenic plants do not adversely impact collembola in the soil.

Carabid beetles are an extensive group of arthropods inhabiting the soil. Their presence could potentially demonstrate whether of Cry proteins in the soil affect the community structure and diversity of soil arthropods. Carabid beetles often are impacted by activities affecting the soil, and can be excellent bioindicators of changes in the soil. As a species, they are phytophagous, detritovores, and predators, and are an appropriate group of insects for evaluating the trophic relationships and the level of diversity in the soil (Lozzia et al.1999, Villa-Castillo and Wagner 2002).

Lozzia (1999) conducted an in-field evaluation of the effects transgenic Bt corn on several non-target arthropods including the carabid beetle. The degree of diversity and community structure was examined and compared in Bt and non-Bt corn. The statistical analysis did not yield any significant differences in the number of carabid beetles in fields where both types of corn were grown. The ground beetle assemblages were very similar, and any differences had no correlation with the presence of Bt corn.

Conclusions

The effects of genetically modified corn and other crop plants engineered to express the insecticidal Cry toxins on agroecosystems are poorly understood. Much of the previous research discussed here indicates that, with a few possible exceptions, these transgenic crops have no noticeable impact on non-target arthropods in the agroecosystem. However, these results could be inadequate. The scale of these tests is small in space and time (often only one or two growing seasons). This reduces the likelihood that any adverse impacts on non-target organisms could be observed. Ecological and evolutionary responses to transgenic Cry toxins are more likely to become more apparent when hundreds of thousand of acres of transgenic crops are planted over many growing seasons. The field studies were often used to evaluate one type of insect or a few limited species in a specific geographic location (Snow and Palama 1997).

The almost permanent availability of the Cry toxins in agroecosystems in addition to their continued expression in the transgenic crops makes it necessary to closely monitor any possible detrimental effects on natural enemies. Enhancing and preserving biological control agents is one of the most important components of modern pest management (McGowan 2001). Sustainable management strategies in agricultural ecosystems, the prevention of crop loss, and general soil health are central to our capacity to maintain the biological productivity of these ecosystems. Arthropods have an important role in crop loss and soil health because they consist of both beneficial and pest species. Cropping systems need to incorporate the relationships between farm practices and the ecosystem to form an equilibrium where farm inputs enhance rather than replace natural processes (Olfert et al. 2002).

The use of transgenic plants to control arthropod pests is still in its infancy. Improving the understanding of any possible hazards of transgenic crops to non-target organisms will require consistent approaches to field studies. The United States Environmental Protection Agency (EPA) is currently developing guidelines for testing the impacts of transgenic plants on non-target arthropods. In December, 1999, a scientific panel met and reviewed the EPA draft of the guidelines, and made the following recommendations:

> “The Agency (EPA) should consider how the data would be used to establish an acceptable level of statistical power. Based on these decisions, appropriate tests and sample sizes can be made.”

No single sample size can fit all cases, although the determination of sample size can be made after the collection of some preliminary data (Snow and Palama 1997).

The primary objective of this research study is to determine, through field evaluations, if the events MON 810 and Bt11, marketed jointly as YieldGard® Bt corn that is engineered to express the insecticidal Bt toxins, detrimentally impact the populations of non-target phytophagous and beneficial arthropods. The data obtained from these experiments will be compared with data from conventional, isogenic non-Bt corn to see if the Cry toxins have any effect on these non-target arthropods.

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CHAPTER 2

IMPACT OF *Bacillus thuringiensis* TRANSGENIC CORN ON NON-TARGET AND PHYTOPHAGOUS ARTHROPOD POPULATIONS [1]

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ABSTRACT Transgenic field corn has been genetically engineered with the gene from the *Bacillus thuringiensis* var. *kurstaki* (Bt), which secretes proteinaceous endotoxins that is toxic to certain species of lepidopteran pests. The effects of transgenic Bt corn containing the MON 810 event on non-target arthropods were compared to those in observed conventional, near isogenic corn at two locations in Georgia. The study was conducted in 2001 and 2002 using visual counts, pitfall traps, and corn ear insect evaluations. The only insect whose numbers and mortality was consistently affected by the Bt corn was the corn earworm, *Helicoverpa zea* (Boddie), a target insect. There were no consistent effects of the Bt toxins on non-target phytophagous and predaceous arthropods observed in the visual counts, nor on those non-target arthropods recovered from the pitfall traps and the corn ear dissection. From these results, transgenic Bt field corn containing the MON 810 event does not have an adverse effect on non-target phytophagous or predaceous arthropods.

INDEX WORDS: *Bacillus thuringiensis*, non-target arthropods, Cry1Ab toxins, soil dwelling arthropods, maize.

Introduction

The *Bacillus thuringiensis* Berliner (Bt) bacterium secretes a proteinaceous crystalline endotoxin (Cry toxin) that is toxic to certain species of pestiferous insects. *Bacillus thuringiensis* var. *kurstaki* targets lepidopteran insects. Susceptibility is determined by specific receptors in the membranous lining of the midgut epithelial cells (Shelton et al. 2002, Snow and Palma 1997). The specific gene in the Bt bacterium coding for the Cry toxins has been isolated and inserted into the cells of certain crop plants, like cotton, potatoes, and corn, where the toxins are expressed continuously throughout the life of the plant (Tenuta et al. 2000). Transgenic Bt corn containing the MON 810 event of the Cry1Ab toxin is the subject of this study. The MON 810 event and Bt11 event are marketed as YieldGard® Bt corn. The main target pests are the European corn borer, *Ostrinia nubilalis* Hübner, and the southwestern corn borer, *Diatraea grandiosella* Dyar. In Georgia, the main lepidopteran pests are the corn earworm, *Helicoverpa zea* (Boddie) and the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Bt corn with the MON 810 event suppresses whorl infestations but only partly controls ear infestations of *H. zea* and *S. frugiperda* (Archer et al 2001, Williams et al.1997, Buntin et al. 2001, Storer et al. 2001).

Numerous other pestiferous arthropods feed on corn and are not targets of the Cry1Ab toxins. Examples include the chinch bugs, *Blissus leucopterus leucopterus* (Say), the corn flea beetle, *Chaetocnema puliceria* Melsheimer, the corn rootworm complex, *Diabrotica* spp., corn leaf aphid, *Rhapalosiphum maidis* (Fitch), and several species of leafhoppers, among others (Steffey et al. 1999). A majority of the arthropod species present in cornfields are not pestiferous. Many are beneficial natural enemies that feed on pestiferous arthropods. Examples of these include numerous species of lady beetles, big-eyed bugs, *Geocoris* spp., insidious flower bug, *Orius insidiosus* Say, and carabid ground beetles (Steffey et al. 1999). Bt corn could potentially have an impact on the populations of non-target arthropod species, both directly and indirectly. Detrimental impacts, if any, could vary widely due to differing levels of sensitivity among the various species present in the corn ecosystem (Wolfenberger and Phifer 2000).

Previous studies have evaluated the potential impact of the transgenic plants that expresses the Bt Cry toxins on non-target phytophagous and beneficial species. Most of these have shown no significant differences in the effect of these toxins on the non-targets (Sims 1995, Pilcher 1997, Lozzia 1998, Riddick et al. 2000). A few studies have indicated the possibility of adverse effects on non-target species, most notably the monarch butterfly, *Danaus plexxipus* (L.) (Losey 1999). Another exception is the Hilbeck et al. (1998a, 1998b, and 1999) studies that found the Bt Cry toxins may enhance the mortality of the green lacewing, *Chrysoperla carnea* (Stephens). When *C. carnea* consumed prey that was reared on a diet containing the Cry1Ab toxins, it developed at a slower rate had a lower rate of reproduction, and a higher mortality rate. However, in these experiments were conducted in a laboratory under artificial conditions, not in field settings. These experiments were not choice experiments in that they only had the choice of consuming prey that had consumed the Bt toxins.

One of the most important components of integrated pest management is the preservation of the natural enemies of pestiferous arthropods on the crop system. There is limited understanding of the impact of the Cry toxins expressed in the transgenic plants on the growth, development, and distribution of non-target arthropods. To assure that transgenic crops are environmentally friendly and sustainable, long-term screenings of these pests management strategies with naturally occurring beneficial arthropods are necessary (Cannon 2000).

The objective of this study is to evaluate the effect of Bt corn expressing the Cry1Ab toxins on field populations of non-target phytophagous and beneficial arthropods. The data obtained from these fields will be compared with those obtained from the populations observed in non-Bt, conventional, isogenic corn plots.

Materials and Methods

Fields studies were conducted at two locations: the Bledsoe Research Farm, Pike County, near Griffin, Georgia, and the Southwest Georgia Experiment Station, Sumter County, Plains, Georgia. The experiments evaluated four corn hybrids at each location in 2001 and 2002. Two of the hybrids (Pioneer brand 31B13 and Pioneer brand 32K64) contain the MON 810 event that expresses the Cry1Ab toxin. The other two hybrids (Pioneer brand 3223 and Pioneer brand 32K61) are the conventional, near isogenic lines that do not express the Cry toxins.

Preparation of the test plots. The experimental design was a randomized complete block design with four replicates at both locations. The hybrids were arranged factorial design. The plots had the dimensions of 21 x 25 m with the rows planted 76 cm apart. Plant population was 66,700 plants per (27,000 plants per acre). The preemergent herbicides atrazine (Aatrex 4L, Syngenta Crop Protection, Greensboro, NC) and pendamethalin (Prowl 3.3 EC, BASF, Research Triangle Park, NC) were applied to control weeds. The fields were fertilized pre-plant at the rate of 112 kg/ha N (100 lbs/acre), 95 kg/ha of P_20_2 (84 lbs/acre), 95 kg/ha of K_20 (84 lbs/acre), and 168 kg/ha

(150 lbs/acre) of N as a side dress. Each plot was irrigated as needed during the growing season.

Seedling Stand and Thrips Counts. Plant stand was measured 14 d after planting by counting two adjacent rows per plot. Thrips populations also were examined 14 to 21 after planting by collecting twelve plants per plot and dipping them into a cup of 70% ethanol in the field. The thrips samples were returned to the laboratory, and the number of thrips per sample cup was counted.

Visual Counts. Visual counts of the arthropods present on ten consecutive plants per plot was conducted weekly beginning when the corn plants entered the six-leaf stage. Upon entering the ear stage of growth, visual counts of arthropods present were conducted in the ear zone (the corn ear plus one ear above and one ear below on corn stalk) in twenty consecutive corn plants. Samples were randomly selected but were not taken within 4 m of the plot edge. These counts were conducted weekly until harvest time.

Soil Arthropod Counts. During the summer of 2001, two pitfall traps were placed in each plot, and three pitfall traps per plot were used in 2002. The pitfall traps were similar to those used by Villa-Castillo and Wagner (2002). They were constructed by placing a 15-cm diameter cup in a drilled hole. A smaller plastic 7.6-cm cup containing propylene glycol as a preservative was placed in the bottom of the larger cup. A small conical cup had its base removed and placed over the larger cup so the insects would fall into the small plastic cup. The pitfall trap was covered with a 30.5 x 30.5 cm wooden cover to exclude rain. Trap contents were emptied weekly during the growing season, and taken to the laboratory for analysis. The contents were removed and placed into vial of 70% ethanol. The contents were examined under a compound microscope, and the arthropods present were identified, counted, and recorded. Pitfall traps were operated from one day after planting until one week before harvesting.

Corn Ear Insect and Damage Samples. When the corn plants entered the green-silk ear stage, 20 ears per plot in 2001 and 15 ears per plot in 2002 were harvested weekly for six weeks. The corn ears were returned to the laboratory where they were dissected, and the insects present were identified, counted, and recorded. The amount of insect damage on the ears were evaluated for twenty corn ears harvested on the last sample date. The amount of damaged tip length on the selected corn ears and the kernel area damaged in cm^2 was measured.

In 2001, all lepidopteran larvae, almost entirely *H. zea*, were collected from the corn ear samples feeding on the Bt or non-Bt corn. Larvae were reared in plastic diet cups containing meridic diet to assess parasitism.

Harvest and Post-Harvest Analysis. The four center rows in each plot were harvested with a four-row commercial combine. The grain collected was weighed on a weigh wagon, and the weights per plot were recorded. A subsample of the grain from each plot was taken to measure moisture content and grain test weight. Grain yields and test weights were adjusted to 15.5% moisture content. A 2-kg subsample also was collected and sent to University of Georgia seed laboratory in Tifton for the measurement of total aflatoxin concentration, as described in Buntin et al. (2001). Kernels were ground to pass through a 20-mesh screen and a 100-g subsample was extracted. The level of aflatoxin contamination was determined using the Vicam immunoaffinity column method

(Truckness et al. 1991) and is reported as total aflatoxin (B_1+B_2+G_1+G_2) in parts per billion.

Statistical Analysis. Insect count data were analyzed by experiment using PROC GLM for split plot design with hybrids as whole plots and sample dates as split plots (SAS Institute 1997). Bt and genotype main effects and interaction were evaluated as a factorial analysis of the whole plots. Insect count data were transformed by using $\log_{10}$ transformation before analysis. The data for each arthropod in the visual counts and the pitfall traps were plotted, as arthropod numbers in Bt versus non-Bt plots using SigmaPlot (SPSS 1998), for the Plains and Griffin locations in both years. Also, the corn earworm numbers and sap beetle numbers in Bt and non-Bt plots were plotted for each location and each year. The data from the harvest, the test weights, yields, corn ear insect damage and aflatoxin analysis were analyzed by two-way ANOVA for a factorial design using GLM (SAS Institute 1997).

Results

Thrips Counts. The thrips collected were mostly corn thrips, *Frankliniella williamsi* Hood (Thysanoptera: Thripidae). Thrips counts were conducted when the corn plants were in the seedling stage, 14 to 21 d after planting (Table 2.). The only sample in which the numbers of thrips that differed significantly was from the Griffin location in 2001 where the mean number of thrips was significantly greater on Bt corn plants than non-Bt corn plots. Thrips numbers were not significantly different in the Bt compared to the non-Bt corn plots in the other three trials.

Visual Counts of Non-Target Phytophagous Arthropods. The mean seasonal numbers of arthropod taxa observed in the visual counts are shown in Table 2.2. The most abundant phytophagous arthropods observed were the chinch bug (Appendix 1.1), corn flea beetle (Appendix 1.2), leafhoppers (Appendix 1.3). All of these taxa were abundant early in the season. Less abundant taxa were stink bugs, *Euchistus servus* (Say) and *Acrosternum hilare* (Say) (Hemiptera: Pentatomidae), dusky sap beetles, *Carpohilus lugribus* Murray (Coleoptera: Nitidulidae), and adult corn rootworms, *Diabrotica undecimpuncta howardi* Barber and *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). Corn leaf aphids were present but not significantly abundant for analysis in any trial.

The only phytophagous arthropod with a significantly difference in abundance between Bt and non-Bt corn in the test of the main Bt effect was chinch bugs from Plains in 2001, where chinch bug numbers were higher in the Bt than the non-Bt plots. Chinch bugs were not significantly different in the other three trials. Also, the Bt x date interactions for chinch bugs was significant in the Plains 2001. However, there were no significant differences in the other Bt x date for interactions chinch bugs in the other trials. The flea beetles were significant in the Bt x date interactions in the Plains 2001 trials, but not in the other three trials. There also was a significant Bt x date interaction for corn rootworm adults at Plains in 2001, but numbers were very low and the differences were not meaningful. There were no significant differences in the mean seasonal numbers in Bt versus non-Bt corn for any other phytophagous arthropod in the visual counts.

Visual Counts of Predaceous Arthropods. The mean seasonal numbers of predaceous arthropods in Bt and non-Bt corn are shown in Table 2.3. The main predaceous non-target taxa observed in the visual counts were damsel bugs, *Nabis* spp., (Hemiptera: Nabidae); big eyed bugs, *Geocoris* spp. (Say) (Appendix 2.1) several coccinellid insects including: *Coleomegilla maculata* DeGeer (Appendix 2.2); Scymnus lady beetle, *Scymnus* spp. (Appendix 2.4); convergent lady beetle, *Hippodamia convergens* Guerin-Meneville and the sevenspotted lady beetle, *Coccinella septempuncta* (L.) ant-like flower beetles, *Notoxus* spp. (Coleoptera: Anthicidae); spiders (many species) (Appendix 2.3); insidious flower bug *O. insidiosus* (Say) (Appendix 2.5). The *Geocoris* spp. were almost entirely *G. punctipes* (say) although a few *Geocoris uliginosus* (Say) were observed late in the season.

A significant difference occurred in the visual counts with the *C. maculata* in the Plains 2002 samples, where the seasonal mean number was higher in the Bt corn plots than the non-Bt corn plots. Bt did not significantly affect *C. maculata* numbers in the other trials. Also, there was a significant difference observed in the visual counts *G. punctipes* at Griffin in 2002 with a significantly higher number in the Bt corn than the non-Bt corn. However, the Bt x date interaction was not significant for either of these cases. The other predaceous arthropods did not differ significantly in mean numbers in the Bt when compared to those numbers in the non-Bt corn plots. Damsel bugs differed significantly in the Bt x date interactions at Plains in 2001, but not in any of the other three trials. There was no significant Bt x date interactions with the other arthropods in the other trials.

Pitfall Trap Samples. The mean seasonal numbers of arthropod taxa recovered in pitfall traps are shown in Table 2.4. The main taxa recovered in the pitfall traps were spiders (many species) (Appendix 3.1); rove beetles, *Lathrobium* spp. and *Sepidophilus* spp. (Coleoptera: Staphylinidae) (Appendix 3.2); anthicids beetles *Notoxus* spp. and *Athicus* spp) (Appendix 3.3); tiger beetles, *Megacephala carolina* (L.) (Coleoptera:

Cicindellidae) (Appendix 3.4); carabid ground Beetles, *Harpalus pennsylvanica* DeGeer and *Calasoma sayi* (Say) (Appendix 3.5); scarab beetles, *Altaneus* spp., *Cyclocephala immaculata* (Oliver), and *Balbocerasoma farctus* F. (Coleoptera: Scarabaeidae) (Appendix 3.6); click beetles, *Conederus* spp. (Coleoptera: Elateridae) (Appendix 3.7); ants, mostly *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Appendix 3.8); crickets, *Gryllus pennsylvaticus* Burmeister and *Allenemobius fasciatus* (DeGeer) (Orthoptera: Gryllidae) (Appendix 3.9); and *Ahasverus advena* (Waltl) (Coleptera: Cucujidae) (Appendix 3.10).

Most of the samples had no significant differences in the mean seasonal number of soil-dwelling arthropods in the Bt corn plots as compared to the non-Bt corn plots (Table 2.4). In the Griffin 2001 trial, significantly more anthicids, rove beetles, and tiger beetles were collected in the Bt corn than the non-Bt corn. These taxa were not significantly different between Bt and non-Bt corn in the other three trials. Conversely, ants, were present in higher numbers in the non-Bt corn plots than the Bt corn plots in the 2001 Griffin trial. Ants were not significantly different between Bt and non-Bt corn in the other trials. There were no other significant differences in the number of arthropods collected in the pitfall traps in the Bt and non-Bt corn plots.

The Bt x date interaction also was not significant for any of the taxa in any of the trials except. The only trial where the Bt x date interactions were significant was with cucujid beetles in the Griffin 2002 trial (Table 2.4). The other trials did not have any significant Bt x date interactions.

Corn Ear Insect and Damage Samples. The main taxa observed in corn ears were corn earworm, larvae and adults of the dusky sap beetles, *Carpohilus dimidiatus*

Murray, and chinch bugs. The seasonal mean numbers of corn earworm larvae were significantly higher in the non-Bt than in the Bt corn ears in three of the four trials (Table 2.5). Corn earworm numbers were not significantly different in the Griffin 2001 trial. The mean number of adult sap beetles was higher in the corn ears obtained from the non-Bt plots than the Bt corn plots at Plains in 2002. Sap beetles in three of the four trials and the larvae in all trials were not significantly different between Bt and non-Bt corn. Corn earworm numbers peaked in early ear development in the brown silk stage, whereas sap beetle numbers increased to a peak when the corn ears reached physiological maturity (Appendix 4.1).

A total of 3968 *H. zea* larvae were collected in ear samples and reared in 2001. No parasitoids were recovered from the corn earworm larvae.

Corn insect kernel damage was caused mostly by *H. zea* in both trials (Table 2.6), and the non-Bt corn had significantly greater levels of corn earworm damage than Bt corn.

Corn Harvest Data. Corn was harvested at Plains in 2001 and at the Griffin Farm in 2001 and 2002 (Table 2.7). The corn in the trials at Plains in 2002 was not harvested because of severe lodging of the corn resulting from a storm. Grain yield was greater in the Bt than the non-Bt corn in all trial, but these differences were not significant in any trial. Grain test weight was significantly lower in Bt than non-Bt corn at Griffin 2001. Bt resistance did not significantly affect test weights from the other trials. Grain aflatoxin levels were not significantly different between the non-Bt and Bt corn in any trial (Table 2.7).

Discussion

Results from this study indicate no consistent effect on non-target arthropod populations from the Bt endotoxins expressed in the genetically modified corn having the Cry1Ab gene when compared to those populations in non-Bt isogenic corn. The only arthropod that was consistently affected by these toxins was the corn earworm, which is susceptible to the Cry1Ab toxins and is a target pest. The overall results are consistent with most of the results of previous studies conducted on the impact of Bt corn on non-target arthropods (Orr and Landis 1997, Pilcher et al. 1997b, Wold et al. 2001), and of studies on transgenic cotton (Sims 1995) and potatoes (Riddick and Barbosa 1998).

The main targets of Bt corn are lepidopterans, including O. *nubilalis* and *H. zea*. Non-target pests of corn are not expected to be affected by the Bt toxins. The field populations of phytophagous insects in the two types of corn observed in the visual counts in all of the trials did not show any consistent, significant differences.

Thrips feed on plant fluids and there is a high probability that they ingest the Bt Cry1Ab toxins while feeding. However, thrips populations were not consistently between Bt and non-Bt corn. Previous research studying effects of Bt toxins on arthropods that feed on plant fluids also did not find any significant differences between non-Bt and Bt corn. Lozzia (2000) examined a sap-sucking mite, the two-spotted spider mite *Tetranychus urticae* Koch, feeding on Bt corn and found no effect of the toxins on mite populations. Hassell and Shephard (2002) evaluated the numbers of several non-target arthropods, including plant fluid feeding like thrips and aphids using D-vac samples on corn plants. They also found no major differences in the number of these insects were found in the transgenic and non-transgenic corn.

Visual counts the predaceous arthropods also revealed no consistent significant population differences between the transgenic and non-transgenic corn, which is similar to the findings of previous research studies. One of the major predators present in cornfields is the *O. insidiosus*. Al-Deeb et al. (2001) conducted studies examining possible effects of Bt corn on *O. insidiosus* nymphs when fed larvae of *O. nubilalis* that had been reared on a meridic diet containing the Bt Cry1Ab toxins or on a diet without the toxin. No significant differences occurred in the developmental time, body weight, or length of mature *O. insidiosus*, nor did they observe significant differences of the number of *O. insidiosus* adults and nymphs on Bt and non-Bt corn in the field. Zwahlen et al. (2000) conducted trials of *O. insidiosus* feeding on the thrips, *Anophrothrips obscurus* (Muller) (Thysanoptera: Thripidae) that had consumed Bt corn or non-Bt corn. They found no significant differences in the total mortality and developmental time from hatch to adult eclosion when *O. insidiosus* was fed both prey types. Field studies conducted by Wold et al. (2001) and Hassell and Shepard (2002) also did not find any significant differences in the field populations of *O. insidiosus* in transgenic and isogenic corn. Riddick and Barbosa (2000) had similar findings with *O. insidiosus* in transgenic potatoes. The data from our studies show no significant differences in the field populations of *O. insidiosus* in the two types of corn. Together these results strongly indicate that *O. insidiosus* populations were not adversely affected by Cry1Ab toxins in Bt corn.

Coccinellids also were prevalent in the corn plots. The most abundant species were *C. maculata* and *Scymnus* spp. No consistent differences in populations of coccinellids were observed between the Bt and non-Bt corn. The only significant difference coccinellid abundance was at Plains in 2002, where *C. maculata* numbers were greater in the Bt corn plots than the non-Bt corn plots. Pilcher et al. (1997) observed no detrimental effects of the Bt corn toxins on *C. maculata* resulting from the feeding of corn pollen from both Bt and non-Bt corn and *O. nubilalis* larvae reared on both types of corn. Wold et al. (2001) examined field populations of several coccinellid insects, including *C. maculata*, *H. convergens, C. septempuncta*, and the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), in Bt and non-Bt sweet corn. This study included both visual counts and caged studies in the field and showed no consistent significant differences in the populations of these predatory coccinellid insects between both types of sweet corn. The data from our studies are consistent with the other research studies indicating that transgenic insecticidal corn expressing the Cry1Ab toxin did not adversely affect on predatory coccinellid populations.

In the southeastern United States, one of the major predatory insects in cornfields is the big-eyed bug, *G. punctipes*. The effect of Bt corn on *G. punctipes* has not been previously reported. In visual observations, there were no consistently significant differences in the number of *G. punctipes* in Bt and non-Bt corn. The populations of hooded beetles, nabids, and spiders also were not significantly different between Bt and non-Bt corn in any of the trials.

The mean number of taxa recovered in pitfall traps did not differ consistently between Bt and non-Bt corn plots. Where significant differences occurred they were in Griffin in 2001. Lozzia (1999) examined populations of carabid beetles in Bt and non-Bt corn by pitfall traps similar in construction to ours, and found no consistent, significant differences in their numbers. The diversity of carabid species was relatively low in the Lozzia study, and the same was true with our study in which only two species, *H. pennsylvanica* and *C. sayi,* accounted for most of the carabids recovered. Nevertheless, no taxa in pitfall traps in any study differed significantly in the abundance between the two types of corn in more than one of the four trials.

Corn earworm numbers were consistently, significantly lower in the corn ears from the Bt corn plants as opposed to those from the non-Bt corn plants (Table 2.5). This is to be expected because corn earworm is a target pest of the Bt corn. The Cry1Ab toxin expressed in the Bt corn usually suppresses *H. zea* growth and development but only partially prevents kernel development by *H. zea* in the corn ears. Storer et al. (2001) examined the life history traits of *H. zea* on Bt and non-Bt corn hybrids. The Bt corn hybrids contained the either MON 810 or Bt11 events. The mortality of *H. zea* was not much higher in the Bt than the non-Bt, but mortality in Bt hybrids increased as *H. zea* progressed through pupation and adult emergence to about 75%. Surviving larvae also developed at a slower rate (Storer et al. 2001). We also found fewer *H. zea* larvae in Bt ears. These larvae also took longer to develop and cause less larval kernel damage in the Bt than the non-Bt hybrids.

A major secondary pest of corn ears is the sap beetle *C. lugribis.* Sap beetles are attracted to corn ears that have been damaged by other insects, especially the activity of corn earworm (Kaster 1999, Dowd 2000). Control of non-lepidopteran pests such as *C. lugribus* by the Cry1Ab toxin is not expected because it is not a targeted pest. However, Bt corn may indirectly affect sap beetle numbers, because reduction in kernel damage by lepidopterans may reduce the attractiveness of Bt corn to sap beetles. The mean number of adult and larval *C. lugribus*, did not consistently differ significantly between Bt and non-Bt corn. The number of sap beetles did increase during the latter part of the growing season as the *H. zea* numbers in the ears decreased (Figure 2.5). Dowd (2000) examined the distribution of sap beetles in Bt and non-Bt corn hybrids at test sites in Illinois in 1998 and 1999. The damage by corn earworm was reduced about 80% in the Bt corn. The sap beetle's numbers were examined by corn ear evaluations, scouting, and specialized sap beetle traps. No consistent significant differences were observed in the number of sap beetles in the Bt versus non-Bt corn. Al-Deeb et al. (2001) also found no significant differences in the number of sap beetles in Bt and non-Bt ears. Studies so far, including our study, indicate that there is no overt effect of the Cry1Ab toxins on *C. lugribus* populations in corn ears.

In summary, there were no consistent effects of the transgenic Bt corn containing the MON 810 even on the non-target phytophagous and beneficial arthropods evaluated in this research study. These results are consistent with those obtained from previous studies. However, the technology of transgenic insecticidal plants is so new that further research over time will be needed to fully assess the impacts on non-target arthropods. In the agroecosystem, preservation of predatory arthropods is one of the most important aspects of integrated pest management. The beneficial arthropods present have a very important role in controlling pestiferous arthropods, and any pest management technique needs to be evaluated on its effect on the beneficial natural enemies. Understanding of interactions between targeted pest, transgenic plants, and beneficial arthropods, is necessary to successfully use transgenic plants in the long run. The understanding of the ecological impacts of transgenic insecticidal plants on non-target arthropods will be difficult and will require a case-by-case approach to address any unintended side effects.

After the article by Losey et al. (1999) on the risks of Bt corn pollen on the monarch butterfly was published, public concern increased about how the Cry1Ab toxins expressed in Bt corn effect non-target arthropods. It may be more of a perceived risk than actual risks. However, even with previous studies by the federal government and universities, the general public and some scientists are cautious about accepting the stated safety assurances associated with the new technology of transgenic crops (Scriber 2001). The growing body of evidence from previous research studies and our study indicate that the effect of Bt transgenic corn has no consistent impact on non-target arthropods. However, work over extended periods is needed to fully assess risks to these nontargets.

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Table 2.1.Mean number of thrips on Bt and non-Bt corn seedlings, twelve plants per plot, obtained 14 days after planting.

Trial	Mean + SE plot/sample date			
Location- Year	Non-Bt	Bt	F-value	P-value
Plains 2001	137.3 ± 21.8	137.4 ± 36.0	0.05	0.5684
Griffin 2001	30.8 ± 16.6	51.9 ± 22.3	7.39	**0.0237***
Plains 2002	70.3 ± 35.2	65.5 ± 32.0	0.31	0.5942
Griffin 2002	34.6 + 12.7	42.6 + 14.6	3.19	0.1076

*Significant P value at alpha=0.05

Table 2.2. Mean seasonal numbers of non-target phytophagous arthropods present on Bt and non-Bt field corn in visual counts (10 consecutive plants per plot up to ear stage, then 20 consecutive plants. (* Significant interaction at the alpha <0.05 level).

	Trial	Mean ± SE/plot/sample date		Bt Main Effect		Bt x Date Interaction	
Taxa	Location- Year	non-Bt	Bt	*F* value	*P* value	*F* value	*P* value
Chinch bugs	Plains 2001	1.63 ± 4.21	3.15 ± 8.40	7.84	***0.0207**	2.10	***0.0248**
	Griffin 2001	0.59 ± 1.63	1.17 ± 2.60	4.36	0.0663	1.35	0.2043
	Plains 2002	0.77 ± 2.03	0.57 ± 1.61	1.90	0.2011	1.05	0.4094
	Griffin 2002	0.05 ± 0.85	0.47 ± 1.67	2.35	0.1594	1.18	0.3016
Flea beetles	Plains 2001	1.69 ± 2.21	1.94 ± 2.47	1.73	0.2212	1.21	0.2871
	Griffin 2001	1.47 ± 1.63	1.86 ± 1.86	3.93	0.0788	0.32	0.9796
	Plains 2002	0.95 ± 1.52	1.02 ± 1.57	0.74	0.4113	0.51	0.9172
	Griffin 2002	3.30 ± 4.75	3.62 ± 5.49	0.01	0.9608	1.87	***0.0425**
Leafhoppers	Plains 2001	0.56 ± 0.27	0.71 ± 1.08	1.07	0.3279	0.56	0.8555
	Griffin 2001	0.46 ± 0.77	0.40 ± 0.70	2.21	0.1716	0.78	0.6640
	Plains 2002	0.70 ± 1.37	0.80 ± 1.20	1.96	0.1945	0.60	0.8515
	Griffin 2002	0.73 ± 1.14	0.64 ± 1.06	0.59	0.4622	1.03	0.4291

Table 2.2 (continued). * Significant interaction at the alpha <0.05 level

	Trial	Mean + SE/plot/sample date		Bt Main Effect		Bt x Date Interaction	
Taxa	Location- Year	non-Bt	Bt	*F* value	*P* value	*F* value	*P* value
Stink bugs	Plains 2001	0.60 ± 0.24	0.05 ± 0.22	0.08	0.7842	0.94	0.5033
	Griffin 2001	0.02 ± 0.07	0.01 ± 0.04	4.50	0.0629	1.23	0.3174
	Plains 2002	0.23 ± 0.50	0.21 ± 0.60	0.98	0.3474	0.70	0.7656
	Griffin 2002	0.01 ± 0.10	0.04 ±0.19	2.25	0.1516	0.95	0.4951
Sap beetles	Plains 2001	0.57 ±2.01	1.09 ± 3.94	1.64	0.2318	1.23	0.2318
	Griffin 2001	0.03 ± 0.23	0.02 ± 0.14	0.06	0.8140	0.69	0.7467
	Plains 2002	0.46 ± 1.36	0.49 ± 1.19	0.42	0.5336	0.78	0.6853
	Griffin 2002	0.13 ± 0.56	0.13 ± 0.41	0.13	0.7253	0.53	0.8945
Corn rootworms	Plains 2001	0	0.02 ± 0.14	0.01	1.0000	2.00	***0.0331**
	Griffin 2001	0.03± 0.17	0.01 ± 0.10	2.25	0.3991	1.85	0.0511
	Plains 2002	0.35 ± 0.26	0.23 ± 0.68	0.78	0.1679	0.58	0.8642
	Griffin 2002	0.25 ± 0.82	0.13 ± 0.50	0.91	0.3658	1.28	0.2359

Table 2.3. (continued). (* Significant interaction at the alpha <0.05 level)

	Trial	Mean + SE/plot/sample date		Bt Main Effect		Bt x Date Interaction	
Taxa	Location- Year	non-Bt	Bt	*F* value	*P* value	*F* value	*P* value
Convergent Lady beetles	Plains 2001	0.70 ± 0.36	0.04 ± 0.25	0.71	0.4213	0.71	0.7247
	Griffin 2001	0.01 ± 0.10	0.03 ± 0.23	0.62	0.7727	1.04	0.4150
	Plains 2002	0.18 ± 0.61	0.16 ± 0.42	0.09	0.4501	0.61	0.8413
	Griffin 2002	0.08 ± 0.33	0.09 ± 0.37	0.03	0.8650	0.33	0.9837
Damsel bugs	Plains 2001	0.73 ± 0.30	0.02 ± 0.14	2.76	0.1307	3.90	***0.0001**
	Griffin 2001	0.06 ± 0.24	0.02 ± 0.14	4.50	0.0629	1.17	0.3174
	Plains 2002	0.17 ± 0.52	0.10 ± 0.36	1.26	0.2899	1.11	0.3530
	Griffin 2002	0.02 ± 0.14	0	3.00	0.1173	0.92	0.5321
Anthicid beetles	Plains 2001	0.05 ± 0.27	0 .04 ± 0.20	0.05	0.8265	0.75	0.6840
	Griffin 2001	0.37 ± 0.17	0.59 ± 1.63	0.68	0.6909	0.55	0.8625
	Plains 2002	0.17 ± 0.53	0.20± 0.73	0.17	0.1414	1.08	0.3780
	Griffin 2002	0.03 ± 0.17	0.05 ± 0.22	0.56	0.4724	0.50	0.9141

Table 2.4. Mean seasonal numbers of non-target arthropods collected in pitfall traps in Bt and non-Bt field corn fields (Two traps per plot in 2001, and three traps per plot in 2002). (* Significant interaction at the alpha <0.05 level)

	Trial	Mean + SE/plot/sample date		Bt Main Effect		Bt x Date Interaction	
Taxa	Location- Year	non-Bt	Bt	*F* value	*P* value	*F* value	*P* value
Spiders	Plains 2001	0.49 ± 1.42	0.74 ± 0.96	1.57	0.2422	0.91	0.5422
	Griffin 2001	0.79 ± 1.30	0.60 ± 1.13	1.74	0.2198	0.47	0.9398
	Plains 2002	1.48 ± 1.58	1.51± 1.70	0.01	0.9479	0.78	0.7004
	Griffin 2002	0.80 ± 1.30	0.80 ± 1.30	0.75	0.4100	0.92	0.5474
Anthicids	Plains 2001	0.70 ± 0.32	0.15 ± 0.92	0.44	0.5245	1.39	0.1768
	Griffin 2001	0.03 ± 0.09	0.01 ± 0.04	22.40	***0.0011**	1.54	0.1094
	Plains 2002	1.35 ± 2.97	1.40 ± 3.54	0.73	0.4136	1.03	0.4305
	Griffin 2002	0.23 ± 0.56	0.32 ± 0.94	0.44	0.5225	0.75	0.8173
Rove beetles	Plains 2001	0.56 ± 1.30	0.54 ± 0.99	0.12	0.7417	0.63	0.8173
	Griffin 2001	0.23 ± 0.55	0.09 ± 0.32	6.68	*** 0.0295**	0.95	0.5067
	Plains 2002	1.15 ±1.40	1.06 ± 1.56	0.62	0.4496	0.90	0.5611
	Griffin 2002	0.85 + 2.25	0.54 +0.96	0.28	0.6102	0.92	0.5376

Table 2.4. (continued). * Significant interaction at the alpha <0.05 level)

	Trial	Mean + SE/plot/sample date		Bt Main Effect		Bt x Date Interaction	
Taxa	Location- Year	non-Bt	Bt	*F* value	*P* value	*F* value	*P* value
Tiger beetles	Plains 2001	0.64 ± 1.42	0.55 ± 1.34	0.89	0.3707	0.39	0.9654
	Griffin 2001	0.42 ± 0.89	0.52 ± 1.26	0.12	0.7380	1.09	0.3757
	Plains 2002	1.76 ± 2.70	1.42 ± 2.44	3.09	0.1128	0.92	0.5457
	Griffin 2002	0.85 ±2.25	1.65 ± 4.06	19.10	***0.0018**	0.85	0.6252
Scarab beetles	Plains 2001	0.21 ± 0.68	0.28 ± 0.83	0.63	0.4480	1.47	0.1419
	Griffin 2001	0.15 ± 0.73	0.13 ± 0.41	0.00	0.9960	0.73	0.7316
	Plains 2002	0.43 ± 1.09	0.98 ± 1.41	0.45	0.9960	0.85	0.6230
	Griffin 2002	0.16 ± 0.78	0.12 ± 0.35	0.01	0.9723	1.01	0.4443
Ants	Plains 2001	1.81 ± 5.18	1.60 ± 4.12	0.01	0.9083	0.66	0.7857
	Griffin 2001	0.15 ± 0.45	0.05± 0.26	5.97	***0.0371**	1.14	0.3293
	Plains 2002	3.79 ± 5.39	3.58± 5.40	0.13	0.7285	1.05	0.4021
	Griffin 2002	1.90 ± 2.25	2.21 ± 3.89	0.02	0.3527	0.54	0.9142

Table 2.4. (continued). * Significant interaction at the alpha <0.05 level

	Trial	Mean + SE/plot/sample date		Bt Main Effect		Bt x Date Interaction	
Taxa	Location- Year	non-Bt	Bt	*F* value	*P* value	*F* value	*P* value
Crickets	Plains 2001	1.16 ± 1.73	1.68 ± 2.27	4.12	0.0730	0.78	0.6737
	Griffin 2001	3.33 ± 4.79	2.39 ± 3 .89	5.00	0.0522	0.75	0.7088
	Plains 2002	6.65 ± 6.02	4.92 ± 4.88	2.23	0.1697	0.61	0.8682
	Griffin 2002	5.73 ± 7.15	5.93 ± 7.73	2.27	0.1688	0.85	0.6210
Cucujid Beetles	Plains 2001	0.02 ± 0.08	0.03 ± 0.09	1.05	0.3332	1.37	0.2152
	Griffin 2001	0.01 ± 0.04	0.83 ± 1.69	0.72	0.4192	0.72	0.4192
	Plains 2002	0.50 ± 1.13	0.83 ± 1.69	1.66	0.4900	1.04	0.4124
	Griffin 2002	0.28 ± 0.63	0.21 ± 0.56	1.40	0.2673	2.78	***0.0007**
Click Beetles	Plains 2001	0.31 ± 0.82	0.31 ± 0.42	4.00	0.0767	1.44	0.0767
	Griffin 2001	0.05 ± 0.23	0.05 ± 0.23	0.01	0.9907	1.67	0.0719
	Plains 2002	1.98 ± 1.32	2.01 ± 1.49	0.05	0.8344	1.11	0.3485
	Griffin 2002	0.15 ± 0.49	0.19 ± 0.51	0.42	0.5327	0.70	0.7812

Table 2.4. (continued). * Significant interaction at the alpha <0.05 level.

	Trial	Mean + SE/plot/sample date		Bt Main Effect		Bt x Date Interaction	
Taxa	Location- Year	non-Bt	Bt	*F* value	*P* value	*F* value	*P* value
Carabid Beetles	Plains 2001	0.81 ± 1.09	0.79 ± 1.32	0.63	0.4480	1.75	0.0620
	Griffin 2001	2.56 ± 3.50	2.79 ± 3.76	0.01	0.9960	0.73	0.7316
	Plains 2002	0.65 ± 0.94	0.98 ± 1.41	3.21	0.1066	0.91	0.5535
	Griffin 2002	0.57 ± 1.33	0.67 ± 1.59	0.29	0.6044	1.35	0.1783

Table 2.5. Mean seasonal numbers of target non-target arthropods observed in harvested corn ears. (20 ears per week for six weeks in 2001, 15 ears per week for six weeks in 2002). * Significant interaction at the alpha <0.05 level.

	Trial	Mean + SE/plot/sample date		Bt Main Effect	
Taxa	Location- Year	non-Bt	Bt	*F* value	*P* value
Corn earworms	Plains 2001	23.8±12.7	18.9±11.5	27.6	***0.0005**
	Bledsoe 2001	15.4± 5.5	15.9±6.7	0.26	0.6220
	Plains 2002	18.5±14.2	12.6±14.3	47.2	***0.0001**
	Bledsoe 2002	16.8±11.8	17.4±18.7	5.06	***0.0511**
Sap beetles	Plains 2001	84.5±96.1	77.8±84.8	0.25	0.6300
	Bledsoe 2001	9.2±13.0	7.8±14.4	0.75	0.4080
	Plains 2002	113.8±108.6	96.8±109.3	6.92	*** 0.0273**
	Bledsoe 2002	30.9±33.0	31.2±39.2	0.63	0.4482
Sap beetle larvae	Plains 2001	0.5±2.89	0.08±0.63	1.06	0.3302
	Bledsoe 2001	5.58±13.0	4.83±10.96	0.91	0.3660
	Plains 2002	9.27±27.3	6.02±15.21	1.92	0.1991
	Bledsoe 2002	0.69±1.64	1.05±3.57	0.04	0.8402

Table 2.5. (continued). * Significant interaction at the alpha <0.05 level.

	Trial	Mean + SE/plot/sample date		Bt Main Effect	
Taxa	Location- Year	non-Bt	Bt	*F* value	*P* value
Chinch bugs	Plains 2001	0.08±0.27	0.08 ± 0.27	0.01	0.9999
	Bledsoe 2001	0.13±0.44	0.31±0.93	0.83	0.3850
	Plains 2002	0.35±1.12	0.06±0.24	0.12	0.7395
	Bledsoe 2002	4.98±27.39	1.75±2.49	0.17	0.6935

Table 2.6. Ear kernel in Bt and non-Bt corn trials in 2001. *Significant differences at alpha <0.05.

Location	Resistance	Ear damaged area (cm_2)	Damaged Tip Length (cm_2)
Plains	non-Bt	6.67 ± 3.36	2.90 ± 1.17
	Bt	4.01 ± 2.59	2.16 ± 1.10
	F value	11.55	5.53
	P-value	*** 0.0079**	***0.0432**
Griffin	non-Bt	5.17 ± 1.75	2.71 ± 0.68
	Bt	3.37 ± 1.06	2.19 ± 0.68
	F value	11.84	11.84
	P-value	***0.0074**	***0.0074**

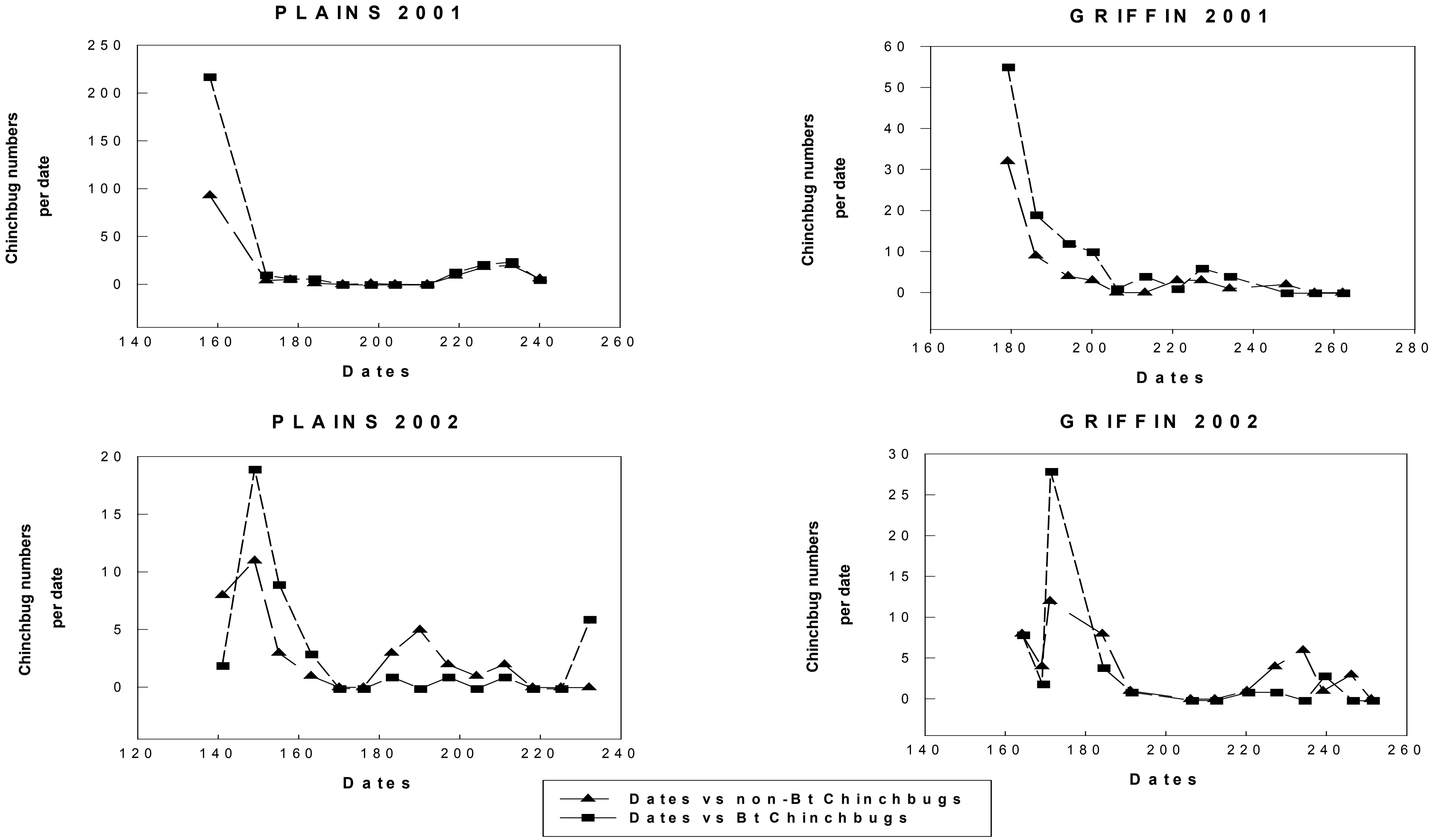
Table 2.7. Corn harvest weight and aflatoxin analysis in non-Bt and Bt corn fields. * Significant interaction at the alpha <0.05 level.

Trial Location- Year	Resistance	Test Weights (Lbs./bu)	Yield (lbs./acre)	Yield (bu./acre)	Aflatoxin Concentrations ppb ± SE
Plains 2001	non-Bt	57.50 ± 0.52	9,917 ± 1,305	177 ± 23.30	6.00 ± 11.35
	Bt	57.50 ± 0.38	10,176 ±1,206	181.72 ± 21.50	3.75 ± 4.57
	F value	1.04	0.89	0.89	0.24
	P value	0.3336	0.3708	0.3708	0.6351
Griffin 2001	non-Bt	60.83± 0.45	8,426± 865	150.50 ± 15.50	3.78 ± 5.29
	Bt	58.85 ± 0.57	9,204± 950	164.40 ±17.00	1.00 ± 1.06
	F value	3.76	3.95	3.95	1.11
	P value	***0.0001**	0.0781	0.0781	0.3204
Griffin 2002	non-Bt	58.25 ± 1.30	6,223 ± 620	111.10 ± 11.10	6.00 ± 2.73
	Bt	56.56 ±3.57	6,612± 990	118.10± 17.70	7.13 ± 3.09
	F value	2.06	0.76	0.76	0.50
	P value	0.1850	0.4096	0.4096	0.4968

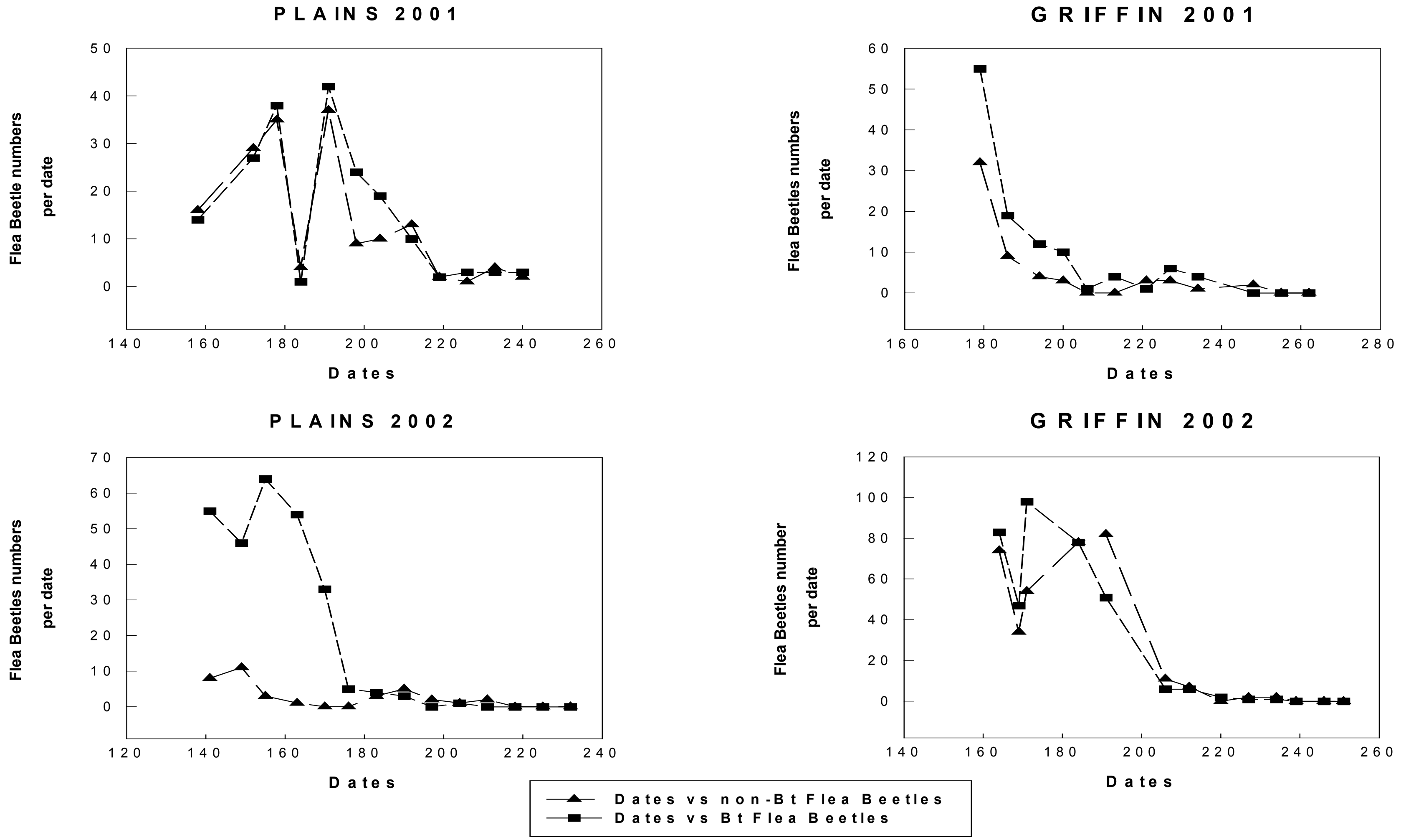
APPENDIX 1

MEAN SEASONAL NUMBERS OF NON-TARGET PHYTOPHAGOUS ARTHROPODS IN BT AND NON-BT FIELD CORN

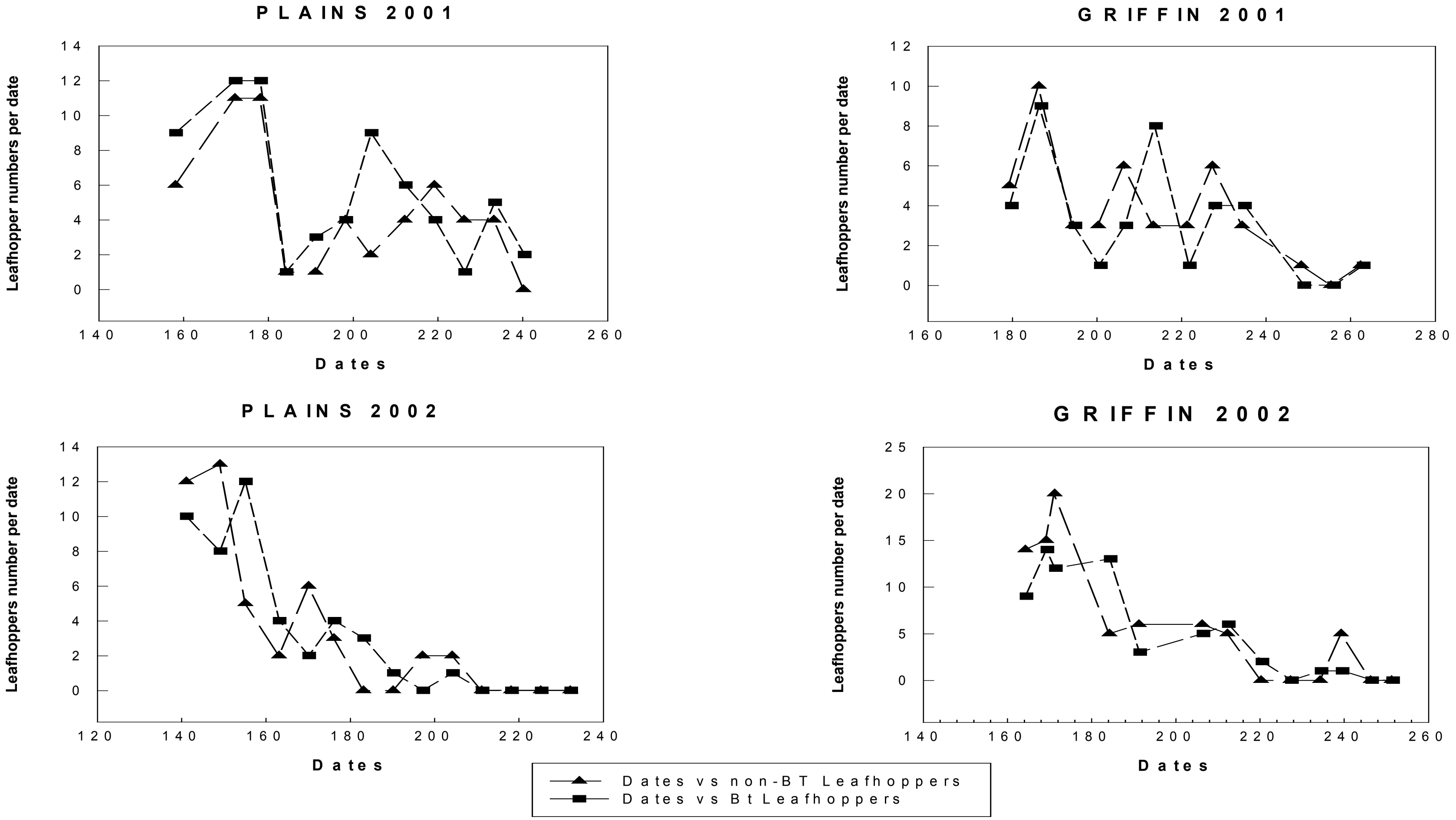
APPENDIX 1.1. Mean seasonal numbers of chinch bugs (*Blissus leucopterus leucopterus*) observered in visual counts of Bt and non- Bt field corn plots. (10 consecutive plants per plot up to ear up to ear stage, then 20 plants per plot).



APPENDIX 1.2. Mean seasonal numbers of flea beetles (*Chaetocnema pulicaria*) observed in visual counts of Bt and non-Bt field corn plots. (10 consecutive plants per plot up to ear stage, then 20 plants per plot).



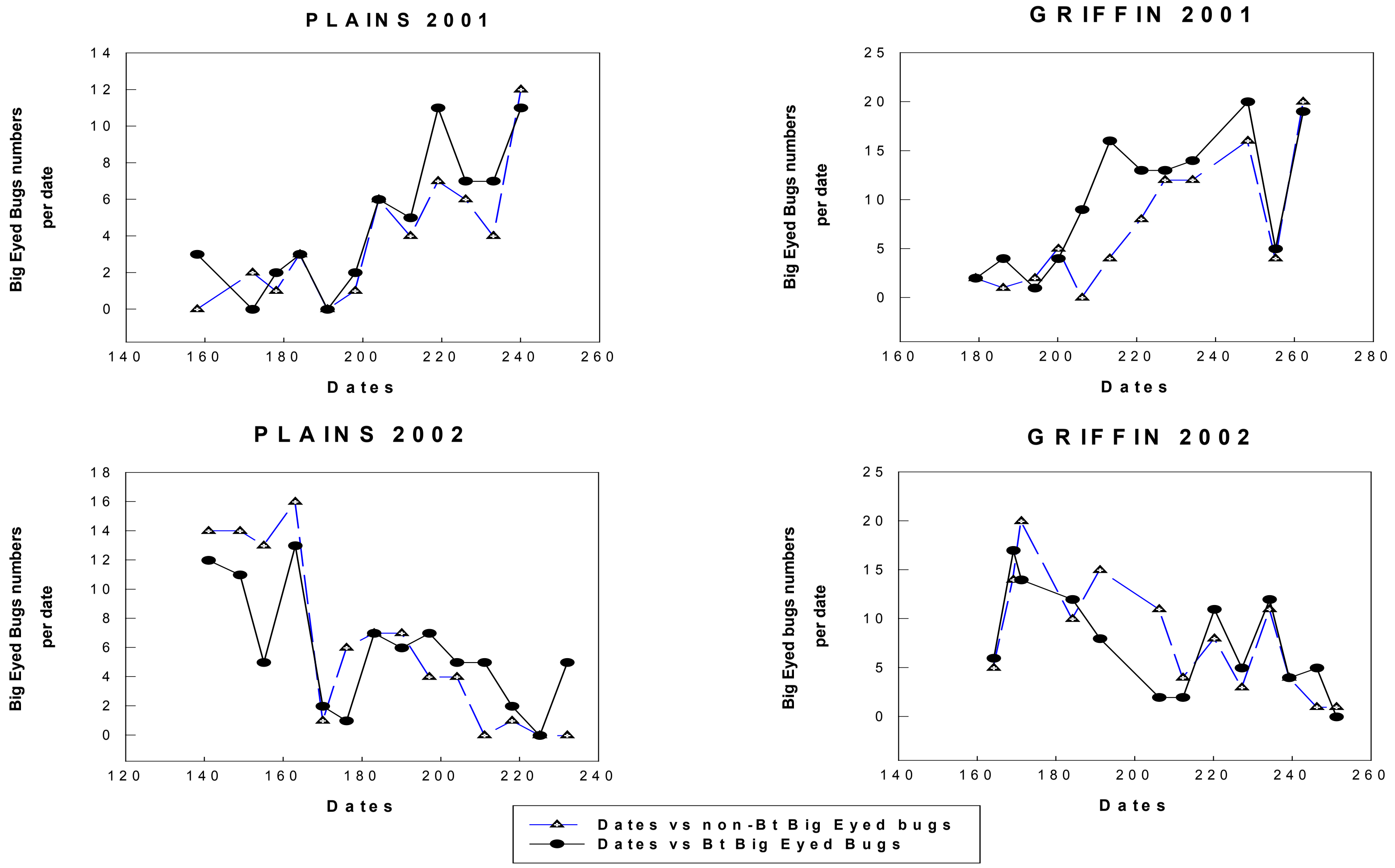
APPENDIX 1.3. Mean seasonal numbers of leafhoppers (many species) observed in visual counts in Bt and non-Bt field corn plots. (10 consecutive until ear stage, then 20 consecutive plants per plot).



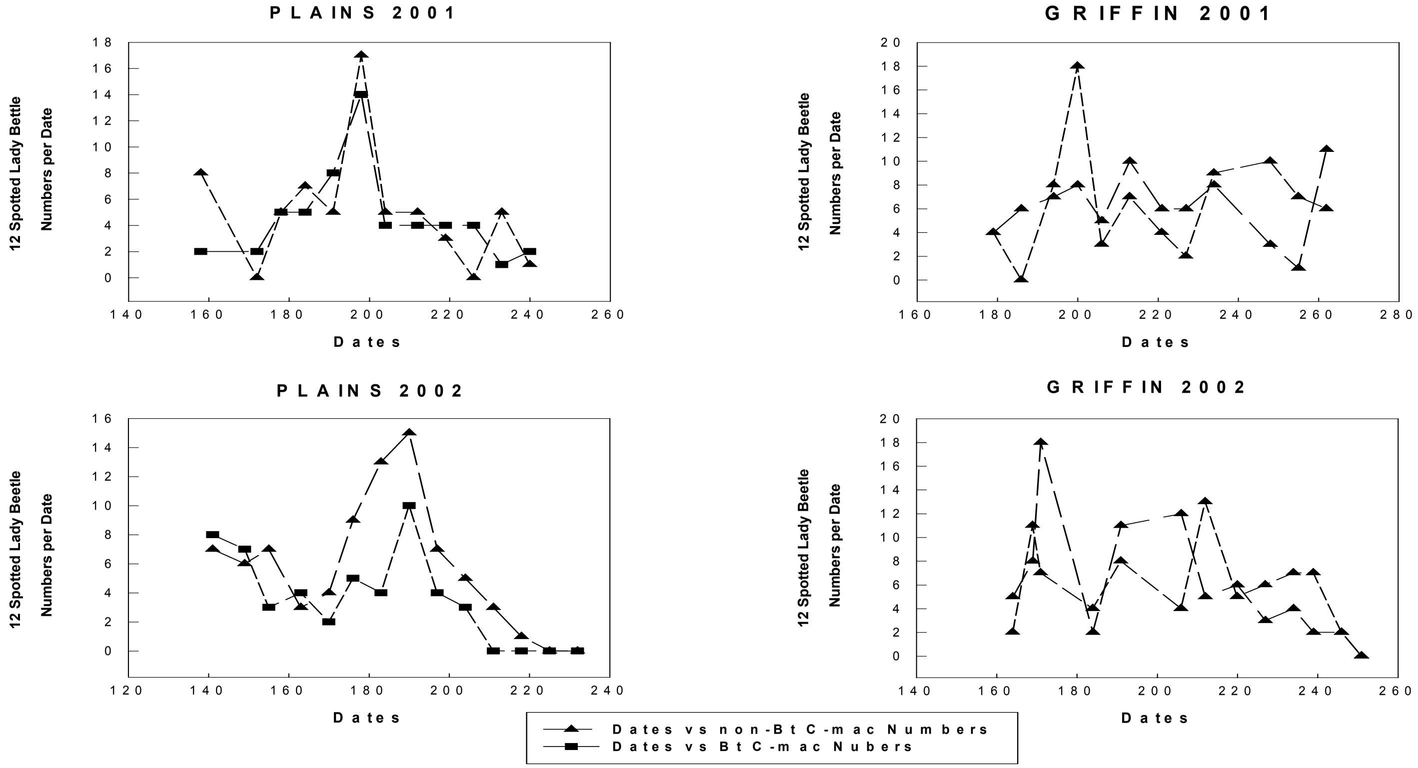
APPENDIX 2

MEAN SEASONAL NUMBERS OF NON-TARGET PREDACEOUS ARTHROPODS IN BT AND NON-BT FIELD CORN

APPENDIX 2.1. Mean seasonal numbers of big-eyed bugs (*Geocoris punctipes*) observed in Bt and non-Bt field corn plots (10 consecutive plants until ear stage, then 20 consecutive plants per plot).

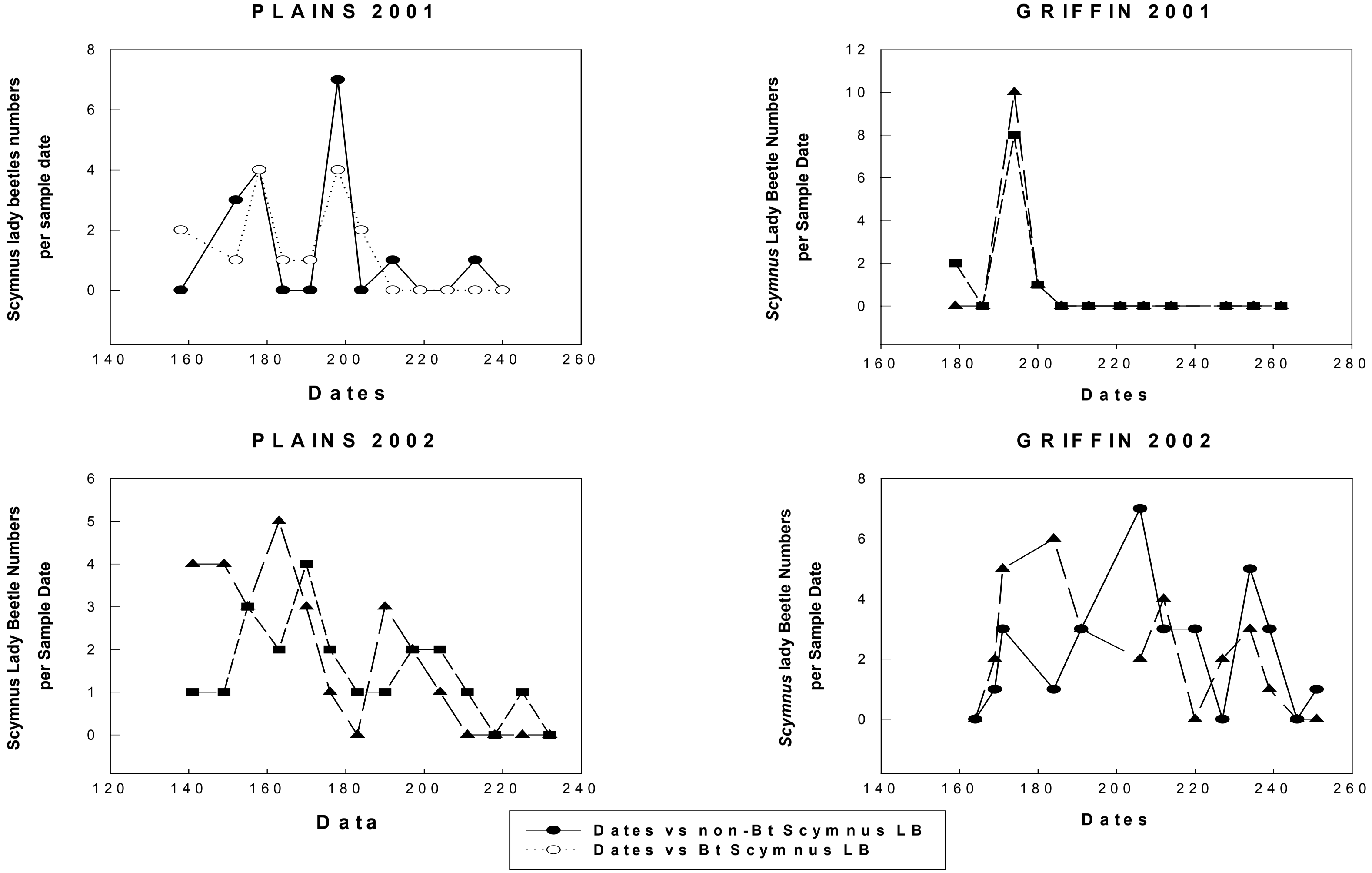


APPENDIX 2.2 . Mean seasonal numbers of twelve spotted lady beetles (*Coleomagilla maculata*) observed in the visual



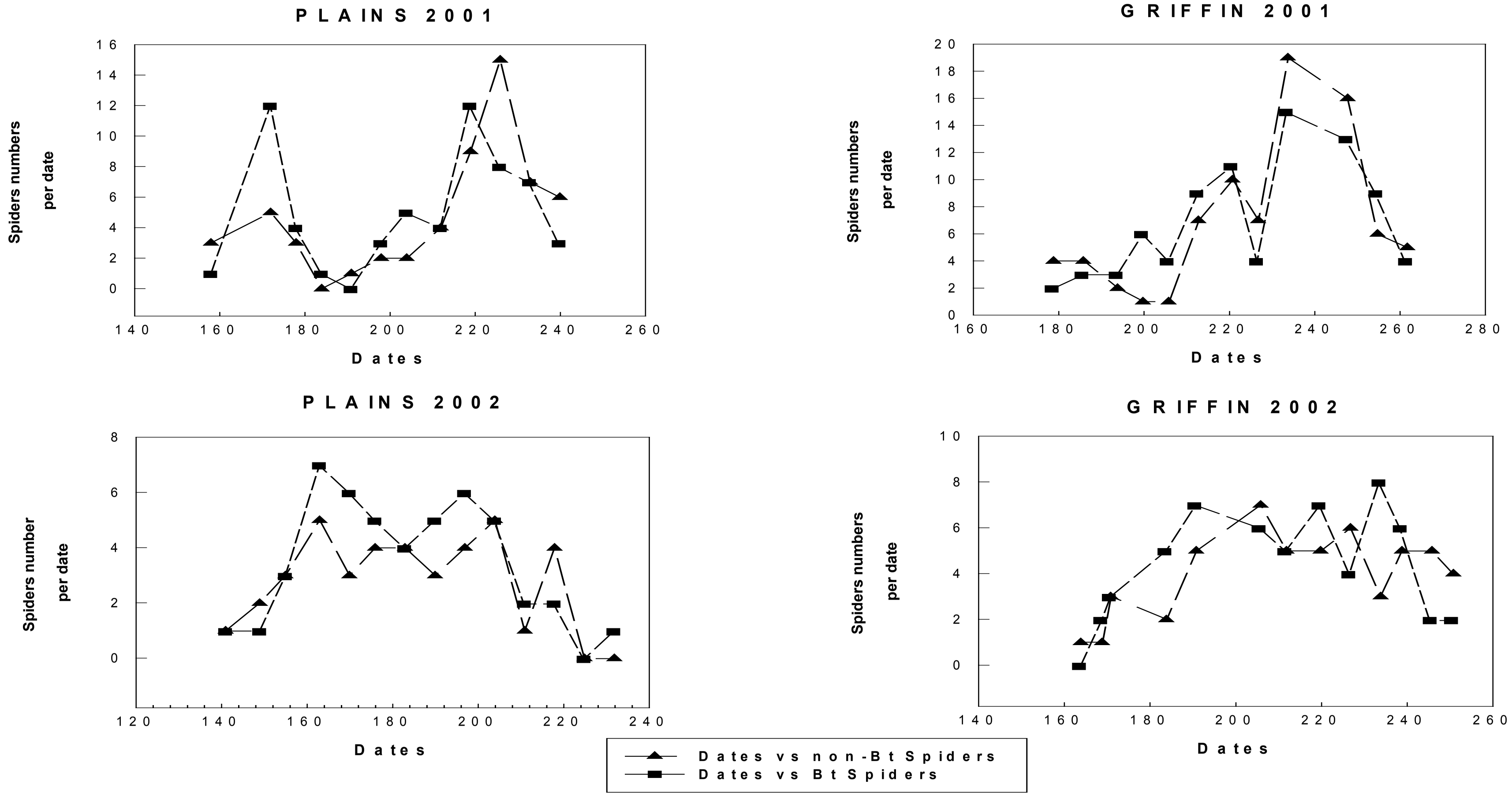
APPENDIX 2.3. Mean seasonal counts of Scymnus lady beetles (*Scymnus* spp.) observed in visual counts in Bt and non-Bt field corn plots (10 consecutive plants per plot up to ear stage, then 20 consecutive plants).

Bt field corn plots (10 consecutive plants counted up to ear stage, then 20 consecutive plants).



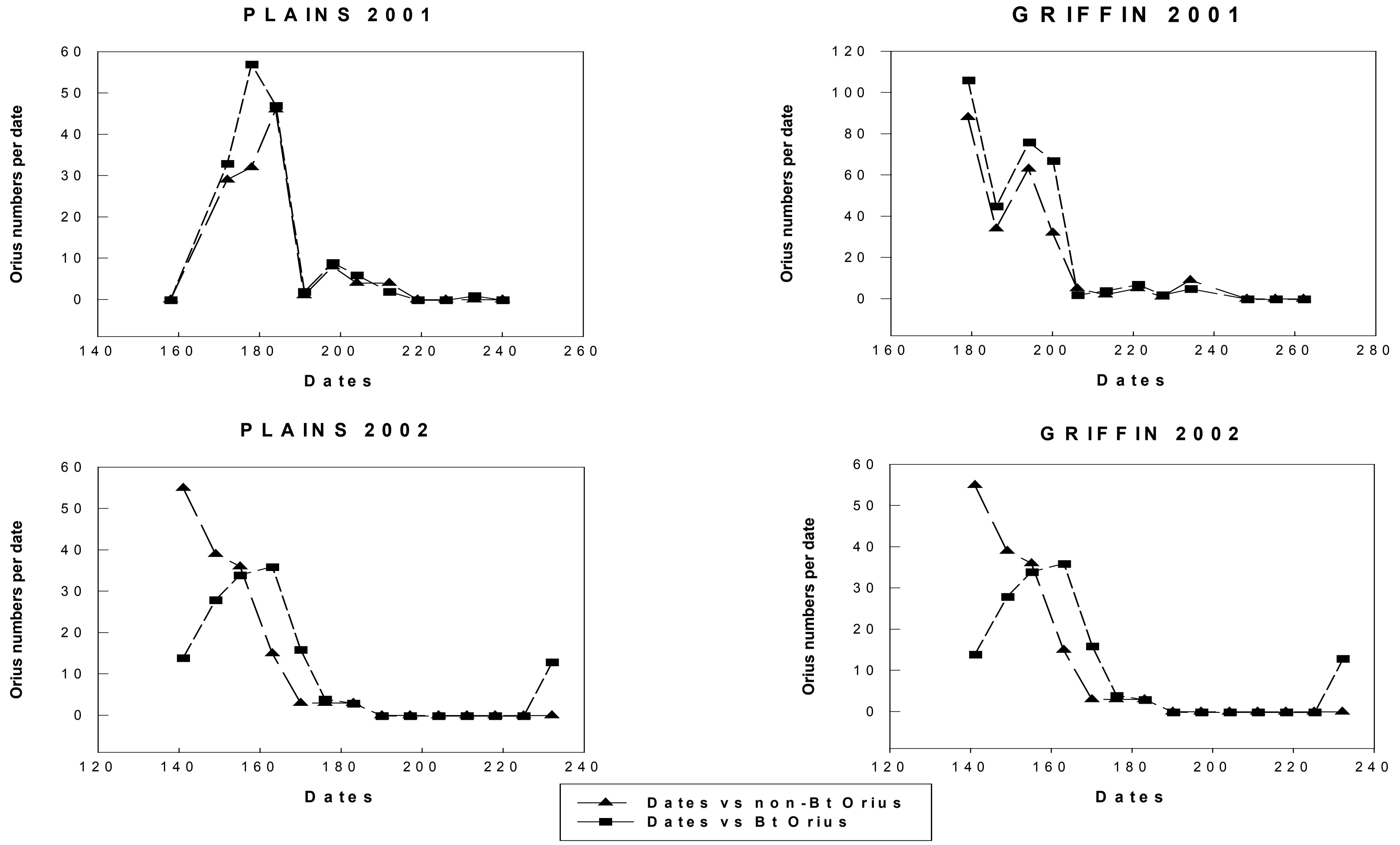
APPENDIX 2.4. Mean seasonal numbers of spiders (many species) observed in the visual counts in Bt

and non-Bt field corn plots (10 consecutive plants per plot up to ear stage, then 20 consecutive plants).



APPENDIX 2.5. Mean seasonal counts of insidious flower bugs (*Orius insidiosus*) observed in visual counts in Bt and non-

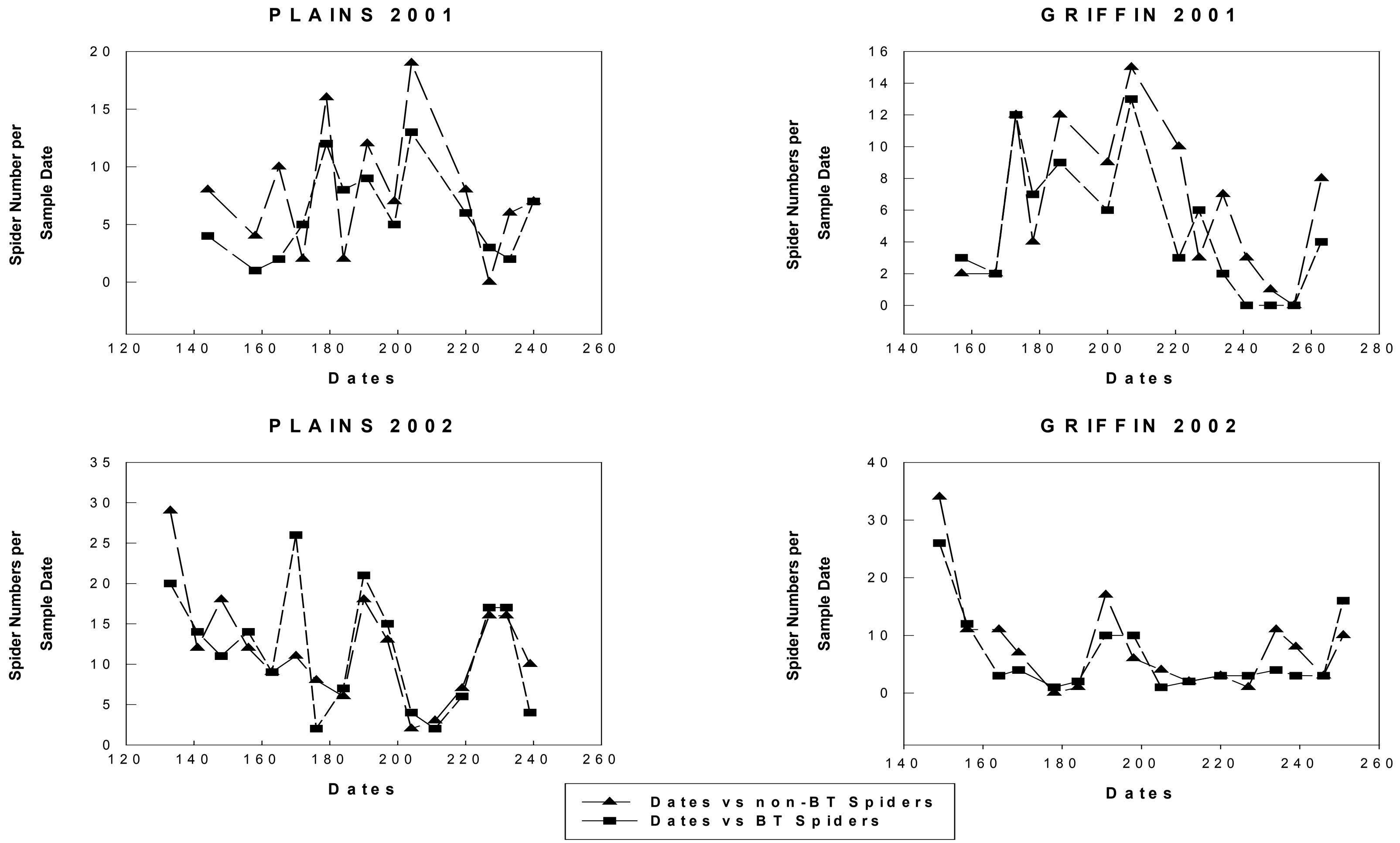
Bt field corn plots (10 consecutive plants counted up to ear stage, then 20 consecutive plants).



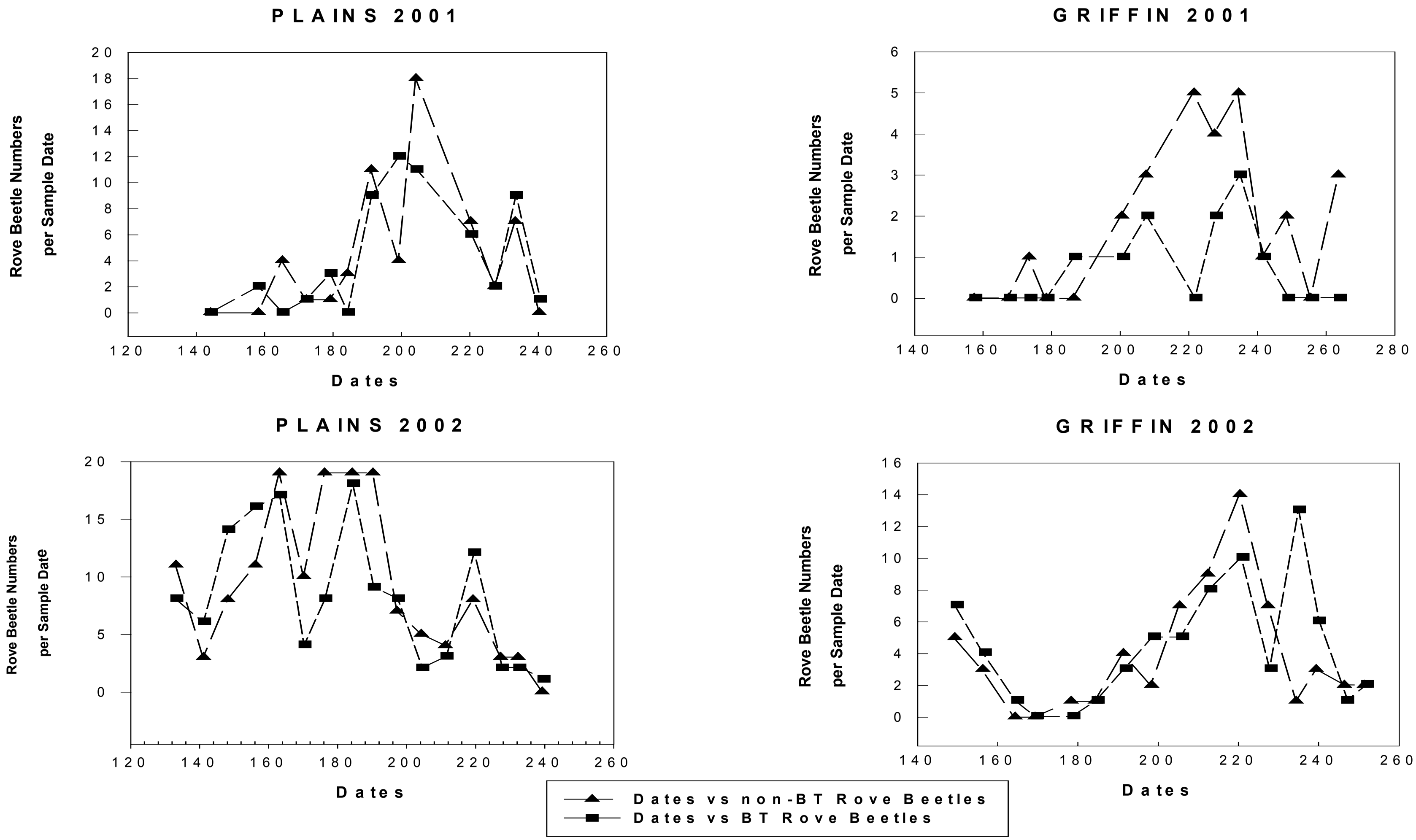
APPENDIX 3

MEAN SEASONAL NUMBERS OF NON-TARGET ARTHROPODS COLLECTED IN PITFALL TRAPS IN BT AND NON-BT FIELD CORN

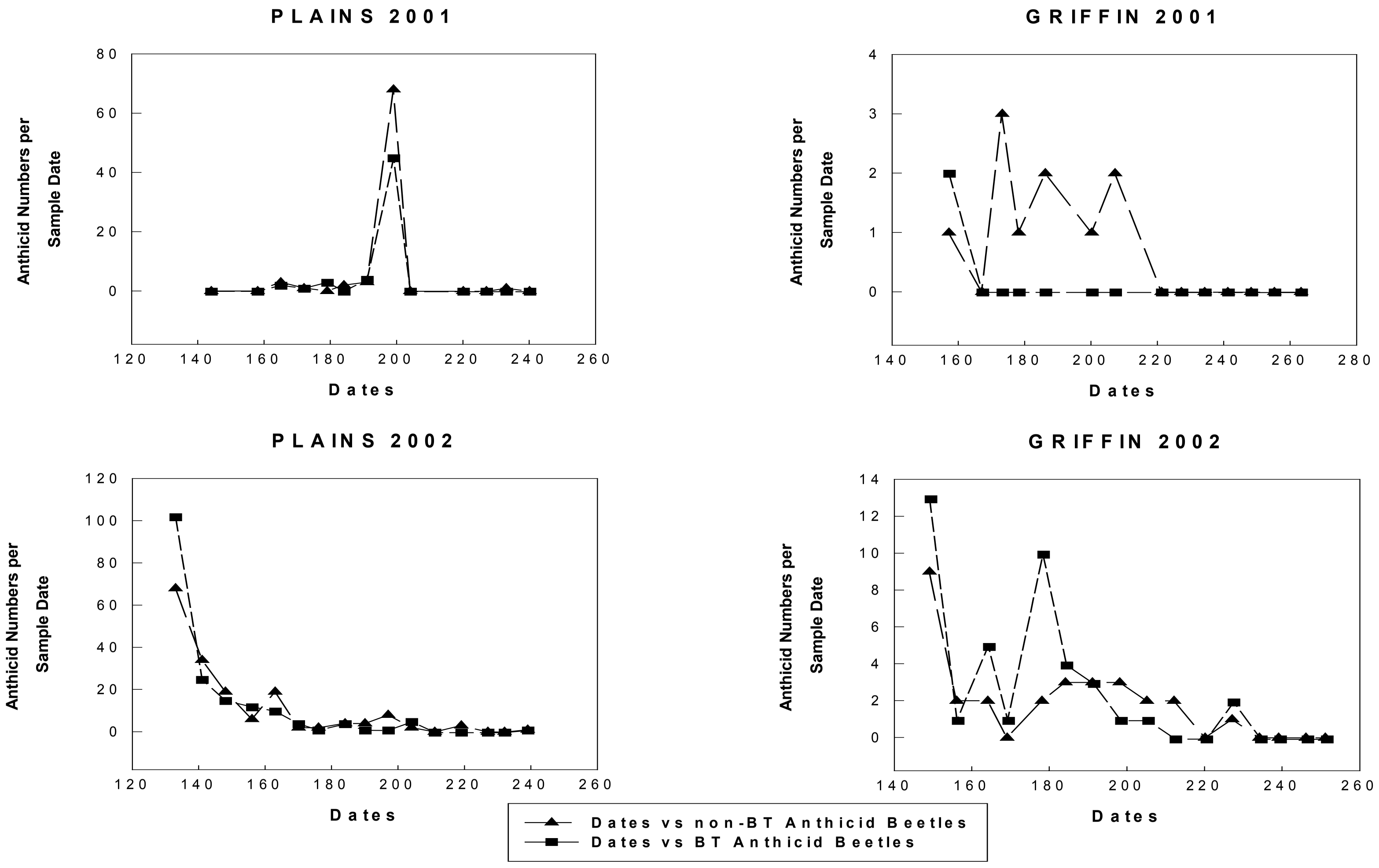
APPENDIX 3.1. Mean seasonal number of spiders (many species) collected in pitfall traps in Bt and non-Bt field corn field plots (Two traps per plot in 2001, three traps per plot in 2002).



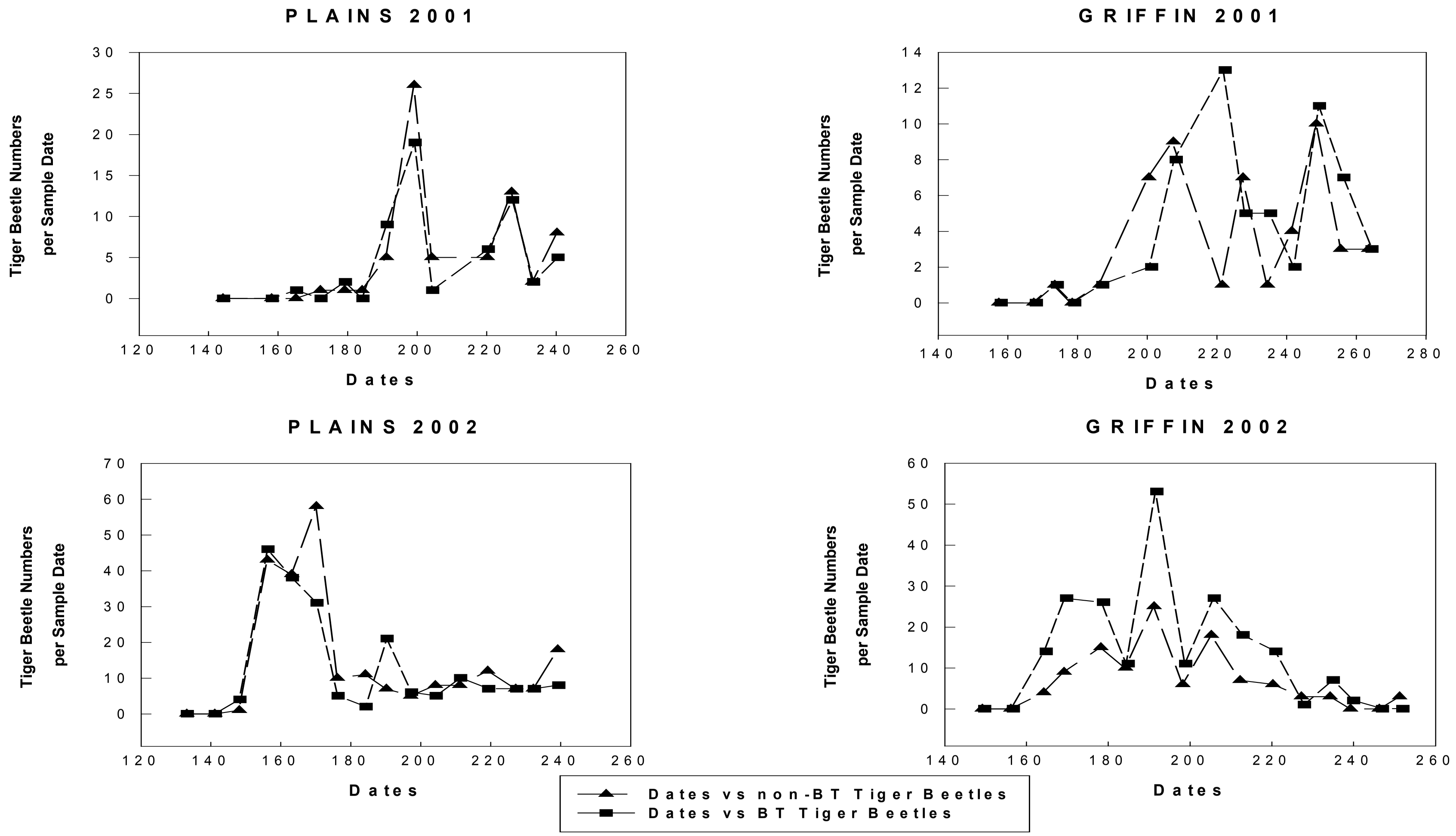
APPENDIX 3.2. Mean seasonal number of rove beetles (*Lathrobium* spp., *Sepidophilus* spp.) collected in pitfall traps in Bt and non-Bt field corn field plots (Two traps per plot in 2001, three traps per plot in 2002).



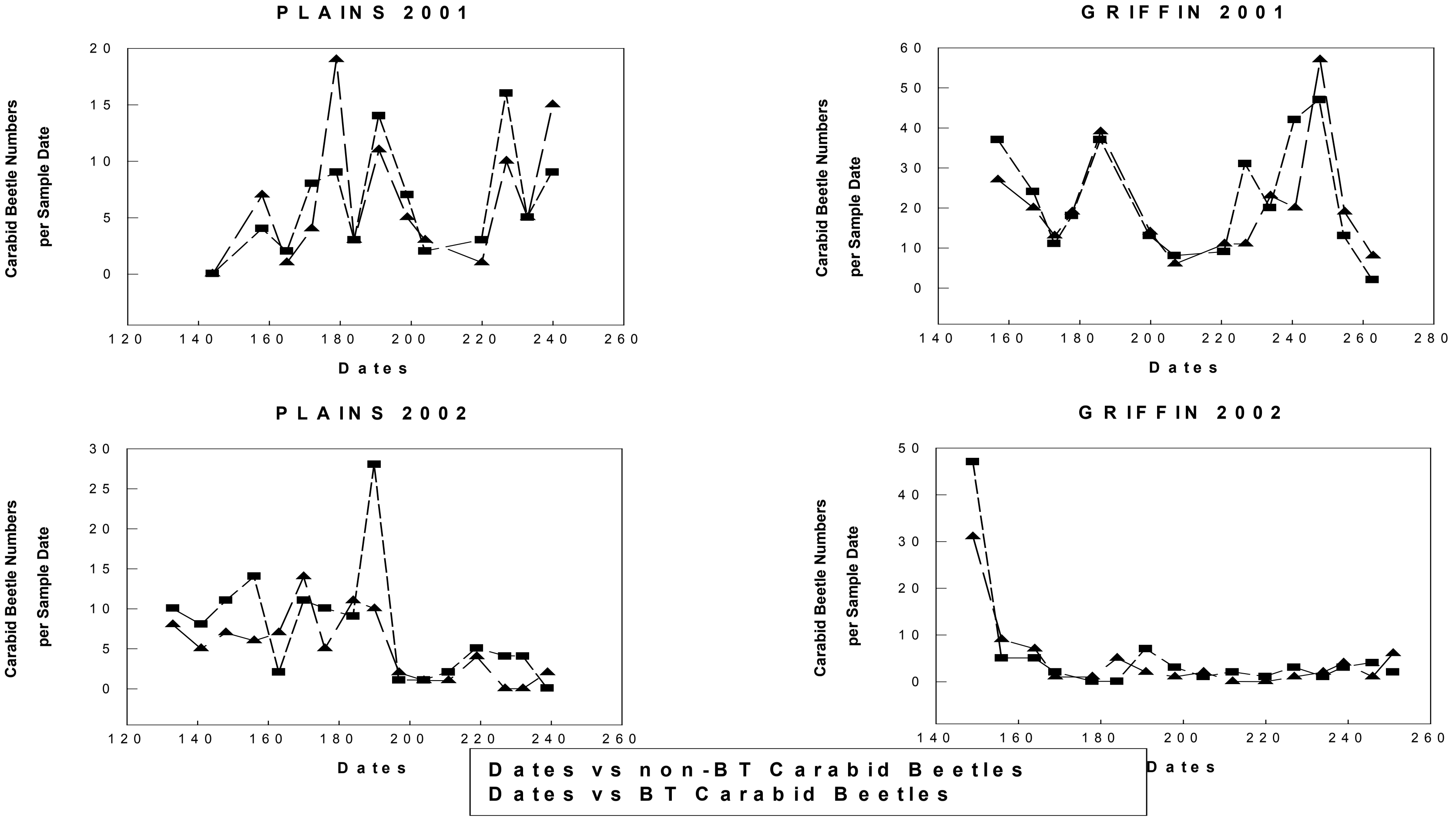
APPENDIX 3.3. Mean seasonal number of anthicid beetles (*Notoxus* spp., *Anthicis* spp.) collected in pitfall traps in Bt and non-Bt field corn field plots (Two traps per plot in 2001, three traps per plot in 2002).



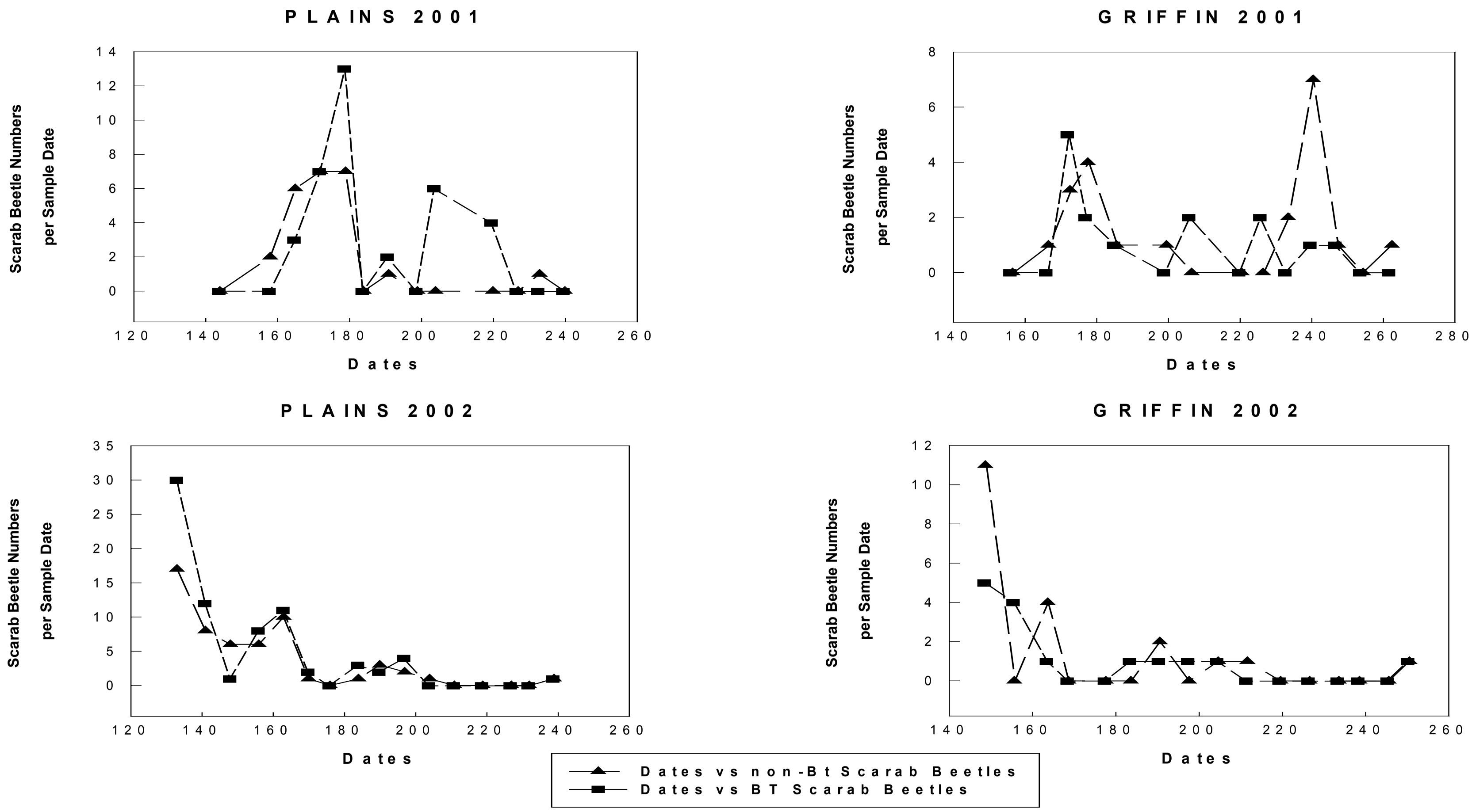
APPENDIX 3.4. Mean seasonal numbers of tiger beetles (*Megacephala carolina*) collected in pitfall traps in Bt and non-Bt field corn plots (two traps per plot in 2001, three per plot in 2002).



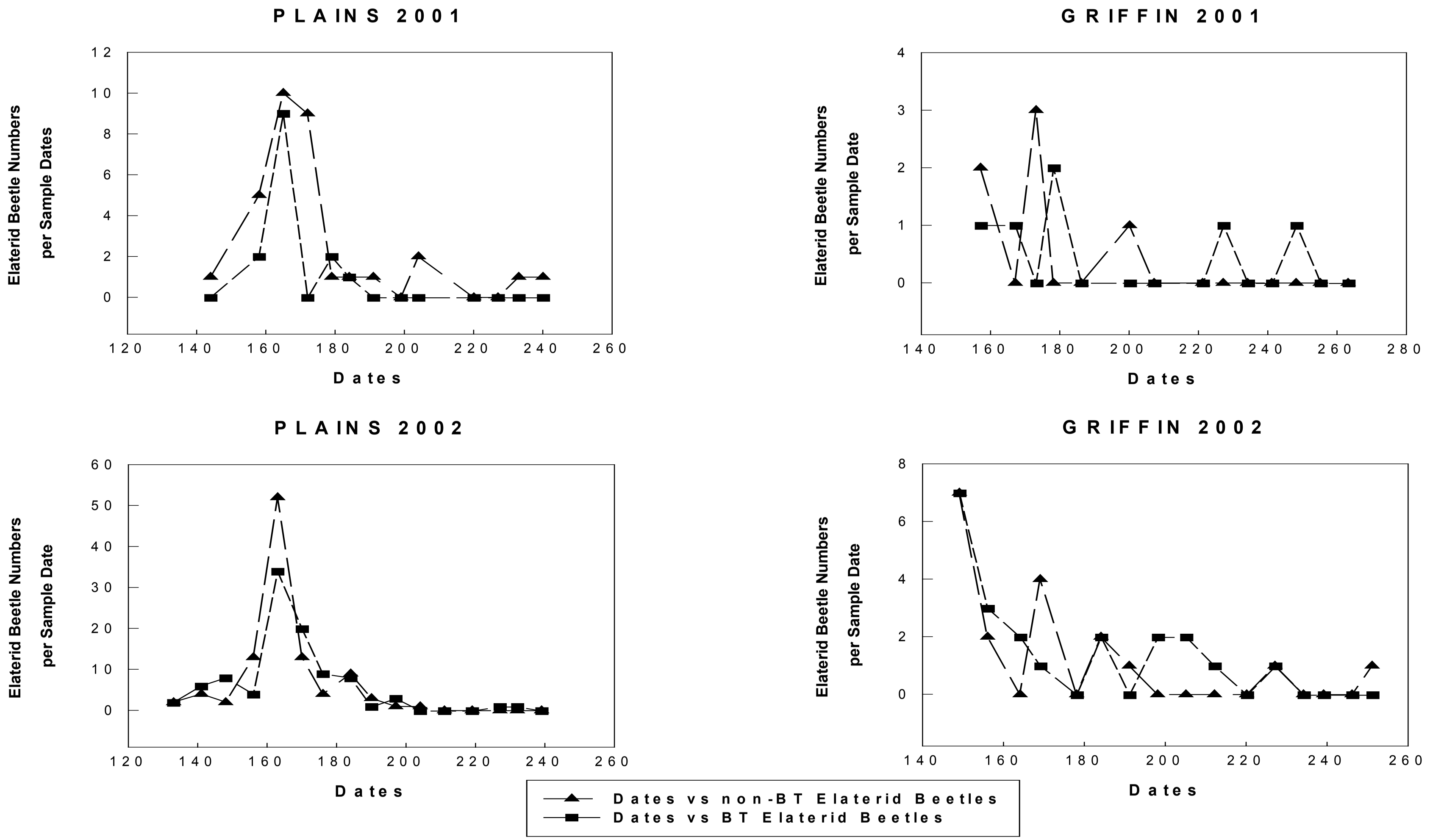
APPENDIX 3.5. Mean seasonal numbers of carabid ground beetles (*Harpalis pennsylvanica, Calasoma sayi*) collected in pitfall traps in Bt and non-Bt field corn plots (two traps per plot in 2001, three per plot in 2002).



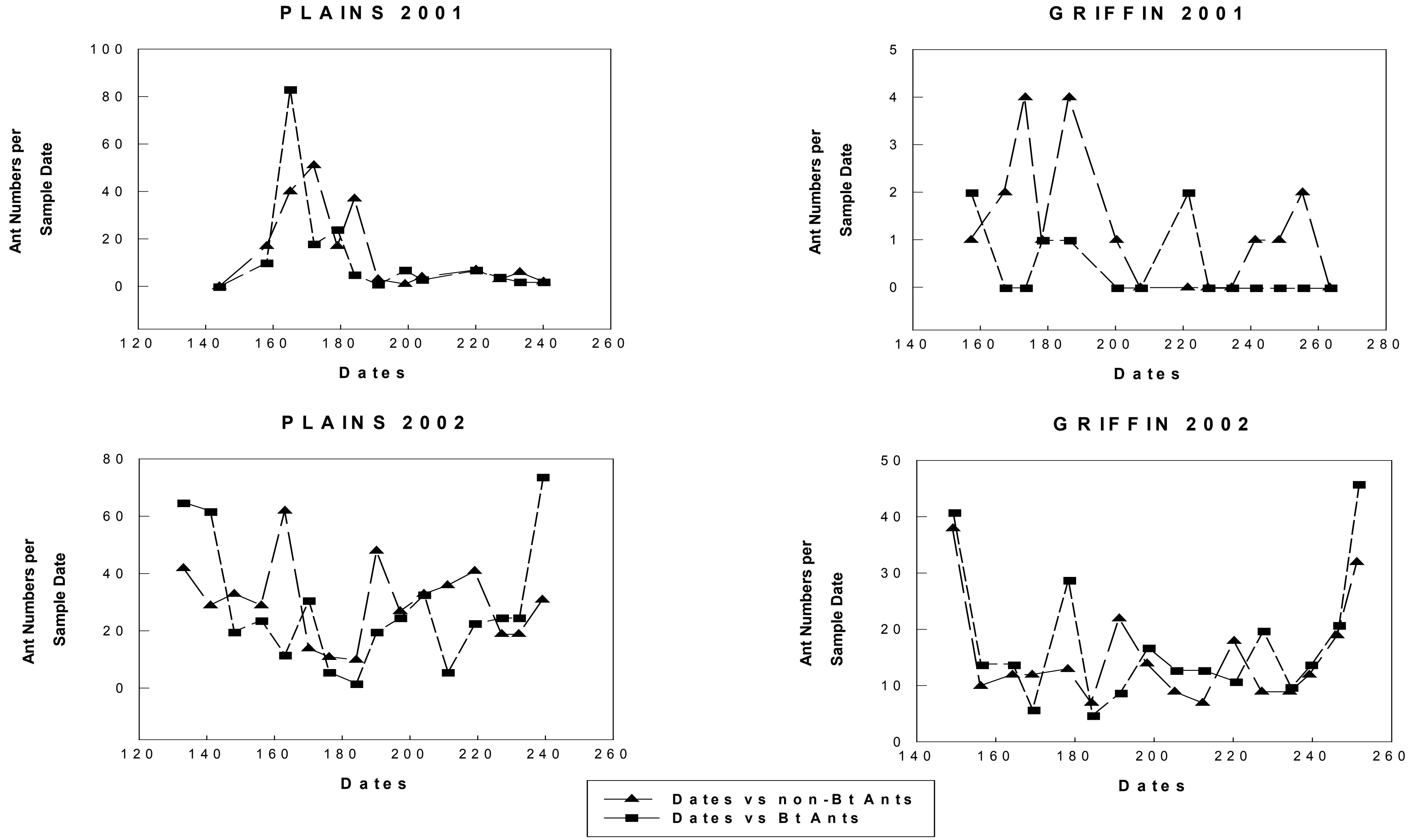
APPENDIX 3.6. Mean seasonal numbers of scarab beetles (*Altaneus* spp., *Cyclosephala immaculata*, *Balbaserasoma farctum*) collected in pitfall traps in Bt and non-Bt field corn plots (two traps per plot in 2001, three per plot in 2002).



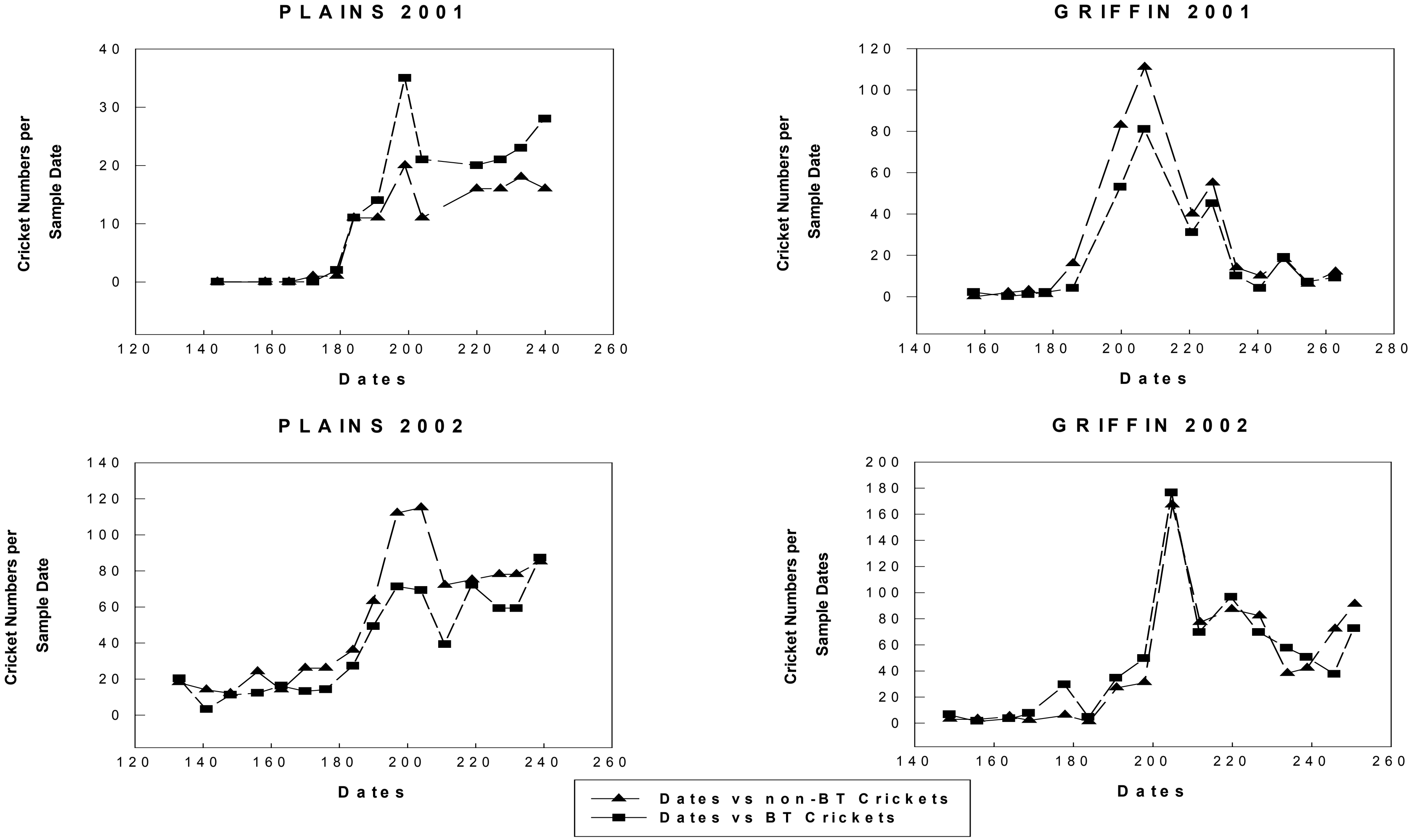
APPENDIX 3.7. Mean seasonal numbers of elaterid beetles (*Conodorus* spp.) collected in pitfall traps in Bt and non-Bt field corn plots (two traps per plot in 2001, three per plot in 2002).



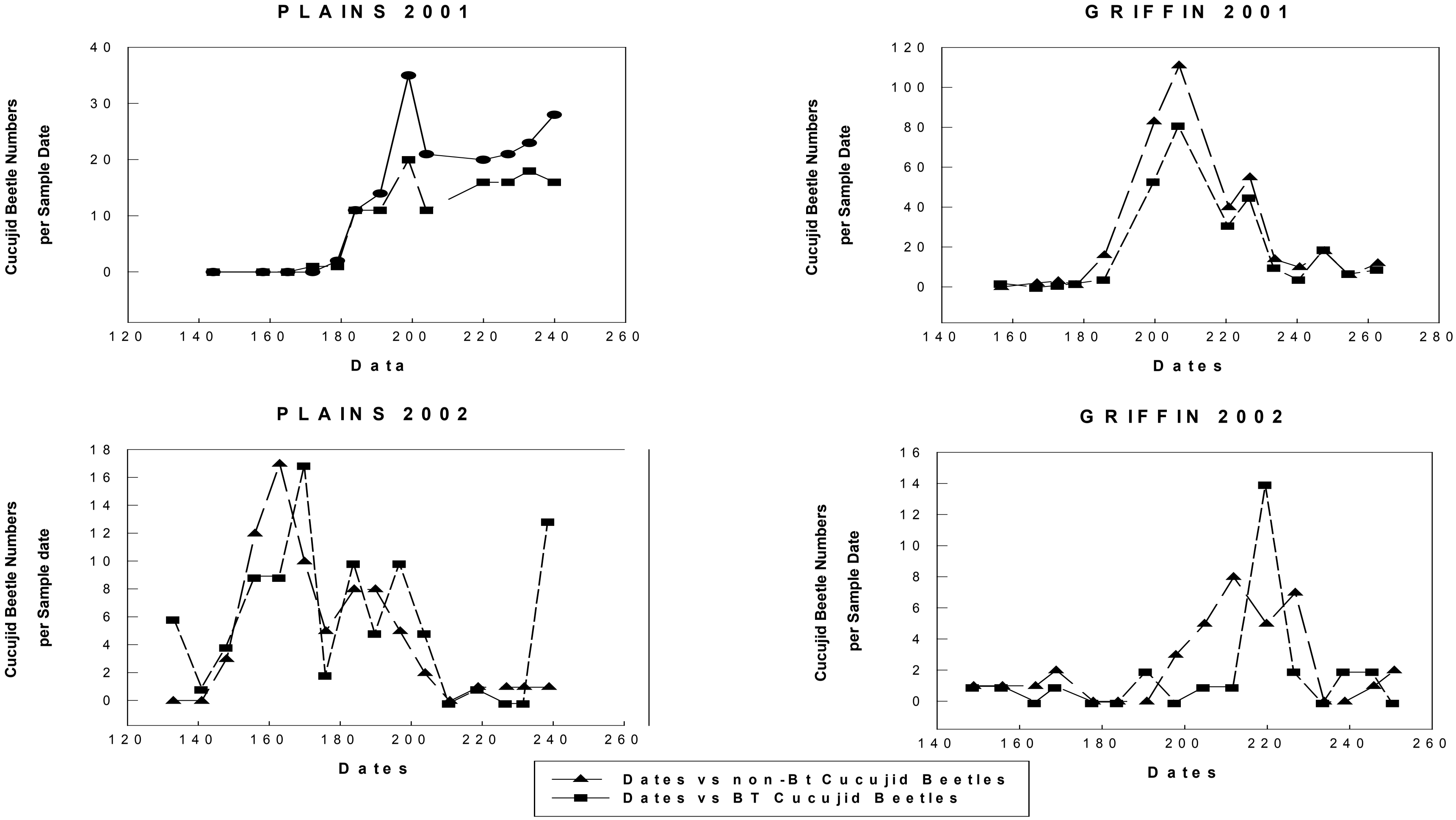
APPENDIX 3.8. Mean seasonal numbers of ants (*Solenopsis invicta*) collected in pitfall traps in Bt and non-Bt field corn plots (two traps per plot in 2001, three per plot in 2002).



APPENDIX 3.9. Mean seasonal numbers of crickets (*Gryllus pennsylvaticus, Allemobius fasciatus*) collected in pitfall traps in Bt and non-Bt field corn plots (two traps per plot in 2001, three per plot in 2002).



APPENDIX 3.10. Mean seasonal numbers of cucujid beetles (*Ahabersus advena.*) collected in pitfall traps in Bt and non-Bt field corn plots (two traps per plot in 2001, three per plot in 2002).



APPENDIX 4

CORN EAR INSECT COUNTS

APPENDIX 4.1. Corn earworms plotted against sap beetles observed in harvested ears obtained from Bt and non-Bt field corn plots (20 ears harvested for six weeks during the ear stage in 2001, 15 harvested in ear stage in 2002).