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FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES

School of Biological sciences

IMPACT OF CRY1AB TOXIN FROM TRANSGENIC MAIZE (MON 810) AND MICROBIAL BT SPRAY (DIPEL) ON THE ECOLOGY OF A NON-TARGET PARASITOID, *COTESIA MARGINIVENTRIS*

By

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ABSTRACT

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IMPACT OF CRY1AB TOXIN FROM TRANSGENIC MAIZE (MON810) AND MICROBIAL BT SPRAY (DIPEL) ON THE ECOLOGY OF A NON-TARGET PARASITOID, *COTESIA MARGINIVENTRIS*

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Cry1Ab toxin derived from *Bacillus thuringiensis* (Bt) has been used for the control of susceptible lepidopteran species throughout the world. Currently, sprayable Bt formulations and transgenic plants have been used for lepidopteran pest control. As plants and insects are part of a complex multitrophic system, using Bt toxin may also affect non-target organisms and thus pose an environmental risk. This research was conducted under controlled laboratory conditions – first tier lab testing on the second (*Spodoptera littoralis*) and third trophic levels (*Cotesia marginiventris*).

Spodoptera littoralis were fed with four different types of maize leaves; non-transgenic isogenic control, transgenic (MON810), and isogenic control plants sprayed with a control spray or Bt spray (Dipel). *S. littoralis* larvae maintained on non-transgenic maize leaves from day 6 to day 20 were significantly heavier when compared to the other maize treatments. No significant effect of Cry1Ab toxin was observed on the survival and pupation time of *S. littoralis* larvae. The groups exposed to transgenic maize were shown to have the lowest weight of parasitized *S. littoralis* larvae at 2 and 5 days after parasitism, and also the lowest weight of parasitoid cocoon.

The behaviour of *Cotesia marginiventris* with *S. littoralis* hosts (same age or same size) in no-choice tests was observed. Time taken to the first attack took significantly longer in the same-age host fed either transgenic maize or Bt spray maize when compared to control maize-fed hosts, however this did not differ in the same-size host. Time to cocoon formation and adult emergence was significantly shorter in the same-age larvae fed on non-transgenic maize when compared to other maize-fed hosts. In the parasitoid developed within same-size hosts, no significant differences in the time to cocoon formation and adult emergence were observed. Moreover, the number of parasitoid cocoons and adults were significantly higher in the same-age host fed on non-transgenic maize, while in the same-size hosts showed no significant difference in these numbers. The results would suggest the attack of parasitoid may be due to the size and age of the hosts. To conclude, the low parasitism and non-emergence of parasitoids observed may be due to the decreased quality of hosts that can not provide sufficient nutrients for development of parasitoid larvae. This

present study has helped to understand the direct or indirect effects of Cry1Ab on the non-target insects in the tri-trophic systems that could lead to the changes on host-parasitoid population dynamics in the ecosystem. The ecological relevance of the present study is discussed within a wider context of risk assessment in the environment.

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Declaration of Authorship

I, **Paiphan Paejaroen**, declare that the thesis entitled '**Impact of Cry1Ab toxin from transgenic maize (MON810) and microbial Bt spray (Dipel) on the ecology of a non-target parasitoid, *Cotesia marginiventris*'** and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this has been clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all my main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

Signed……..

Date……..

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Chapter 1

General Introduction

1.1 Overall Aim

The present study investigates the effect of *Bacillus thuringiensis* (Bt) toxin (Cry1Ab) from both transgenic maize and microbial Bt spray on the ecology of a non-target parasitoid. As this insect is part of the multi-trophic interactions in the ecosystem, the effects of Bt toxin may influence the insect population dynamics, which could have environmental implications.

Previous research has either focused on risk assessment or the ecology of the multi-trophic interactions. In this study an attempt is make to link these two approaches.

The present study consists of two parts;

1) to investigate the ecological risk assessment of Cry1Ab on the non-target insects, the herbivores *Spodoptera littoralis* and the parasitoids, *Cotesia marginiventris*) – a controlled laboratory condition (first tier test) was developed (Classical risk assessment), and
2) to identify possible effects of host feeding on Bt toxin on parasitoid fitness – the behaviour of parasitoids with their hosts in no-choice tests were investigated (Ecological interactions).

The present study could therefore links the ecological risk assessment of the Bt toxin (Cry1Ab) on the non-target insects and the general study of the multi-trophic ecology, thus integrating two disciplines frequently used separately in similar studies.

1.2 Introduction

1.2.1 *Bacillus thuringiensis* toxin

Bacillus thuringiensis (Bt) is a gram-positive, rod-shape, aerobic and spore-forming soil bacterium (for example - Hofte and Whiteley, 1989, Schnepf et al., 1998, Joung and Cote, 2000, Whalon and Wingerd, 2003, Chattopadhyay et al., 2004, Federici, 2005). It forms a parasporal crystal, which performs as an insecticide, during the stationary phase of its growth cycle (Hofte and Whiteley, 1989, Schnepf et al., 1998, de Maagd et al., 2001). The crystals are made of proteins, named δ-endotoxins, that have specific toxicity in a variety of pest species (Hofte and Whiteley, 1989, Keller et al., 1996). As chemical insecticides have known to have toxic effects beyond their target pests (including toxic effects to animals and human) (Joung and Cote, 2000), Bt is a useful alternative to synthetic chemical insecticide applications in agriculture and its safer, more selective, and biodegradable biocontrol agents can provide important ecological benefits (Schnepf et al., 1998, Joung and Cote, 2000, Whalon and Wingerd, 2003, Chattopadhyay et al., 2004, Federici, 2005).

The mode of action of Cry toxicity in the insect host results from the solubilization of the crystal proteins in the complex environment of the insect's midgut lumen and on the surface of the midgut epithelial cells (Hofte and Whiteley, 1989, Schnepf et al., 1998, Joung and Cote, 2000, Whalon and Wingerd, 2003). The crystal proteins, comprised of protoxins, cause the lysis of midgut epithelial cells, which leads to gut paralysis. Digestion of the crystal proteins by the insect, which leads to the proteolytic processing of the protoxin by the midgut proteases to become an activated toxin (Schnepf et al., 1998). For most lepidopterans, protoxins are solubilized under the alkaline conditions of the insect's midgut producing the toxic fragment (toxin) (Schnepf et al., 1998, Joung and Cote, 2000, Whalon and Wingerd, 2003, Chattopadhyay et al., 2004). The protoxins become active and then binds the Cry toxin to midgut receptors. The insertion of the toxin into the apical membrane creates ion channels or pores (as a result of pore formation). The cells die; eventually leading to the insect's death through starvation (Figure 1.1). It can be

concluded that there are four parameters involved in crystal protein function; (i) effectiveness of solubilization, (ii) efficiency of protoxin-toxin conversion, (iii) specific membrane receptor binding, and (iv) membrane pore formation (Schnepf et al., 1998, Joung and Cote, 2000).

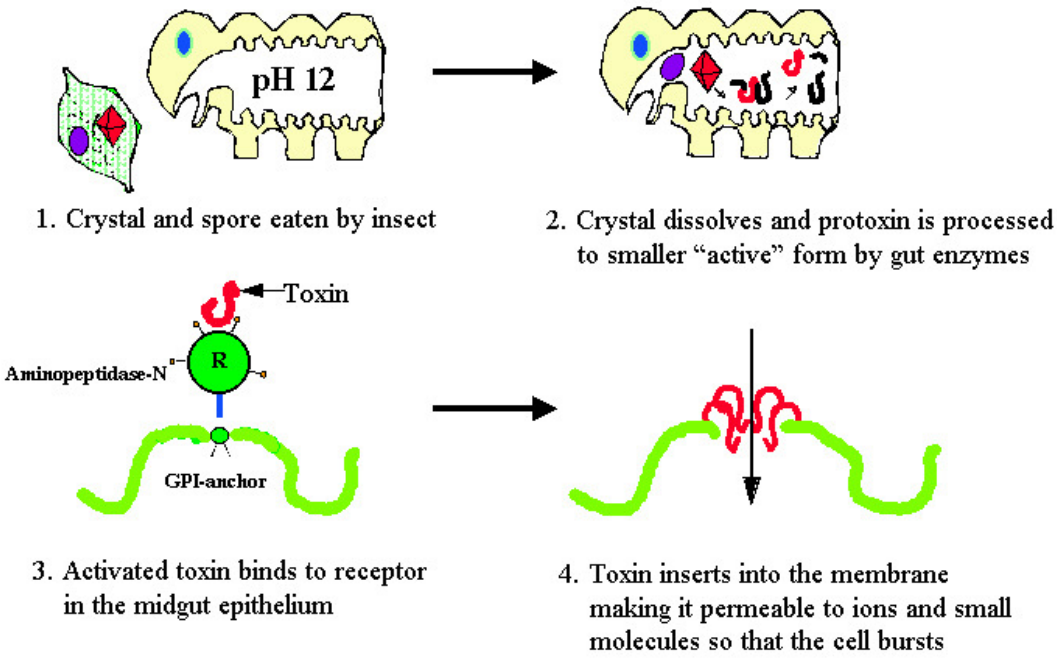


Figure 1.1. Bt toxin - Mode of action (Martinez et al., 2004) (http://www.bioc.cam.ac.uk/~dje1/).

The first commercial formulations of Bt were used in the field testing in the United States in 1958 and then as the biopesticide to control of lepidopteran pests in 1961 (Joung and Cote, 2000). It is widely used in the spray form which is composed of mixtures of δ-endotoxin crystals and Bt spores (Joung and Cote, 2000) alongside other typical agricultural formulations such as wettable powders, liquid concentrates, baits, dusts, and time-release rings (Chattopadhyay et al., 2004). This Bt product has been considered to a safe option for pest control and is used in many biological and integrated pest management control (Joung and Cote, 2000, Dutton et al., 2003a,

Chattopadhyay et al., 2004). For example, Dipel is the most often used Bt product (Dutton et al., 2003a) for controlling over 100 species of lepidopteran pests including the European corn borer (ECB), *Ostrinia nubilalis* (Hubner) in maize (Dutton et al., 2003a). However, the longevity of Bt in the form of an insecticidal spray is short, being rapidly degraded in ultraviolet light and washed away by rains or irrigation (Table 1.1) (Joung and Cote, 2000, Chattopadhyay et al., 2004).

Table 1.1. Comparison between Bt spray and transgenic plant.

Bt spray	Bt plant
• Not activated toxin (need cleaving in high pH environment)	• Partially activated toxin (no need to fully cleave)
• Multiple toxins and Spores	• Single toxin • By inserting truncated gene

• Degrade in environment
  • Wash away by rain or irrigation
	• Continuously produce protein toxin
	  • Bt gene introducing in plant cells

• UV sensitive
  • 24-48 hour degraded
	• Weather-independent protection
	  • Toxin synthesis in plant cells

1.3 Multi-trophic systems – the model organisms involved

1.3.1 Microbial Insecticide

Microbial insecticides are an alternative method of insect pest management which can replace some hazardous synthetic chemical insecticides (Joung and Cote, 2000, Dutton et al., 2003a). Bt spray, consisting of insecticidal crystal proteins from the bacterium *Bacillus thuringiensis* Berliner, is one example of microbial insecticides and is highly compatible with natural enemies and other non-target organisms. Due to the narrow host specificity and biodegradability in the environment of the Bt sprays, they are highly compatible with other forms of pest control such as natural enemy (Joung and Cote, 2000). Because the Bt Spray gives a limited level of exposure, uneven distribution on plants and rapid degradation (Dutton et al., 2005), it is interesting to undertake a comparison between the effect of Bt toxin using transgenic plants and the sprayable formulation, keeping the concentration of Bt toxin the same in both on the non-target insects in the tri-trophic system.

1.3.2 Transgenic maize

Transgenic plants have been genetically modified by inserting Bt δ-endotoxin genes into the plant cells to confer new characteristics (Schuler et al., 1998, Joung and Cote, 2000, Liu et al., 2005). Cotton, corn and soybean are engineered for insect resistance by introducing a single isolated gene from *Bacillus thuringiensis*, into the plant tissue (Zangerl et al., 2001, Zhang et al., 2004, Morse et al., 2005). This gene codes for the protein toxin (Cry or δ-endotoxin) which severely disrupts the digestive system of the insects (Schuler et al., 1998). However, it is not possible to use the complete toxin genes in the plants because these are not sufficiently soluble in the plant cells (the protoxins are only soluble at higher pH ≈ 9, whereas the pH in plant cells is around 7). To solve this problem, truncated genes are used to produce almost fully activated toxin molecules which reside in the plant cell in a solubilized form (Schuler et al., 1998). When the insect consumes parts of the toxin-expressing plant, the toxin undergoes proteolytic cleavage giving rise to the actual toxin.

The active toxin binds to the receptors located in the insect gut leading to the formation of pores and destruction of ion gradients. The gut wall breaks down and normal gut bacteria invade the body cavity. The insect stops feeding and dies of septicemia (Whalon and Wingerd, 2003). The advantages of the transgenic plants are concluded in Table 1.1 (Chattopadhyay et al., 2004, Federici, 2005).

The spread of transgenic plants has been very rapid; most of these plants are either herbicide or insect resistant, which lead to a reduction in pesticide use in intensive crop systems (Lovei and Arpaia, 2005). The first insect-resistant crop, transgenic maize, *Zea mays* (L.), was commercialized in 1996 (EPA, 2000 [from Dutton et. al., 2005]). The transgenic maize expressing the Cry1Ab protein has highly specific insecticidal effects on lepidopterans and protects the plants against the corn borer larvae such as the European corn borer, *Ostrinia nubilalis* (Meissle et al., 2005) (Figure 1.2). Some types of the transgenic maize also provide protection against other lepidopteran pests that feed on maize, including the Southwestern corn borer (*Diatraea grandiosella*), corn earworm (*Helicoverpa zea*) and fall armyworm (*Spodoptera frugiperda*).

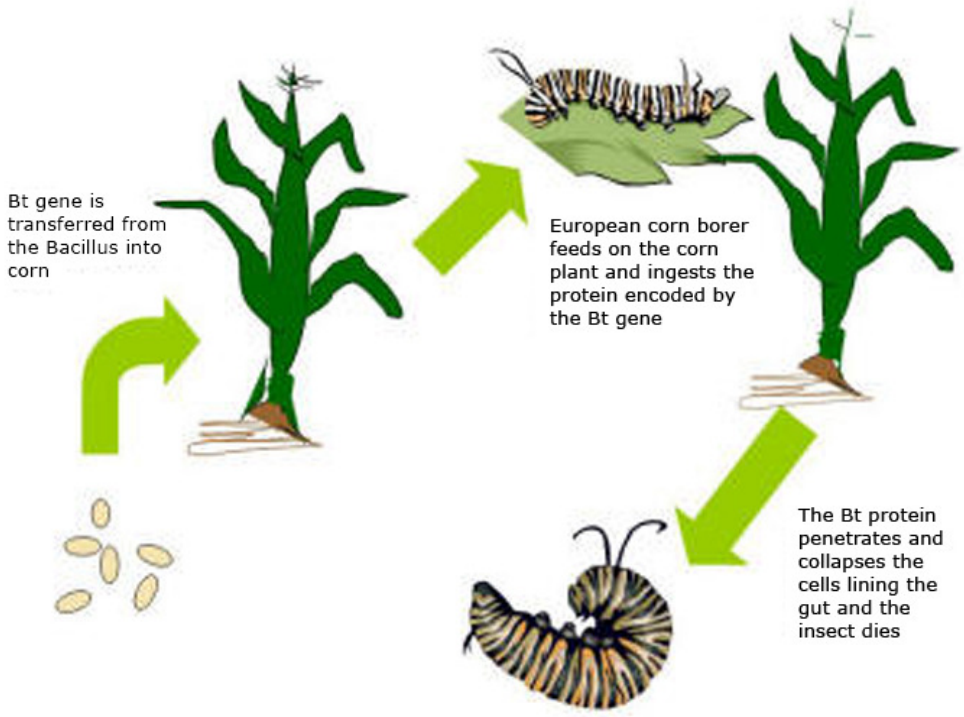


Figure 1.2. Transgenic maize. (http://www.apsnet.org/education/k-12plantpathways/teachersguide/Activities/PlantBiotechnology/text/fig37.htm)

Each of the transgenic maize cultivar includes the insertion of a Bt gene, a promoter gene and a marker gene. The gene promoter regulates the tissue-specific and developmental stage-specific expression of the Bt gene (Schuler et al., 1998, Magg et al., 2001). However, the levels of gene expression have been reported to vary in different parts of the plant (Table 1.2) (Schuler et al., 1998). For example, the MON810 cultivar uses the gene promoter, resulting in a season-long expression of the Bt toxin in all plant tissues (Magg et al., 2001). In contrast, event 176 contains two promoters, one regulating Bt gene expression exclusively in the green plant tissues and the other in the pollen (Magg et al., 2001). The selectable marker genes are introduced alongside the insect-resistance gene to allow separation of the plant cells that have

incorporated the new genes from untransformed cells. In the transgenic maize, herbicide-tolerance genes have been used as the selectable markers (Schuler et al., 1998).

Table 1.2. Promoters used with insect-resistance genes in maize (Schuler et al., 1998).

Promoter	**Expression site**	**Insecticidal protein**
Maize metallothionein-like promoter (MT-L)	Root preferred	Cry1Ab
Maize phosphoenolpyruvate-carboxylase promoter (PEPC)	Green tissue	Cry1Ab
Maize pollen-specific promoter	Pollen	Cry1Ab
Maize tryptophan-synthase α-subunit promoter (trp A)	Pith preferred	Cry1Ab

1.3.3 Non-target insects

a) non-target herbivore; Spodoptera littoralis

Spodoptera littoralis Boisduval (Lepidoptera, Noctuidae) is considered to be a very destructive pest in subtropical and tropical agriculture (Salama et al., 1990). The caterpillar attacks Solanaceae, Cruciferaceae, artichokes, strawberries, fodder crops, maize, cotton, tomatoes and capsicum (www.defra.gov.uk; www.inra.fr). This pest is a highly polyphagous species and it is one of the most serious cotton pests in Egypt as well as in North Africa. It is mostly found in glasshouses where it causes damage to the leaves leading to reduced photosynthetic activity in the plants (www.inra.fr) (Fig. 1.3).

Figure 1.3. *Spodoptera littoralis* on tomato leaf (http://www.inra.fr/internet/Produits/HYPPZ/IMAGES/7033081.jpg)

Spodoptera littoralis larvae are susceptible to Cry1C and Cry1E, whereas other Cry proteins such as Cry1Aa, Cry1Ab and Cry1Ac have less effect (Keller et al., 1996, Regev et al., 1996). The study by Sneh et al. (1981) reported that young larval stages (1st and 2nd) of *S. littoralis* are the most sensitive to Cry1C and Cry1E whereas the 3rd and 6th instar larvae are resistant to these endotoxins as the high proteolytic activities in the gut juice lead to the complete degradation of Cry1C protein (Keller et al., 1996). Due to its susceptibility to Cry1C and Cry1E, this insect of interest has been used as a model to indicate the effects from Cry1Ab protein as a second trophic level in a worst case scenario test, as they are a non-target herbivore in the tri-trophic level. Several studies (Hemerik and Harvey, 1999, Dutton et al., 2002, Dutton et al., 2003a, Dutton et al., 2005, Meissle et al., 2005, Vojtech et al., 2005) used *S. littoralis* as the non-target insect that ingested the Cry1Ab protein (from both the transgenic plants and the insecticidal spray) and then passed the toxin to predatory insects on a higher trophic level. For example, Dutton et al. (2002) and Vojtech et al. (2005) showed that the survival rate and the time required to reach the second instar were affected significantly when *S. littoralis* larvae were reared on the transgenic plants compared with the larvae reared on the non-transgenic plants.

b) non-target parasitoid; Cotesia marginiventris

Cotesia marginiventris Cresson (Hymenoptera: Braconidae) or armyworm parasitoid is a polyphagous, solitary endoparasitoid of several species of Noctuidae (http://edis.ifas.ufl.edu; www.ivia.es) (Fig. 1.4). This insect plays an important role in the biological control of noctuid pests of vegetable crops (Vojtech et al., 2005, Riddick, 2006). This insect is an endoparasite of a wide range of insect pests such as *Heliothis virescens* (F.), the tobacco budworm; *Spodoptera eridania* (Cram.), the southern armyworm, *S. exigua* (Hubner), the beet armyworm; *S. frugiperda* (Smith), the fall armyworm (http://edis.ifas.ufl.edu).

Figure 1.4. *Cotesia marginiventris*. (http://i27.photobucket.com/albums/c198/olguis/blog/avispaorugasmaiz.jpg)

The mated adult females parasitize only young larvae (first to second instar) of noctuid pests. The single oval-shaped egg is laid in each host then the larva hatches two days after oviposition. The larva emerges from the posterior end of the host and immediately begins spinning a tight silky cocoon, which hatches in seven to 10 days. The host, which feeds less throughout its life, dies within a

day of the parasitoid emerging, all the organs inside having consumed by the parasitoid (http://edis.ifas.ufl.edu). *C. marginiventris* adults prefer to oviposit in the early instar hosts (1^{st} - 2^{nd} instar) and the parasitoid larvae complete their whole development on a single host individual and are therefore closely connected with their hosts (Meissle et al., 2004). This makes the parasitoid important in biological control (Riddick, 2001), especially in the control of secondary pests in the Bt crops and it is useful in quantifying the risk assessment of transgenic crops (Vojtech et al., 2005). Vojtech et al. (2005) showed the negative effects on *C. marginiventris* when parasitizing Bt maize-feeding *S. littoralis* larva, including a delayed development, higher mortality or reduced pupal weight. These parasitoids are appropriate model systems for examining the possible direct and indirect impacts of transgenic crops on the non-target Lepidoptera and their parasitoids in the agro-ecological system.

1.4 Effects of Bt toxin on non-target insects

Since the introduction of Bt toxin to agricultural pest management, it is increasingly employed instead of synthetic insecticides. Recently, the bacterial toxin genes have been engineered into the plants to protect against insect pests. Although the Bt toxins are most targeted against insect pests, both the Bt spray and Bt plant may be had the adverse effects on non-target organisms (Hails, 2000, Dutton et al., 2002).

Bt insecticides are the most widely used in agriculture, given their specificity and mode of action. Bt products have been considered a safe option for a pest control. With this wide-spread use, there are concerns about the environmental impact of Bt toxins on non-target beneficial arthropods as there are may be two pathways of non-target exposure: (i) direct exposure (e.g. by eating leaves, litter, or the uppermost layer of the soil) and (ii) indirect exposure (e.g. by eating caterpillars which have been infected with Bt) (Joung and Cote, 2000, Dutton et al., 2003a). Studies showed less effect of the Bt insecticidal sprays,

perhaps due to the low persistence of Bt in the environment (Hofte and Whiteley, 1989) with 50% of its insecticidal activity in 1-3 days (Joung and Cote, 2000). However, the studies by Dutton et al. (2003b) and by Dutton et al. (2005) showed that the Bt insecticidal spray (Dipel), which has the Cry1Ab toxin had the negative effect on *Spodoptera littoralis* and *Chrysoperla carnea* including the prolonged developmental time, high mortality and decreasing weight.

Transgenic maize may affect non-target arthropods including herbivores, natural enemies, and pollen feeders (Lovei, 2001, Dutton et al., 2005, Romeis et al., 2006) as its Bt proteins are produced in relatively high levels in a large proportion of the plants throughout most of their growing period until the plants senesce (Hilbeck et al., 1998c). The larvae of Monarch butterfly (*Danaus plexippus*) fed on pollen (from transgenic maize event 176) deposited on milkweed leaves can influence the larval survival and the weight gains (Hellmich et al., 2001). The pollen from event 176 did produce pollen that has very harmful effects on the Monarch larvae (Hellmich et al., 2001) such as eating less, slow development and high mortality (Losey et al., 1999, Jesse and Obrycki, 2000). It produces 40-fold higher concentrations of the endotoxins compared with the MON810, owing to the use of a different promoter (Hellmich et al., 2001). This result shows that the levels of the Bt expression in the pollen are very important for subsequent toxicity of the transgenic maize pollen (Gatehouse et al., 2002). Similarly, the study by Zangerl et al. (2001) showed that the swallowtail (*Papilio polyxenes*) also had high mortality when fed on the pollen from event 176 deposited on wild parsnip (*Pastinaca sativa*).

The Bt maize expressing Cry1Ab toxin has the negative effect on *S. littoralis* larvae maintained on transgenic maize. These effects are a delay in development and a higher mortality rate. Hilbeck et al. (1998a) showed higher mortality of the predatory lacewing *C. carnea* when the larvae were supplied by the Bt-fed prey, *S. littoralis*. Vojtech et al. (2005) observed that there was a negative effect by the Bt maize on the survival, developmental time and larval

weight of *S. littoralis* larvae. The survival, developmental time and cocoon weight of *Cotesia marginiventris* were adversely affected when developing within host fed on Bt maize (Vojtech et al., 2005). Meissle et al. (2004) showed that when *S. littoralis* larvae were fed Bt maize, its parasitoid *Compoletis sonorensis* (Cameron) had a longer development time. This result is similar to the study of Bt maize expressing Cry1Ab on the generalist predator *Poecilus cupreus* (Meissle et al., 2005). It showed that newly hatched *P. cupreus* larvae were affected when feeding on Bt maize-fed prey (*S. littoralis*) when compared to the 10-day old *P. cupreus* larvae. It is also shown in the study by Manachini and Lozzia (2004) that the European corn borer larvae fed on transgenic maize displayed a lower level of parasitism both in percentage and in absolute numbers of parasitoids (*Lydella thompsoni* Herting). It would be interesting to investigate the effect of Bt toxin both from the Bt spray and the transgenic maize on the non-target insect *S. littoralis* and on the natural enemies *C. marginiventris*.

1.5 How the quality of host has an effect on the parasitoid fitness

A parasitoid is an organism that spends a part of its life history attaches to or within a single host organism, ultimately killing the host (Godfray, 1994). Koinobiont parasitoids allow the host to continue its development and often do not kill or consume the host until the host is about to either pupate or become an adult; this therefore typically involves living within an active, mobile host (Harvey et al., 1994). Since parasitoids lay their eggs in or on other insects, these insects of interest are used in biological control (Hemerik and van der Hoeven, 2003, Meissle et al., 2004, Morales et al., 2007). The rate of finding hosts, quality of hosts, number and sex of the eggs, are representative of the fitness of a female parasitoid (Hemerik and Harvey, 1999). As the host is the finite resource, the quality of host influences three correlates of fitness in the parasitoids: (1) parasitoid survival until the adult stage; (2) parasitoid size and

fecundity as an adult mate and; (3) parasitoid development time (Godfray, 1994, Hemerik and Harvey, 1999, Couty et al., 2001a). Hosts must find sufficient nutrients to permit their normal growth and development (Collier et al., 1994, Thompson, 1999, Bede et al., 2007, Urrutia C et al., 2007). If a host is unable to survive, then the developing parasitoid is doomed to follow the same fate.

Host size also has a major impact on parasitoid fitness, including longevity, fecundity, and host-finding ability (Vinson and Iwantsch, 1980b, Thompson, 1999) as it determines the maximum amount of food available for the developing parasitoid (Godfray, 1994). When the parasitoid develops in a small host which has insufficient nutrients, this may reduce the development time and also leads to a reduction in the adult size (Godfray, 1994, Hemerik and Harvey, 1999). Urrutia C et al. (2007) stated that the fitness of parasitoid depends on the size and age of the host. Older hosts may provide less nutrition for host-feeding and for parasitoid offspring development (King, 1998). Though a larger host provides the better food source for the parasitoid larvae, they may be better defended. Khafagi and Hegazi (2004) showed that the development of a parasitoid, *Microplitis rufiventris*, was clearly affected by *Spodoptera littoralis* host instar and ages within same instar. Third instar-parasitized larvae produced significantly more parasitoid cocoons than in the fourth instar. This may be due to the endocrine system in different ages of the host.

Godfray (1994) stated that host condition can also influence parasitoid fitness as poor condition host contain reduced nutrients for the developing immature parasitoid. This reduced quality of host leads to increase parasitoid development time (Godfray, 1994). Transgenic plants may also affect natural enemies by reducing the quality of the herbivore as a host source. Several studies (see reviews - Manachini and Lozzia, 2004, Meissle et al., 2004, Vojtech et al., 2005, Sanders et al., 2007) observed that hosts fed on Cry1Ab toxin have smaller size and a delay development. This smaller host could cause

an indirect affect on the parasitoid development such as smaller cocoon and a prolonged development time as the host provides less nutritional resources for the developing parasitoid (Romeis et al., 2006). Parasitoids are very sensitive to changes in their hosts after toxin ingestion compared with predators which are generalists and feed on different prey species, as they complete their development on one single host (Vojtech et al., 2005). Therefore, the studies using herbivores that are targeted by Bt toxin are relevant to assess the risks for natural enemies. *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) is a polyphagous, solitary endoparasitoid and can be considered a generalist as it attacks many lepidopteran species. This makes the parasitoid potentially important to study the ecological impacts of Bt toxin as it belongs to the third trophic level in the food chain.

1.6 Risk assessment of transgenic crops

Ecological risk assessment (ERA) is a process that evaluates the likelihood that adverse ecological effects are occurring or may occur as a result of exposure to one or more stressors (USEPA, 1992). A risk does not exist unless: (1) the contaminant has the ability to cause an adverse effect and (2) a plant or animal can come in contact with a contaminant long enough and at a high enough concentration that the contaminant causes an adverse effect (USEPA, 1992). Ecological risk assessments can help identify environmental problems, establish priorities, and provide a scientific basis for regulatory actions (USEPA, 1992). Risk assessment can be described in terms of: assessment endpoints, hazard, exposure, an estimator of risk and a trigger value of the estimator (Figure 1.5) (Poppy and Wilkinson, 2005). Assessment and measurement endpoints may involved ecological components from any level of biological organization, ranging from individual organisms to the ecosystem itself (USEPA, 1992). In general, the use of a suite of assessment and measurement endpoints at different organizational levels can build confidence

in the conclusions of the risk assessment, ensuring that all important endpoints are evaluated (USEPA, 1992).

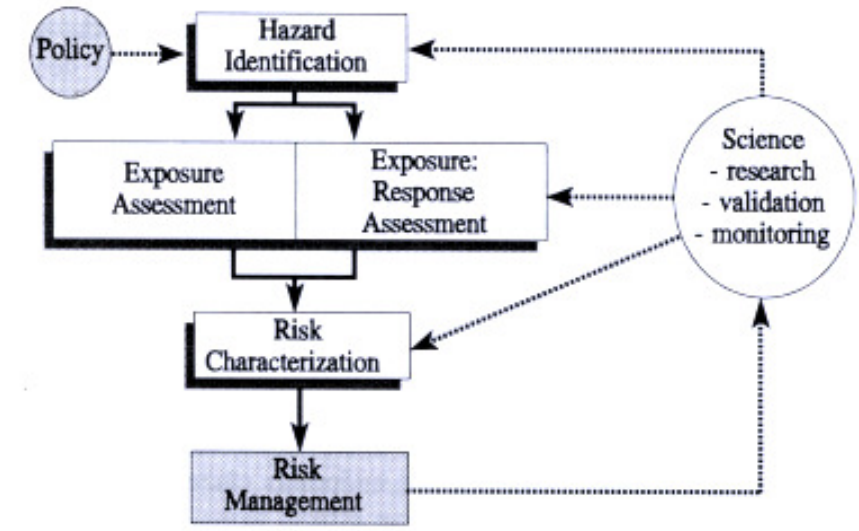


Figure 1.5. A risk assessment framework (USEPA, 1992)

Risk is defined in a mathematical form as: "Risk = Hazard x Exposure" (Gatehouse et al., 2002, Conner et al., 2003, Wilkinson et al., 2003, Poppy and Sutherland, 2004, Poppy and Wilkinson, 2005). Thus, even if the potential hazard is great, if the exposure is effectively zero, so will be the risk (Gatehouse et al., 2002). The efficient strategy to collect the relevant data to assess risk is part of a tiered test (Figure 1.6), which begin by assessing risk from measurements of hazard and exposure under worst-case conditions – the first tier test (Poppy and Wilkinson, 2005). This first tier test identifies the direct toxic effects to an organism within the laboratory. For example, in the case of risk assessment of transgenic crops, early tier tests are conducts to determine whether an organism is susceptible to the toxin under worst case conditions, that is, organisms are directly exposed to high dosed of the toxin (Romeis et al., 2006). The purpose of this test is to aid efficient decision to prevent higher tier testing of substances that present very low hazard.

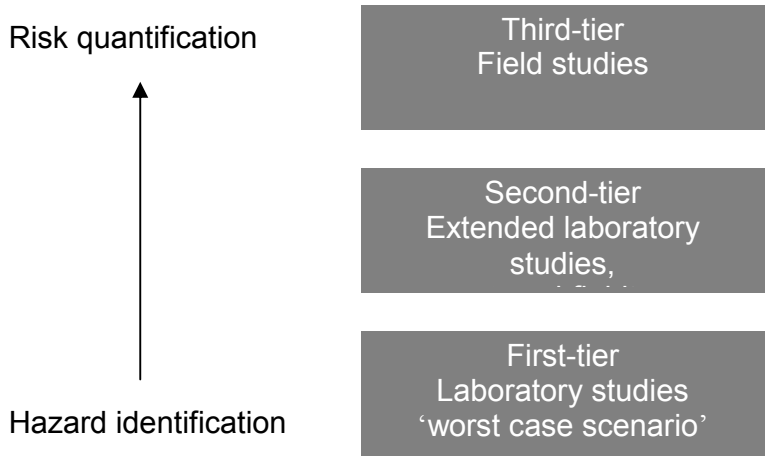


Figure 1.6. Tier risk assessment (Wilkinson et al., 2003).

There are concerns over possible environmental effect from transgenic plants. Concerns include assessing the significance of gene flow from the transgenic crops to wild relatives and the effect on the non-target organisms in the environment (Poppy, 2000, Dale, 2003, Poppy, 2004, Poppy and Wilkinson, 2005). For GM risk assessment, it is not always clear whether gene flow is concerned as a hazard or as a component of exposure. If the presence of a transgene in a wild plant or a non-transgenic crop is undesirable, regardless of the effect of the gene, then gene flow is a hazard (Raybound, 2004). Mono- and bitrophic interactions are the interaction between the GM recipients and other organisms which could cause hazard realization whose interactions can be plant-plant or plant-animal (Figure 1.7) (Gatehouse et al., 2002, Wilkinson et al., 2003), for example, between Bt pollen and monarch butterfly (Losey et al., 1999), or Bt crops on non-target pests and natural enemies (Walker et al., 2007). Affected herbivores by the toxin are often smaller and develop slower compared to healthy individuals. It is questionable if studies using herbivores that are targeted by the toxin in Bt crops are relevant to assess the risks for natural enemies. Surviving, sublethally affected herbivores are likely to be altered in nutritional quality and this will have potential consequences for higher trophic levels (Romeis et al., 2006). Therefore, the environmental risk assessments in ecological interactions between transgenic plants, herbivores

and its natural enemies continue to be improved to provide a better understanding of the possible risks involved in the release of transgenic crops into the environment.

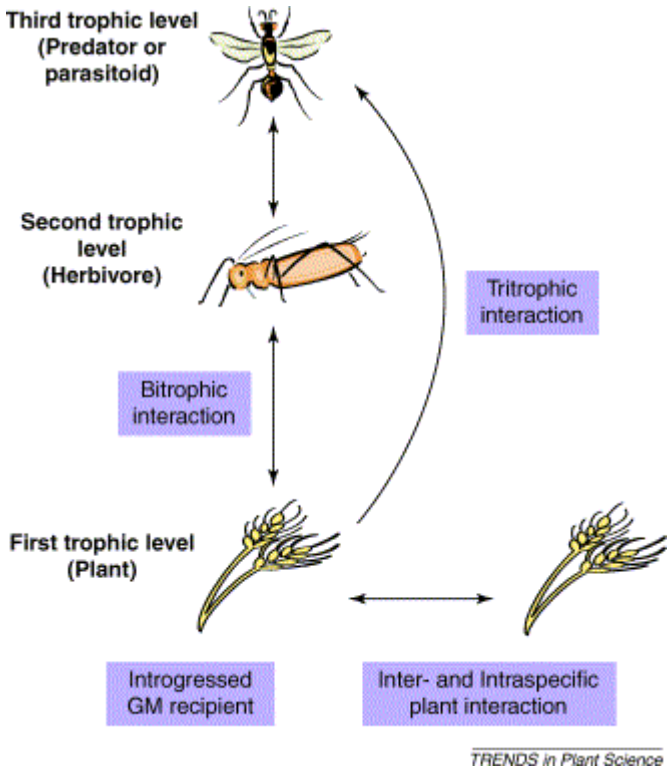


Figure 1.7. Possible levels of interaction between genetically modified (GM) recipient wild species and other organisms. (Wilkinson et al., 2003).

1.7 Summary

The main aim of this study is to investigate the effects of Cry1Ab toxin from both transgenic maize MON810 and Bt spray (Dipel) on the non-target insects in the tri-trophic level. The findings from this study are relevant to the ecological risk assessment of the Bt toxin (Cry1Ab) on the non-target insects (Chapter 2) and on the study of ecology of interactions of higher trophic level, – the parasitoid (Chapter 3).

The study was conducted under controlled laboratory conditions – first tier lab testing on the second and third trophic levels. The experimental insects were the cotton worm, *Spodoptera littoralis* at the second trophic level and its parasitoid, *Cotesia marginiventris* representing the third trophic level (Figure 1.8).

Bt toxin used in insect pest control is an alternative to synthetic chemical insecticides (Federici, 2005). This toxin is widely used in the sprayable form and recently it has been incorporated into the plant cells.. Measurement endpoints such as mass, size, survival, growth and development, and parasitoid behaviour were observed in this study, which is part of a risk assessment framework. Also, by addressing changes in nutritional quality of the host, general ecological interactions theories can be considered.

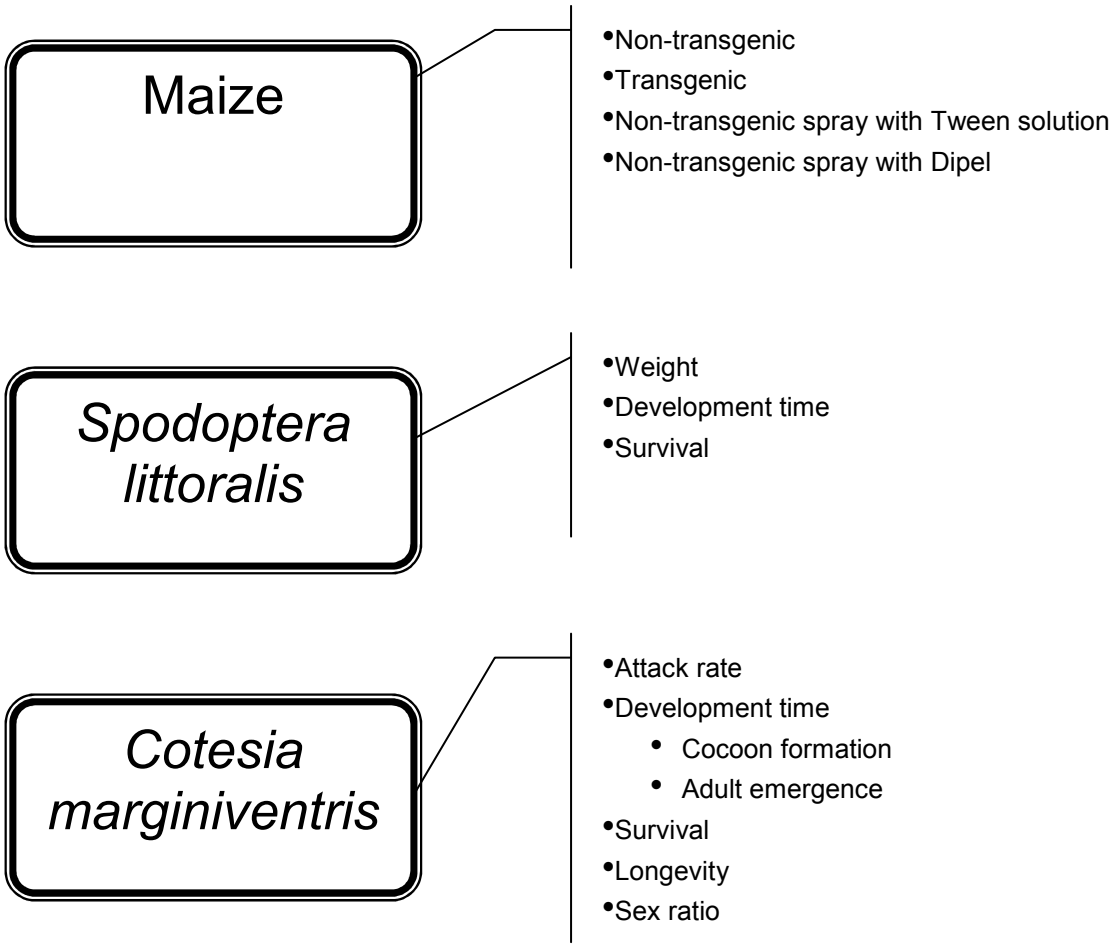


Figure 1.8 . Summary of tri-trophic interaction experiment protocols

The second trophic level in this study is *S. littoralis* (Lepidoptera: Noctuidae) used as a host and directly exposed to the Cry1Ab toxin. This insect is a non-target pest. It is insensitive to Cry1Aa, Cry1Ab and Cry1Ac endotoxin (Keller et al., 1996, Regev et al., 1996) but it is susceptible to Cry1C and Cry1E. In this study, *S. littoralis* was used to access the effect of the toxin as a second trophic level in a tri-trophic interaction.

The third trophic level in this study is *C. marginiventris*. It is a polyphagous, solitary endoparasitoid of several species of Noctuidae. This makes the parasitoids important in biological control of pests. Widespread use of the Bt

toxin may reduce host populations and affect the quality of hosts for the parasitoid fitness.

The summary of this study is in the Figure 1.9. This summarizes all the experimental works from the baseline data given the parameters to be used and suggested for the further experiments.

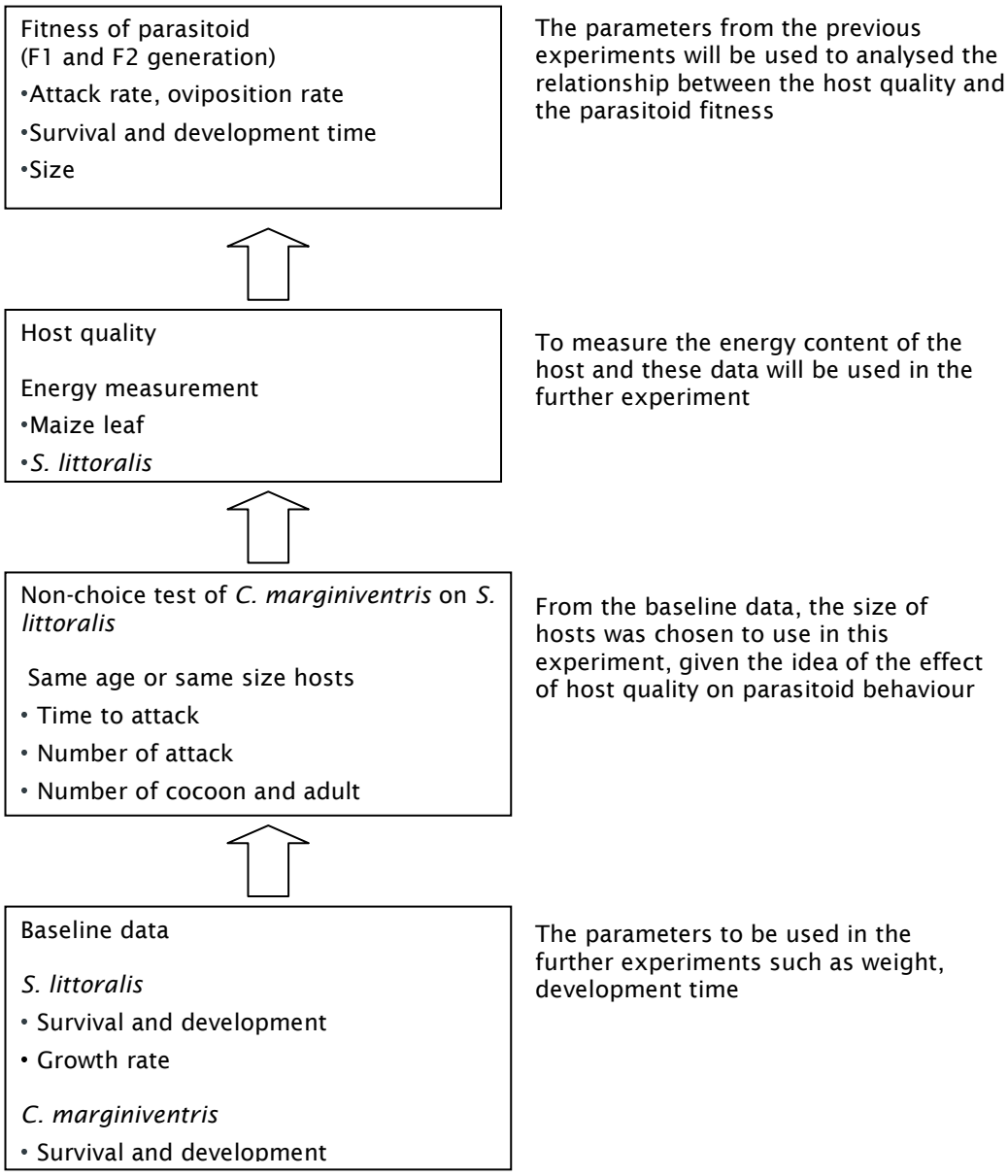


Figure 1.9. Road map for the study

Chapter 2

Effects of Bt toxin from Bt maize and Dipel spray on tri-trophic interactions

2.1 Introduction

Cry toxins from *Bacillus thuringiensis* (Bt) can be a useful alternative to synthetic chemical insecticides. Parasporal crystals are formed by bacteria during the stationary phase (Schnepf et al., 1998) and these crystals are comprised of protoxins which become active when susceptible insects ingest them (Schnepf et al., 1998) (see chapter 1).

This form of microbial insecticide is the most widely used biological insecticide in agriculture because of the specificity and mode of action of the Cry toxins (Joung and Cote, 2000, Dutton et al., 2003a). The degradation of microbial Bt sprays occurs relatively quickly (Dutton et al., 2005) due to sunlight exposure (UV radiation) which inactivates 50% of Bt spores within 30 minutes (Joung and Cote, 2000). Dipel, which is the product of *B. thuringiensis* ssp. *kurstaki* (HD-1 strain), is sprayed for controlling lepidopteran pests including the European corn borer (*Ostrinia nubilalis* Hubner) (Dutton et al., 2003a). There are many studies investigating the effects of Bt spray on non-target insects; for example feeding *Spodoptera littoralis* larvae with sprayed leaf resulting in higher mortality and reduced weight (Dutton et al., 2003a, Dutton et al., 2005).

Currently, genetically modified (GM) plants expressing insecticidal proteins are an alternative to chemical insecticide against insect pests (Schuler et al., 1998, Dutton et al., 2005). This new technology has many advantages over the spray, such as more effective targeting of insects protected within plants, greater resilience to weather conditions, fast biodegradability, reduced operator exposure to toxins and financial savings (Schuler et al., 1998). Transgenic plants can lead to a decrease in the need for insecticide spraying and reduce ecological damage (Schuler et al., 1998, Poppy and Wilkinson, 2005, Romeis et al., 2006). Many studies reported the effects of transgenic plants on non-target insects (see reviews - Dutton et al., 2005, Vojtech et al., 2005, Walker et al., 2007). For example, Dutton et al. (2005) and Vojtech et al. (2005) showed

that *S. littoralis* larvae fed with transgenic maize had a significantly higher mortality, reduced weight and a prolonged development to pupation. Thus, the time to pupate and adult emergence in the parasitoid (*Cotesia marginiventris*) were significantly longer when parasitoid larvae developed in hosts fed with transgenic maize (Vojtech et al., 2005). There is a concern that growing transgenic plants may pose a negative impact on biodiversity, as insect pests and natural enemies are part of a multi-trophic system. A reduction in an insect pest population adversely affects the population of natural enemies. For instance, natural enemies will frequently encounter *B. thuringiensis*-containing prey or hosts, in particular, in areas where different *B. thuringiensis* crop plants are grown either next to each other or following each other in rotation (Hilbeck et al., 1998c). Therefore, long-term bioassays with natural enemies are more realistic indicators of possible population-level effects in a system with transgenic plants.

Spodoptera littoralis is a polyphagous pest and is one of the most serious cotton pests in Egypt. It affects a number of important crops such as tomato, capsicum, cotton and maize (Salama et al., 1990). Due to its significance as a pest and its partial susceptibility to Bt, this insect has become a model species for studying the effects of *B. thuringiensis* endotoxin proteins. When fed Dipel-sprayed maize leaves expressing Cry1Ab toxin, higher mortality and a prolonged development time were observed in *S. littoralis* larvae (Dutton et al., 2005). Dutton et al. (2005) and Vojtech et al. (2005) reported the significantly negative effects of transgenic maize expressing Cry1Ab protein on the survival and development time of *S. littoralis* larvae. *S. littoralis* shares similar morphology and behaviour to *O. nubilalis*. Whilst *O. nubilalis* larva is highly susceptible to Cry1Ab toxin, *S. littoralis* is susceptible to Cry1C and Cry1E but has partial susceptibility to Cry1Ab. The youngest larval stages (1^{st} – 2^{nd} instar) of *S. littoralis* are more sensitive to the Bt toxin than the advanced instar larvae (Keller et al., 1996). This can be explained by the gut juice of advanced *S .littoralis* larval instars (3^{rd}-5^{th}), which exhibits very high proteolytic activities leading to a complete degradation of Cry proteins (Keller et al., 1996, Dutton et

al., 2005). Therefore, *S. littoralis* is a powerful model with implications for high trophic level studies. In order to investigate the effects of Cry toxins on non-target insects, development, the sensitivity of different stages to toxin effects and host quality to parasitoids were observed.

Cotesia marginiventris is a polyphagous, solitary endoparasitoid of several species of Noctuid pests (Vojtech et al., 2005). It plays an important role in biological control of a wide range of lepidopteran pests such as fall armyworm (Ruberson et al., 1994). Mated females only parasitize young larvae of noctuid pests and oviposit one egg into each host. Parasitoid larvae develop inside the host, which makes the host feed less throughout its life, and it dies within a day of the parasitoid emerging from the host. Parasitoids are very sensitive to changes in their hosts after toxin ingestion, as they complete their development on one single host individual (Godfray, 1994). Therefore, parasitoids are the most important in biological control by playing a role in insect pest prevention. The effects of Bt toxin (from both spray and transgenic plant) cause a reduction of the host population and thus it might influence parasitoid population dynamics in the environment. Vojtech et al. (2005) reported significantly higher survival of *C. marginiventris* until cocoon formation found in the group exposed to non-transgenic maize-fed larvae than in the group exposed to transgenic maize-fed larvae. Cocoons of *C. marginiventris* developing in *S. littoralis* larvae that had fed on transgenic Bt maize were smaller and developmental times longer (Vojtech et al., 2005). It is important to study the effects of Bt toxin on this parasitoid, whether negative effects are caused indirectly, via a low quality host, or directly via the Bt toxin. Therefore, this insect of interest is used as a model for studying the impact of Bt toxin on the natural enemies in the ecosystem.

The present study investigated the effects of Bt toxin on the non-target insects in the tri-trophic level. There are many published works studied on the effect of Bt toxin both from spray and plant on non-target insects. Several studies have evaluated the effect of Bt spray on the non-target insects by using the

concentration recommended in the field for maize against *O. nubilalis* (between 0.035 and 0.094 kg a.i./ha in which 100,000 plants/ha are grown) (Dutton et al., 2003a, Dutton et al., 2005). Dutton et al. (2003b) and Dutton et al. (2005) showed that the Bt insecticidal spray (Dipel) which has Cry1Ab toxin had a negative effect on *S. littoralis* and *Chrysoperla carnea*: namely prolonged developmental times, high mortality and decreased weight. There are many studies on the effect of Bt maize expressing Cry1Ab toxin on the non-target pests and natural enemies. The insects suffered from the Bt toxin from transgenic plants with a reduced weight, longer development and high mortality (see reviews - Hilbeck et al., 1998a, Hilbeck et al., 1998b, Dutton et al., 2003a, Manachini and Lozzia, 2004, Meissle et al., 2004, Dutton et al., 2005, Meissle et al., 2005). However, various amounts of Bt toxins are found in different commercial transgenic plants. For example, MON810 uses the gene promoter, which results in a season-long expression of the Bt toxin in all plant tissues. The plants express 1.597 μg/g Cry1Ab fresh per weight (Vojtech et al., 2005). There has been little research undertaken on the comparison between the Bt spray and transgenic maize (Dutton et al., 2003a, Dutton et al., 2005). This research did not use the same amount of Bt toxin in the spray and in the plants. In order to compare the effect of the Bt toxin in different applications, the same amount of toxin in Bt spray formulation (Dipel) and transgenic maize (MON810) are applied to these non-target herbivores and parasitoids. The amount of the Bt toxin (both spray and plant) in this study is compatible with the amount in the MON810 maize cultivar (1.597 μg/g Cry1Ab fresh per weight). The survival, development and weight of *S. littoralis* and *C. marginiventris* are observed. A good understanding of the effects of Bt toxin on the second trophic level provides important knowledge for studies of effects at the third trophic level. Therefore, these effects of Bt toxin on *S. littoralis* and *C. marginiventris* are an important example in the context of biological control. Bt toxin may reduce the population of *S. littoralis* and negatively affect the quality of surviving individuals as hosts for the parasitoid, *C. marginiventris*.

2.2 Aim

The aim is to conduct a first tier study under controlled laboratory condition to determine the effects of Cry toxins, and their modes of delivery (spray and plant) on the tri-trophic interactions involving *Spodoptera littoralis* (second trophic level) and *Cotesia marginiventris* (third trophic level). To compare mass and consumption of *S. littoralis* when reared on non-transgenic, transgenic and Bt spray maize and then to provide the baseline data for the future experiments on the higher trophic level i.e. parasitoid on host fed with these Bt toxin maize.

2.3 Hypotheses

1. Bt transgenic maize and Bt spray have negative effects on hosts *(Spodoptera littoralis*)
2. Bt transgenic maize and Bt spray have a negative effect on parasitoids *(Cotesia marginiventris*)
3. There is no difference between Bt transgenic maize and Bt spray on hosts (*S. littoralis*) and parasitoids (*C. marginiventris*)

2.4 Materials and Methods

2.4.1 Maize

Transgenic maize (*Zea mays*) plants (event MON810, Monsanto) expressing Cry1Ab protein and the near-isogenic non-Bt maize (DK315, Monsanto) were used in all experiments. The maize line MON810 (trade name YieldGard®) was developed through a specific genetic modification to be resistant to attack by the European corn borer (ECB, *Ostrinia nubilalis*). The MON810 contains the cauliflower mosaic virus (CaMV) 35S promoter which expresses the toxin

throughout the season in leaves, stalk, roots, and kernels. The plants were cultivated in 15 cm plastic pots (3 grains per pot), using vapogro soil (Vapogro Ltd, Glastonbury, Somerset, UK), and then were kept in a greenhouse. New plants were sown weekly and were used for experiments when 4-5 weeks old. No fertilizers were applied. Three leaves were excised from each plant these being leaves 3, 4 and 5 from the base of the plant.

The plants were grouped as follows:

- transgenic maize plants (Bt maize):
- non-transgenic plants (non Bt maize):
- non-transgenic plants which had been sprayed with Dipel (Abbotts Laboratories, North Chicago, IL, USA) (Bt spray maize) as follows:
    a) Prepare 20 mg Dipel in 20 mL Tween solution (0.5 mL in 1 L distilled water) for spraying the non-transgenic maize plants;
    b) Spray the plants with a manual sprayer (amount: 0.8 mL per leaf);
    c) Spray only once and allow the plants to dry before being infested by *S. littoralis* larvae;
- non-transgenic plants which were sprayed with Tween solution (Control spray maize) as follows:
    a) Prepare the Tween solution (0.5 mL in 1 L distilled water);
    b) Spray the plants with a manual sprayer (amount: 0.8 mL per leaf);
    c) Spray only once and allow the plants to dry before being infested by *S. littoralis* larvae.

N.B. Detection of the Cry1Ab toxin expressed in the experimental transgenic maize (MON 810) and the Dipel spray maize was carried out using enzyme-linked immunosorbant assays (ELISA) Pathoscreen kit (Cry1Ab/1Ac) (Agdia, USA). Four to five weeks old plants were selected. Three leaves were excised from each plant these being leaves 3, 4 and 5 from the base of the plant. Five leaf disks of 5 mm diameter were removed from each leaf and placed into a 1.5 ml eppendorf tube with 300 µl of 5% mPBS Tween buffer solution. Two stainless steel ball bearings were added to each sample eppendorf. The samples

were the loaded into a macerator and agitated for 1.5 minutes, then rotated and agitated for a further 1.5 minutes. Samples were centrifuged at 13 rpm for 5 minutes to spin down any remaining plant tissue. All samples were then maintained on ice prior to analysis. Samples were diluted to 1/100 to be within the optimal range of sensitivity compared to the standards. Samples were assayed according to the standard protocol of the immunosorbant assays (ELISA) kit and plates read at 450 nm (Anthos reader 2001, Anthos labtech instruments). (see Appendix I).

2.4.2 Insects

a) *Spodoptera littoralis*

Eggs of *S. littoralis* were supplied on netting by Syngenta, Bracknell, UK, each week. The eggs were left on the netting and placed in 250mL pots (Roundstone, Catering Equipment Ltd, Melksham, Wiltshire, UK) with ventilation. The eggs were maintained in a Thermo Electron A/013 cooled incubator at 25 ^{o}C ± 1.0 ^{o}C, photoperiod 14 hrs light/ 10 hrs dark.

In the experimental trials, the eggs were maintained in a 1.5 L plastic box and were allowed to develop into first instar larvae. The plant material (Non-Bt (DK315), Bt (MON810), control-spray and Bt-spray maize leaves) were changed every day until day 6. Larvae were individually transferred to 250mL pots (Roundstone, Catering Equipment Ltd, Melksham, Wiltshire, UK) containing the plant materials. The experimental groups were set up with one larva per pot (n = 20 for each four treatments). On day 11 a 1 cm layer of vermiculite was added to the pots to act as a pupation substrate as well as to avoid excessive moisture. The plant materials were changed and weighed every two days, using this weight maize value computed for maize consumption. Vermiculite was changed every other day. Larvae were weighed every two days until adult emergence. The pupae were weighed two days after pupation because of high sensitivity to disturbance during this stage. Using these weight

values, several growth and nutritional parameters were computed according to Deml et al. (1999)as followed;

Relative growth rate (RG) = weight change of larvae/(time period x mean larval weight).

Relative food consumption (RC) = food consumed/(time period x mean larval weight).

Efficiency of food conversion (ECI) = weight change of larvae x 100/food consumed.

The larval survival rate and developmental time were recorded.

b) *Cotesia marginiventris*

Cocoons of *C. marginiventris* were obtained from the Laboratoire d'Entomologie Evolutive, Institut de Zoologie, Université de Neuchâtel, Switzerland. The insect adults were maintained in 30x30x30 cm Perspex cages in standardized conditions. Adults were reared on a 20% honey/water solution. This was provide on soaked cotton wool which was changed every 48 hours. For general culturing, 35 second instar *S. littoralis* were removed from large emergence groups and placed in 250mL pots (Roundstone, Catering Equipment Ltd, Melksham, Wiltshire, UK) with ventilation containing an excess of artificial diet (Beet Armyworm diet F9220B Bio-Serve, Frenchtown, NJ 08825). *S. littoralis* larvae were offered to two mated *C. marginiventris* females (2-6 days old); pots also contained a small ball of damp cotton wool dipped in honey/water solution. The pots were observed until at least one female attacked a host. The *C. marginiventris/S. littoralis* groups were then left to parasitize for 24 hours. After 24 hours, the adult *C. marginiventris* and honey cotton ball were removed. *C. marginiventris* larvae took between 8-12 days to emerge during which time the pots were monitored and any large unparasitized *S. littoralis* were removed and the fresh artificial diet added as required.

In the experimental trials the *S. littoralis* eggs were maintained in a 1.5 L plastic box and were allowed to develop into first instar larvae. The plant materials (non-transgenic (DK315), transgenic (MON810), control-spray and Bt-spray maize leaves) were changed every day until day six. Twenty-five 2nd instar larvae were offered to one mated *C. marginiventris* female (2-6-days old) until parasitization was observed. The parasitized larvae were kept until cocoon formation. During this time the plants were changed daily. The parasitized larvae were weighed 2 and 5 days after parasitization to cover the time between parasitization and the earliest day when the parasitoid larvae might leave their host. The cocoons were weighed one day after formation, to prevent damage to the fragile freshly-spun cocoon. The time until cocoon formation and the time until adult emergence of parasitoids were recorded.

2.4.3 Statistical analysis

All the data were analysed using SPSS for Windows version 14.0 (SPSS Inc., Chicago, USA). All data except the survival data were tested by the Kolmogorov-Smirnov test, for a background to test whether the population had a normal distribution.

The weight and maize consumption of the individual caterpillar were measured every other day from day 6 until day 20. The repeated data (mass of *Spodotpera littoralis* larvae, maize consumption of *S. littoralis,* and the weight at 2 and 5 days after parasitization of *S. littoralis* larvae) were analysed using GLM (General linear model), repeated measures ANOVA.

One-way analysis of variance (ANOVA) was used for the non-repeated data (weight of *S. littoralis* before death, the relative growth rate, the relative food consumption, the efficiency of food conversion and the time to pupation of *S. littoralis,* the weight gain in the parasitized *S. littoralis*, and the weight of *Cotesia marginiventris* cocoon). Mean values between treatments were compared with Tukey HSD tests. The data on the relative food consumption

were logarithmically transformed prior to analysis, as the data did not conform to a normal distribution.

Linear regression was used to analyse the relationship between the relative growth rate of *S. littoralis* larvae and the total maize biomass consumption.

Survival of *S. littoralis* larvae until pupation and the survival of *C. marginiventris* developing in *S. littoralis* were analysed using the Kaplan-Meier procedure and Breslow (Wilcoxon) test. Survival data were recorded until all *S. littoralis* or *C. marginiventris* larvae were either dead or pupated. All pupated individuals were considered as "surviving until the last recorded day".

2.5 Results

2.5.1 Effect of Bt toxin on *Spodoptera littoralis* survival and development

The survival of the larvae was assessed from 6 days old until pupation and was analysed by Kaplan-Meier procedure. No significant differences of the survival of 2nd instar *S. littoralis* larvae until pupation were observed between the non-transgenic, transgenic, control spray and Dipel spray maize treatments (Fig. 2.1, Kaplan-Meier, Breslow, $\chi^2 = 0.154$, df = 3, p = 0.985).

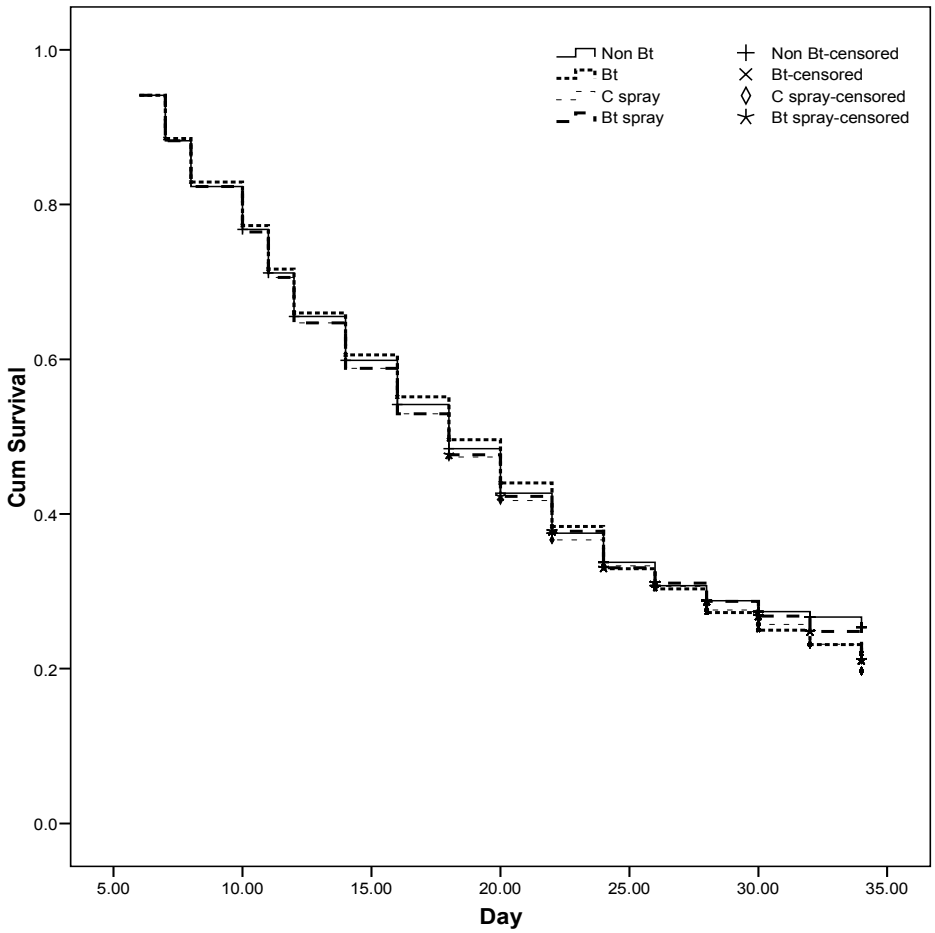


Figure 2.1. Survival of 2nd instar *S. littoralis* larvae until pupation as calculated in the Kaplan-Meier procedure. Larvae were reared either on non-transgenic maize (Non Bt), transgenic maize (Bt), control spray maize (C spray) or Dipel spray maize (Bt spray). Treatments are not significantly different (p=0.985).

The time to pupation of *S .littoralis* was analysed by one-way ANOVA. No significant difference in the time to pupation was found among the four maize treatments (Fig. 2.2, $F_{3,4} = 2.667$, $p = 0.184$).

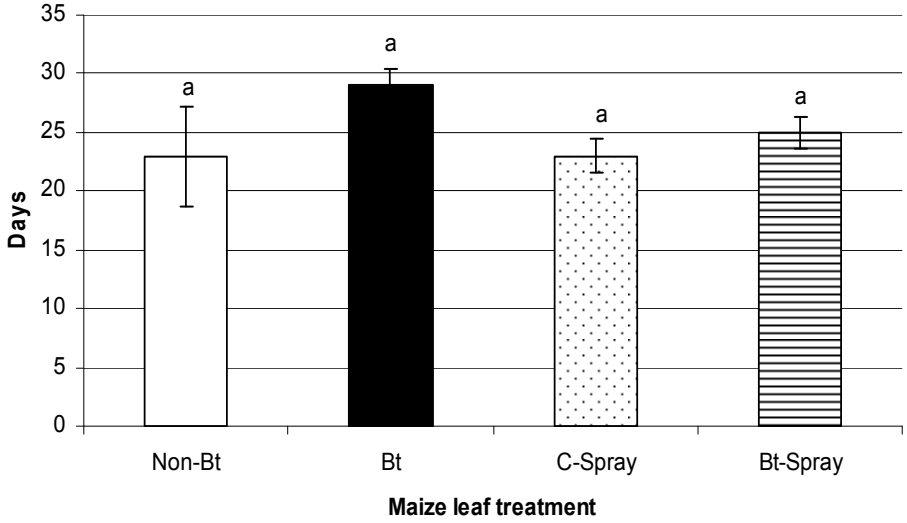


Figure 2.2. Mean of time to pupation (day) of *S. littoralis* fed non-transgenic (Non Bt), transgenic (Bt), control spray (C-Spray) or Dipel spray (Bt-spray) maize leaves. Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different (p=0.184).

A repeated measures one-way ANOVA revealed that there were significant differences in the weight of *S. littoralis* larvae from day 6 until day 20 between the four treatments (Fig. 2.3, $F_{3,21} = 6.732$, $p = 0.002$). Mean weight of the caterpillars from day 6 until day 20 in the transgenic treatment was the lowest compared with those reared on non-transgenic maize ($p = 0.008$), control spray maize ($p = 0.031$) and those reared on Dipel spray maize ($p = 0.038$). Larvae maintained on non-transgenic maize leaf were heavier than those maintained on transgenic leaf ($p = 0.008$) and larvae maintained on Dipel spray leaf ($p = 0.004$). No significant differences in the larval weight were observed between the non-transgenic group and the control spray group ($p = 0.900$). Thus, larvae fed on control spray maize showed no significant difference in weight when compared with larvae fed on Dipel spray maize ($p = 0.104$).

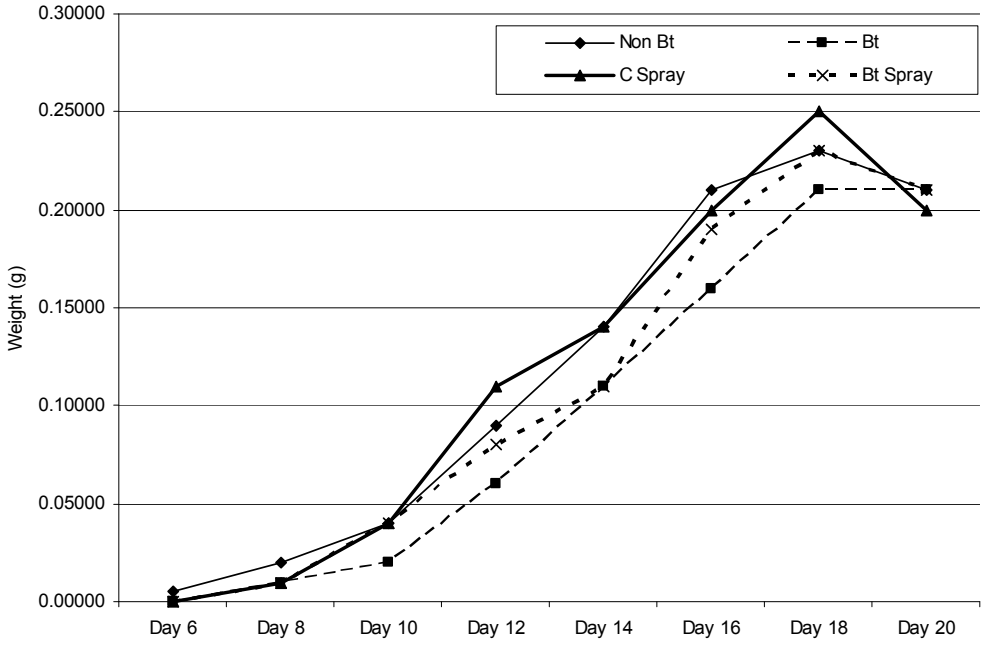


Figure 2.3. Mean weight of *S. littoralis* larvae from day 6 until day 20. Larvae fed either non-transgenic maize (Non Bt), transgenic maize (Bt), control spray maize (C spray) or Dipel spray maize (Bt spray). Significant differences in weight of the larvae from day 6 until day 20 were found between these treatments ($p=0.002$).

For individual larvae, the weight before pupation and before death, maize consumption, relative growth rate, relative food consumption and efficiency of food conversion were assessed. The relationship between the relative growth rate and maize consumption was also assessed.

The weight of the individual larva before pupation of the four treatments is shown in Figure 2.4. (Fig. 2.4). The maximum weight of the larvae before pupation was found in the larva maintained on non-transgenic maize (0.41261 g) while the minimum weight was in the larva maintained on transgenic maize (0.09717 g)

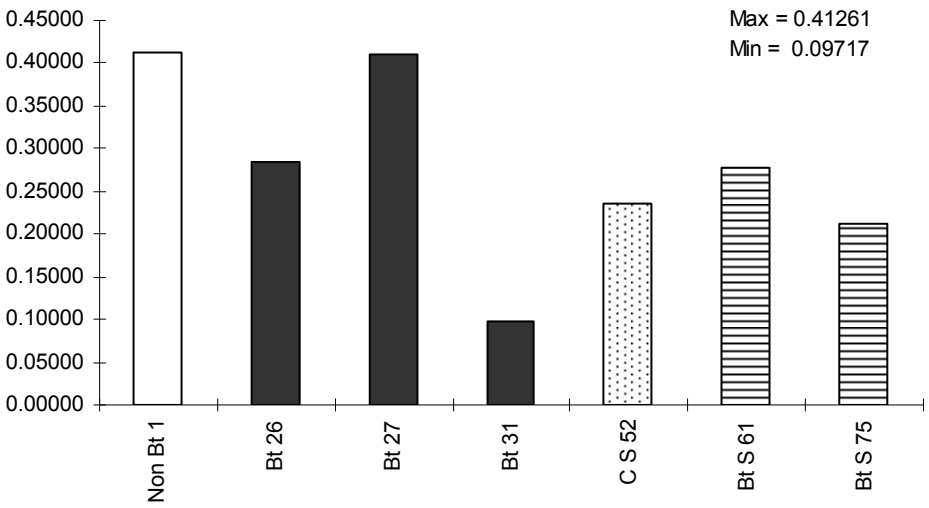


Figure 2.4. Weight of *S. littoralis* individual before pupation. Larvae either fed on non-transgenic (Non Bt – white), transgenic (Bt – black), control spray (C S - dotted) or Dipel spray maize (Bt S - horizontal). Different numbers represent each larva individual in the four treatments.

The weight of the individual larva before death is shown in Figure 2.5. The maximum weight of the larvae before death was observed in the larva maintained on the control spray maize, whereas the minimum weight of larvae before death were observed in the larva fed on transgenic maize.

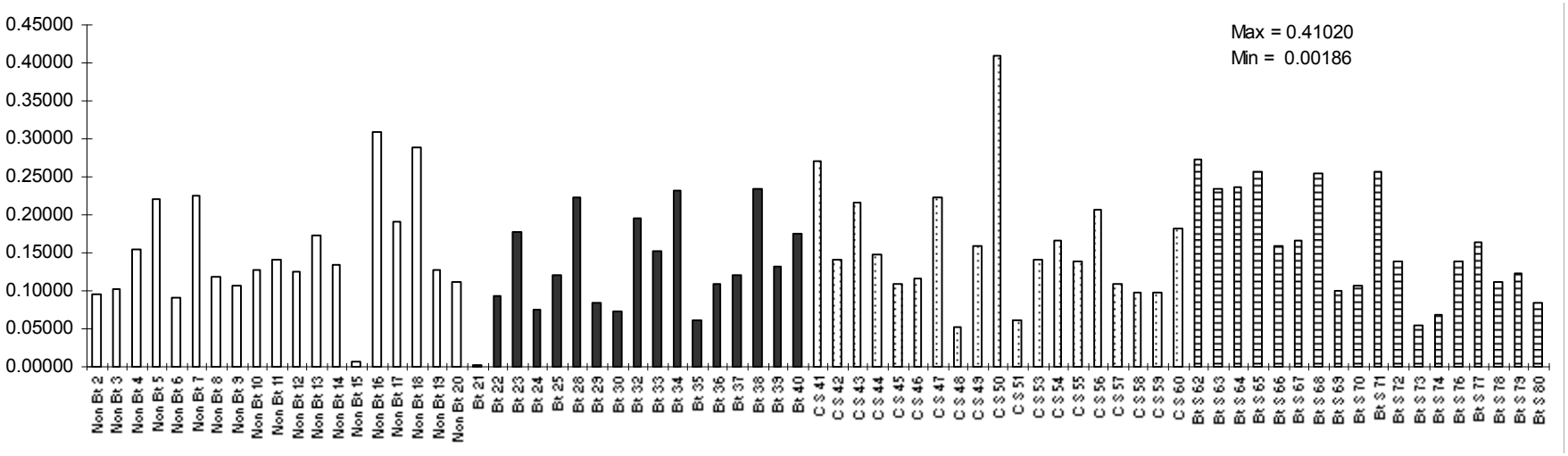


Figure 2.5. Weight of *S. littoralis* individual before death. Larvae either fed on non-transgenic (Non Bt – white), transgenic (Bt – black), control spray (C S - dotted) or Dipel spray maize (Bt S - horizontal). Different numbers represent each individual larva in the four treatments.

One-way ANOVA showed that the average weights of the larvae before death in these treatments had no significant differences (Fig. 2.6, $F_{3,40} = 2.111$, $p = 0.114$).

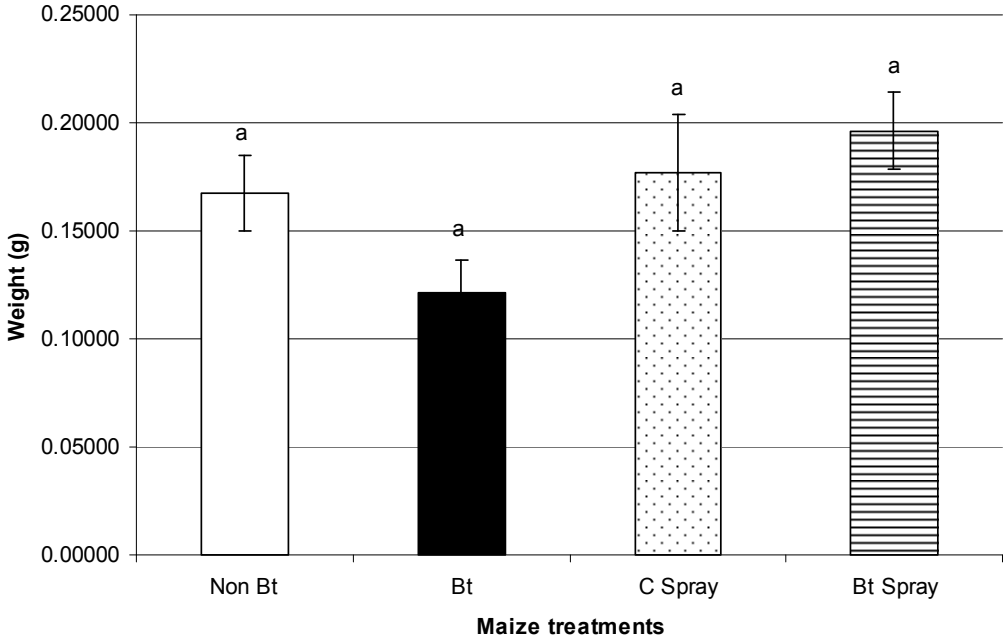


Figure 2.6. Mean weight of the larvae before death. Larvae fed non-transgenic (Non Bt), transgenic (Bt), control spray (C-Spray) or Dipel spray (Bt-spray) maize leaves. Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different ($p = 0.114$).

One-way repeated measures ANOVA showed that the maize consumption of *S. littoralis* in these treatments was significantly different between treatments (Fig. 2.7, $F_{3,21} = 3.417$, $p = 0.036$). The mean maize consumption from day 6 until day 20 in the non-transgenic treatment was significantly higher than that in the Dipel spray treatment ($p = 0.007$). No significant differences in the maize consumption were observed either between the non-transgenic and transgenic treatments ($p = 0.305$) or the non-transgenic and control spray treatments ($p = 0.055$). There were no significant differences in the maize consumption either between the transgenic and control spray groups ($p = 0.253$) or the transgenic and the Dipel spray groups ($p = 0.085$). Control spray and Dipel spray treatments showed no significant difference in the maize consumption from day 6 until day 20 ($p = 0.816$). At day 20, larvae stopped consuming and excrete large amount of faeces and prepared to pupate.

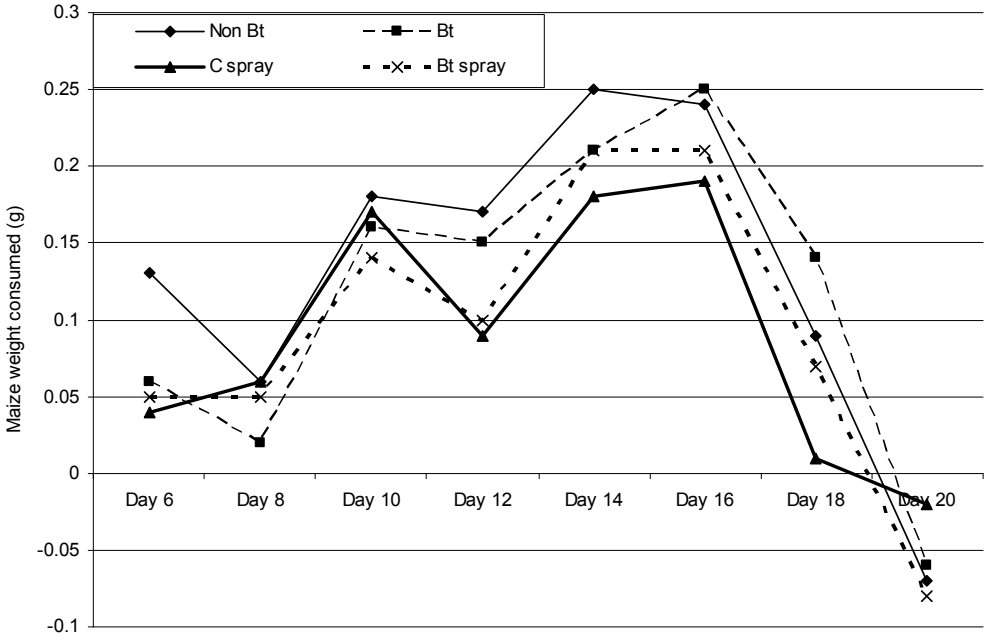


Figure 2.7. Mean maize consumption of *S. littoralis* from day 6 until day 20 in the non-transgenic (Non Bt), transgenic (Bt), control spray (C spray) and Dipel spray (Bt spray). Significant differences in the maize consumption of the larvae from day 6 until day 20 were found between these treatments (p=0.036).

Figure 2.8 represents the mean relative growth rate of *S. littoralis* larvae fed on four different maize leaves. One-way ANOVA showed that there was a significant difference in the mean relative growth rate of *S. littoralis* between these non-transgenic, transgenic, control spray and Dipel spray treatments (Fig. 2.7, $F_{3,76} = 12.554$, $p < 0.001$). Larvae fed on non-transgenic maize had the lowest relative growth rate (29.69 ± 3.93) when compared to the group reared on transgenic maize (102.42 ± 14.50) ($p < 0.001$), the highest. The relative growth rate in the transgenic group is significantly higher than those in the control spray group ($p = 0.001$). Dipel spray maize-fed larvae had significant higher relative growth rate when compared with the non-transgenic group ($p < 0.001$). However, no significant difference was observed between the relative growth rate of the non-transgenic and control spray treatments ($p = 0.286$), nor in the transgenic and Dipel spray maize treatments ($p = 0.485$).

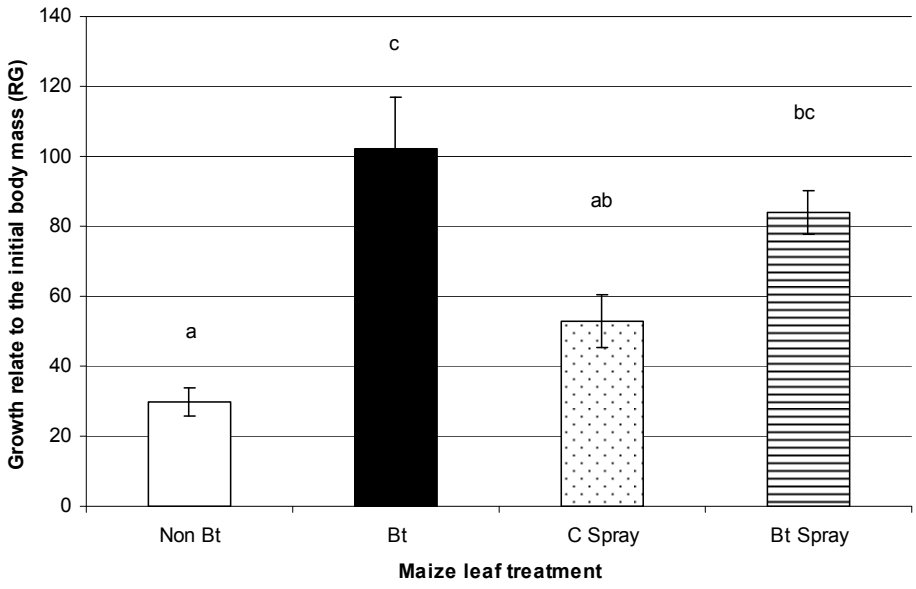


Figure 2.8. Mean relative growth rate (RG) of *S. littoralis* fed either non-transgenic maize (Non Bt), transgenic maize (Bt), control spray maize (C spray) or Dipel spray maize (Bt maize). Columns with different letters represent treatment means that are significantly different at $p<0.001$.

The relative food consumption of the larvae was analysed by one-way ANOVA. Data were logarithmically transformed prior to analysis, as the data did not conform to a normal distribution. The relative food consumption of *S .littoralis* larvae was significantly different among these non-transgenic, transgenic, control spray and Dipel spray treatments (Fig. 2.9, $F_{3,76} = 7.244$, $p < 0.001$). The highest relative food consumption was observed in the group exposed to transgenic maize (2.74600 ± 0.12785) when compared with the non-transgenic group ($p < 0.001$), the lowest. The transgenic maize treatment group had significantly higher relative maize consumption than the control spray maize group ($p = 0.002$). However, there were no significant differences in the relative food consumption in the group exposed to non-transgenic and those in the control spray group ($p = 0.925$). The relative maize consumption did not differ significantly between the non-transgenic and the Dipel spray treatments ($p = 0.339$).

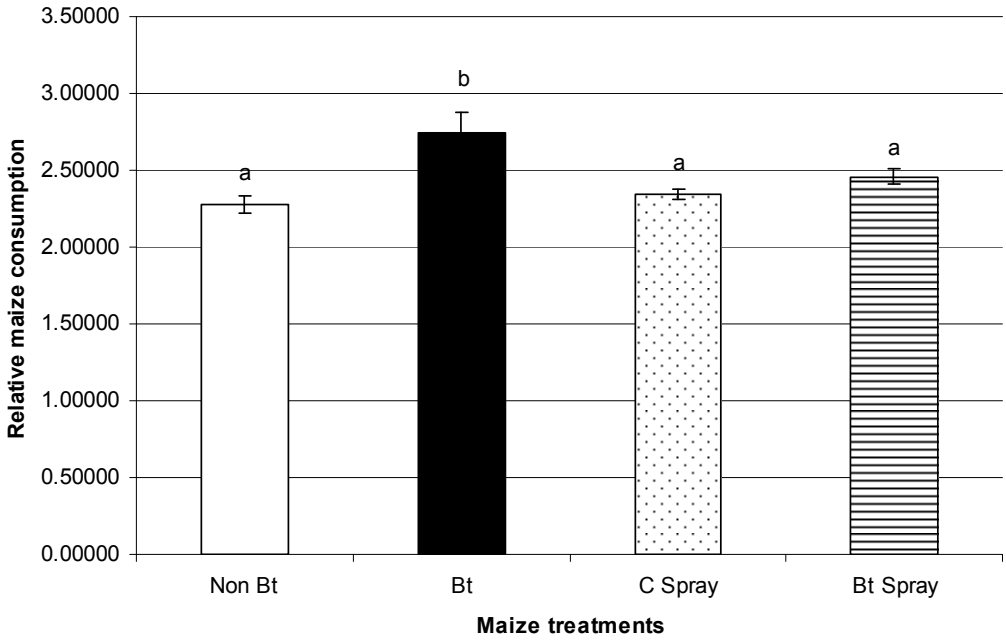


Figure 2.9. Mean relative food consumption (RC) of *S. littoralis* fed either non-transgenic maize (Non Bt), transgenic maize (Bt), control spray maize (C spray) or Dipel spray maize (Bt maize). Columns with different letters represent treatment means that are significantly different at $p<0.001$.

One-way ANOVA showed that no significant differences were observed in the efficiency of food conversion of *S. littoralis* among the four treatments (Fig. 2.10, $F_{3,76} = 0.154$, $p = 0.927$).

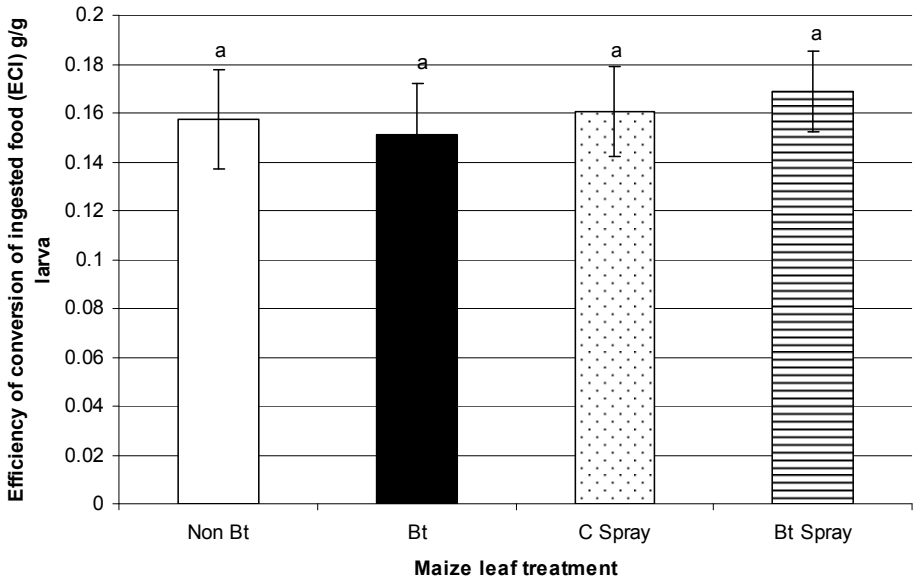


Figure 2.10. Mean efficiency of food conversion (ECI) of *S. littoralis* fed either non-transgenic maize (Non Bt), transgenic maize (Bt), control spray maize (C spray) or Dipel spray maize (Bt spray). Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different (p=0.927).

Regression analysis was carried out to assess whether there was a correlation between the relative growth rate and the maize consumption of *S. littoralis* larvae (Fig. 2.11). There was no significant relationship between the relative growth rate and the maize consumption of *S. littoralis* ($F_{1,77} = 1.329$, $p = 0.253$).

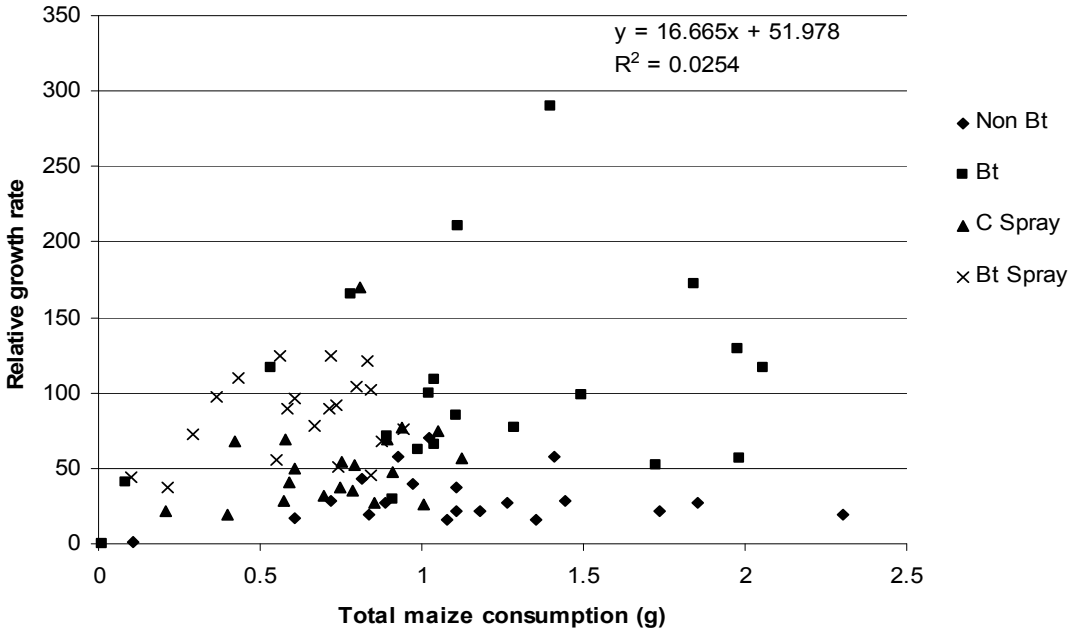


Figure 2.11. Relationship between the relative growth rate and the maize consumption of *S. littoralis* reared on non-transgenic maize (Non Bt), transgenic maize (Bt), control spray maize (C spray) or Dipel spray maize (Bt spray).

According to the result above (Fig. 2.11), the regression analysis was carried out to assess the relationship between the relative growth rate and maize consumption in the four treatments; non-transgenic, transgenic, control spray and Dipel spray treatments (Fig. 2.12-2.15, respectively).

Figure 2.12 revealed no relationship between the relative growth rate and the maize consumption in *S. littoralis* reared on non-transgenic maize ($F_{1,18}$ = 0.076, p = 0.786).

Figure 2.12. Relationship between the relative growth rate and the maize consumption of *S. littoralis* reared on non-transgenic maize.

No relationship between the relative growth rate and the maize consumption was found in the larvae maintained on the transgenic maize leaf (Fig. 2.13, $F_{1,17} = 0.636$, $p = 0.436$).

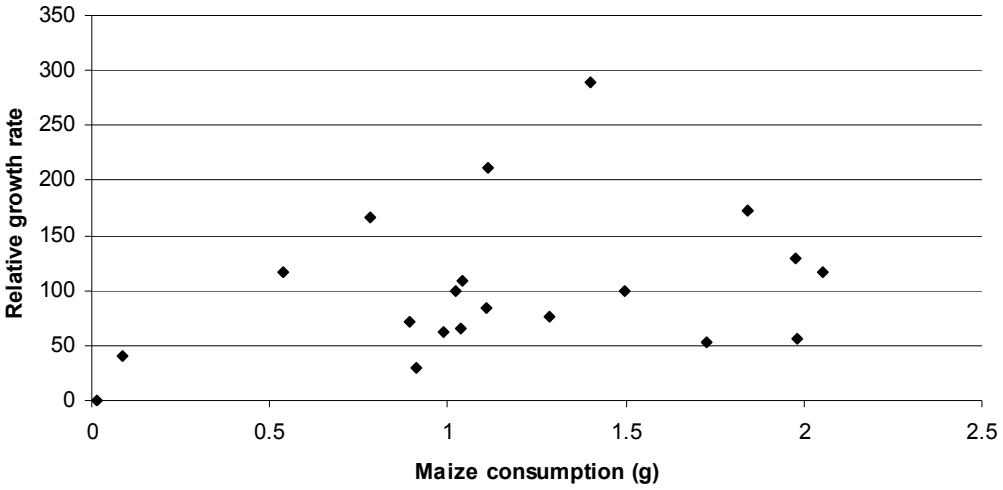


Figure 2.13. Relationship between the relative growth rate and the maize consumption of *S. littoralis* reared on transgenic maize.

Regression analysis showed no relationship between the relative growth rate and the maize consumption in *S. littoralis* larvae fed on control spray maize (Fig. 2.14, $F_{1,18} = 1.436$, $p = 0.246$).

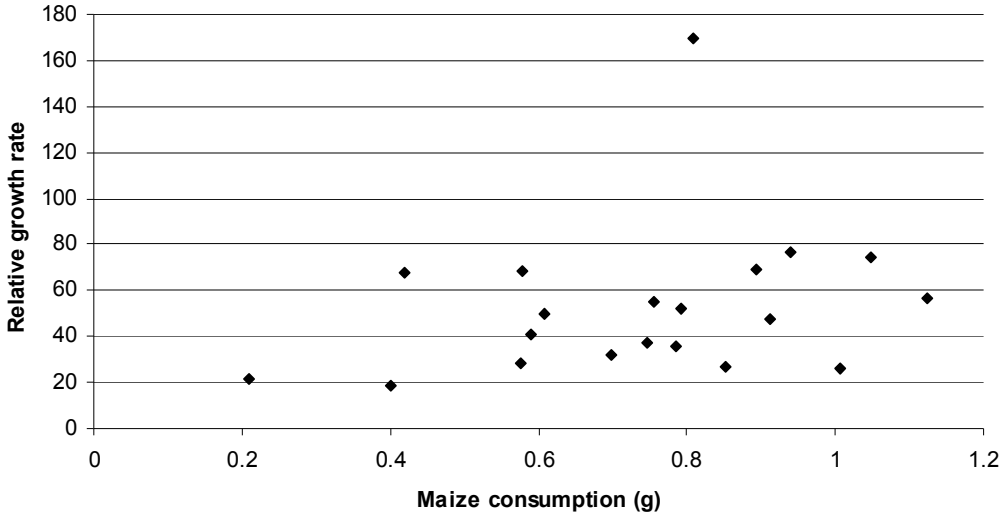


Figure 2.14. Relationship between the relative growth rate and the maize consumption of *S. littoralis* reared on control spray maize.

Figure 2.15 showed no relationship between the relative growth rate and the maize consumption in the group fed on Dipel spray maize ($F_{1,18} = 1.734$, $p = 0.204$).

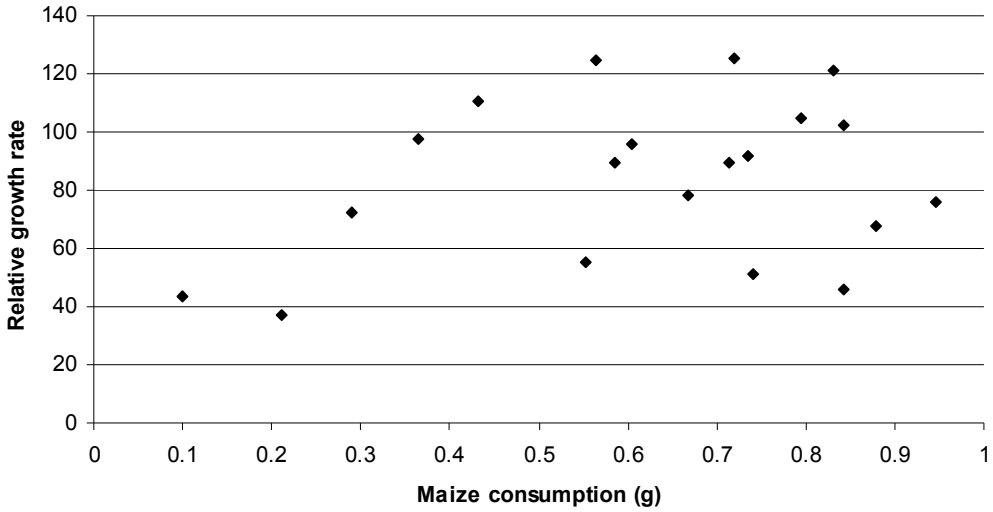


Figure 2.15. Relationship between the relative growth rate and the maize consumption of *S. littoralis* reared on Dipel spray maize.

2.5.2 Effect of Bt toxin on survival and development of parasitized *S. littoralis* and *C. marginiventris*

Survival of *C. marginiventris* developed within *S. littoralis* hosts from parasitization until adult emergence was analysed by the Kaplan-Meier procedure. There was significantly lower survival in the group exposed to transgenic maize (Fig. 2.16, Kaplan-Meier, Breslow, $\chi^2 = 516.98$, df = 3, $p < 0.001$). At day 16, there was only 50% survival in parasitized larvae reared on transgenic maize when compared to other groups.

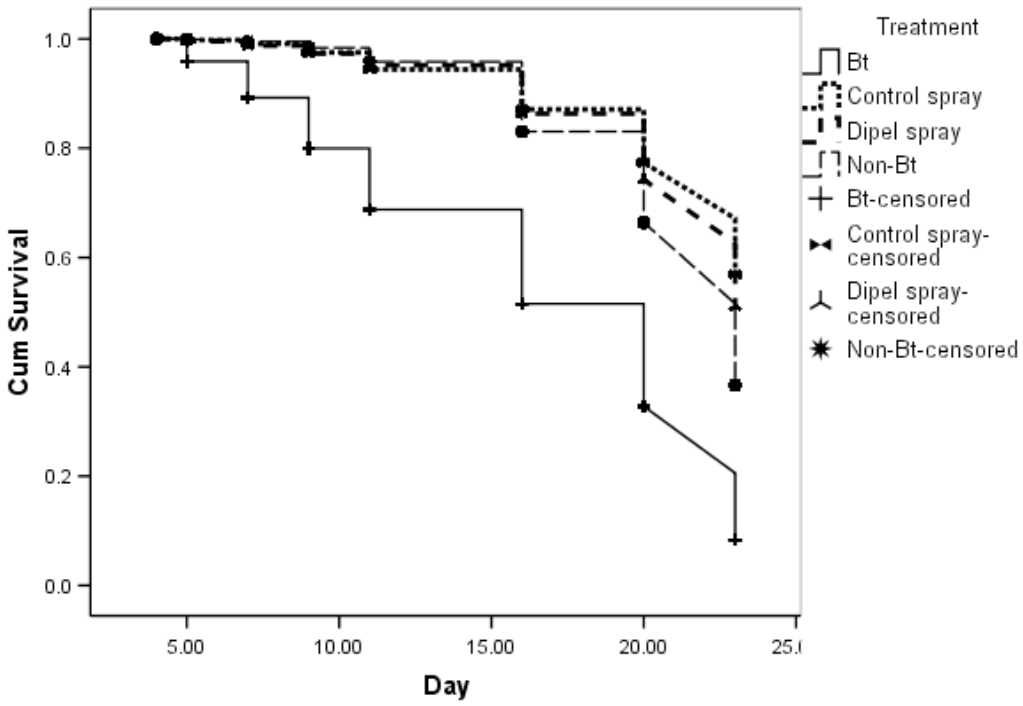


Figure 2.16. Survival of *C. marginiventris* developing in *S. littoralis* larvae from parasitization until adult emergence. ($p<0.001$).

Two-way ANOVA (treatments and day of larval weighing with repeated measures of day of larval weighing) showed that there was a significant interaction in the weight of parasitized *S littoralis* between the number of days after parasitisation and the treatments (non-transgenic maize, transgenic maize, control spray maize, Dipel spray maize) (Fig. 2.17, $F_{3, 397} = 538.423$, $p < 0.001$).

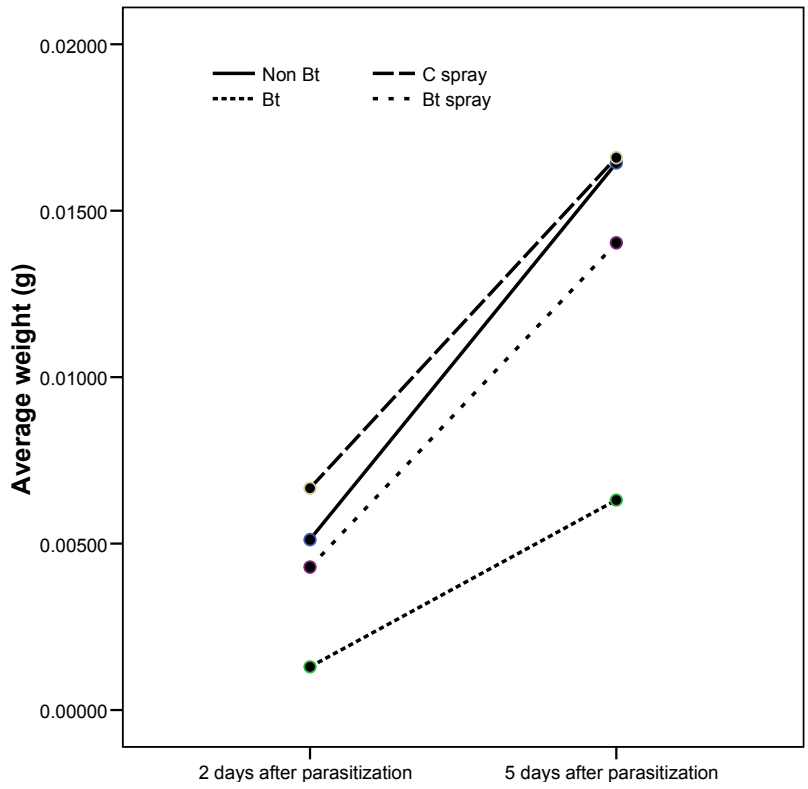


Figure 2.17. Mean weight of parasitized *S. littoralis* larvae at 2 and 5 days after parasitization. Larvae fed either non-transgenic maize (Non Bt), transgenic maize (Bt), control spray maize (C spray) or Dipel spray maize (Bt spray). Treatments are significantly different ($p<0.001$).

The significant interaction was investigated by considering the weight gain between day 2 and day 5 after parasitization. One-way ANOVA showed a significant difference in the weight gain of the parasitized larvae (Fig. 2.18, $F_{3,397} = 538.423$, $p < 0.001$). It showed that the weight gain for the transgenic group was significantly less than for the other groups ($p < 0.001$), that the weight gain for the non-transgenic group was significantly greater than for the other groups ($p < 0.001$), and that there was no significant difference in weight gain between the two spray groups ($p = 0.468$).

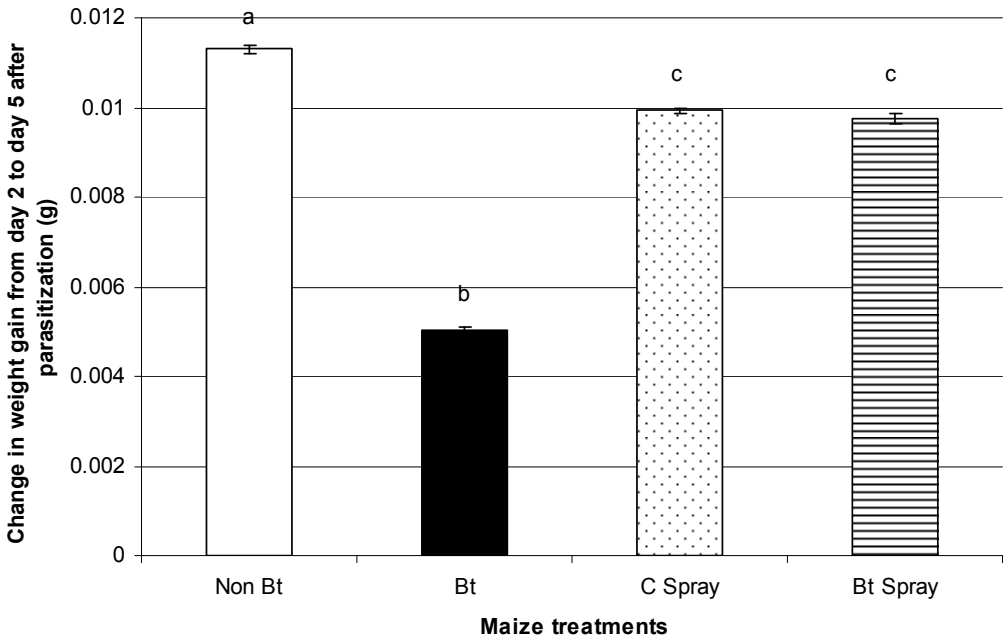


Figure 2.18. Changes in weight of *S. littoralis* from day 2 to day 5 after parasitization. Larvae fed either non-transgenic maize (Non Bt), transgenic maize (Bt), control spray maize (C spray) or Dipel spray maize (Bt maize). Columns with different letters represent treatment means that are significantly different at $p < 0.001$.

One-way ANOVA showed that there was a significant effect of maize treatments on the weight of *C. marginiventris* cocoon (Fig. 2.19, $F_{3, 289}$ = 221.316, $p < 0.001$). *C. marginiventris* cocoons weighed significantly less when developing in transgenic maize-fed hosts (0.00196 ± 0.00003 g) than cocoons from non-transgenic (0.00251 ± 0.00001 g), control spray (0.00251 ± 0.00001 g) and Dipel groups (0.00216 ± 0.00002 g), respectively ($p < 0.001$). The mean weight of cocoon in the transgenic group was significantly lighter than in the Dipel spray group ($p < 0.001$). There were no significant differences in the weight of cocoons from the non-transgenic and control spray groups ($p = 1.000$).

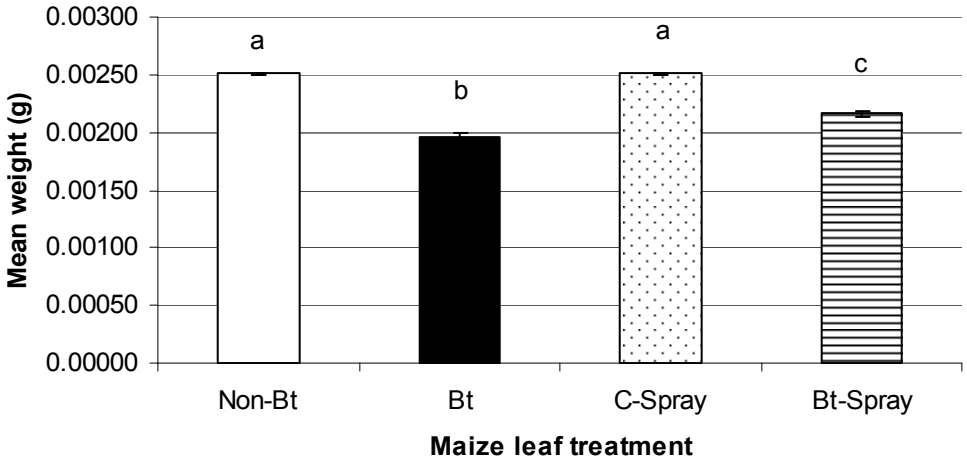


Figure 2.19. Mean weight of *C. marginiventris* cocoon. Columns with different letters represent treatment means that are significantly different at $p<0.001$.

2.6 Discussion

2.6.1 Effect of Bt toxin on *Spodoptera littoralis*

The present findings showed that there were significant differences in the weight of *S. littoralis* larvae from day 6 until day 20 when reared on the transgenic maize (MON810) expression Cry1Ab toxin and Dipel spray contains Cry1Ab toxin from the *B. thuringiensis*, Berliner, when compared to the controls (larvae reared on the non-transgenic and the control spray maize). Larvae maintained on transgenic and Dipel spray maize had significant lower weight compared to the non-transgenic and the control spray maize. This finding is similar to the previous research, (see reviews - Bokonon-Ganta et al., 2003, Dutton et al., 2005, Vojtech et al., 2005, Sanders et al., 2007), which showed that Cry1Ab toxins expressed in the transgenic maize and in the microbial Bt spray (Dipel) had negative effects on the development of the insects at the second trophic level. Vojtech et al. (2005) studied the effect of MON810 transgenic maize on *S. littoralis* larvae. They investigated the lower weight of larvae exposed to the transgenic maize when compared to larvae maintained on the non-transgenic maize. In Bokonon-Ganta et al. (2003), the authors used the transgenic MON810 maize and fall armyworm, *S. frugiperda*, and observed that the 10-day old larvae reared on the MON810 maize had the lowest weight compared to those reared on conventional maize. Dutton et al. (2005) compared the effect between non-transgenic maize (N4046), transgenic maize (N4046Bt) and Dipel spray maize on *S. littoralis* caterpillars. They observed significant differences in the weight of 3rd instar *S. littoralis* larvae from the transgenic and Dipel spray maize groups when compared to the non-transgenic maize group. Moreover, the larvae fed on Bt maize weighed significantly less than larvae fed on Dipel spray maize (Dutton et al., 2005). In a study by Sanders et al. (2007) on the transgenic maize event Bt 176 on the fall armyworm, larvae reared on the transgenic maize were significantly smaller and lighter at a younger instar than those reared on the conventional maize.

In the present study, the ranges of weight of the *S. littoralis* individuals reaching pupation and weight before until death were observed in these treatments. These findings could be used to predict that if each larva can not pass through these critical weight ranges, then the larvae are unsuccessfully pupated or survived. Thus, larval weight provides an indicator of host quality given that smaller hosts represent reduced resources for parasitoid growth and development (Couty et al., 2001a). Maize consumption of *S. littoralis* was monitored from day 6 to 20. Larvae raised on transgenic maize had higher relative growth rate than those raised on either maize over their juvenile life cycle. However, the larvae maintained on transgenic maize consumed more than the control spray or Dipel spray maize. It could be that the insects can not digest the maize leaf material and this could be caused by the mode of delivery of the Cry1Ab toxin. In the transgenic plant, the toxin is partially activated and the stability of gene expression provides a continuous exposure of toxin to herbivores (Schuler et al., 1998, Dutton et al., 2002). In the Dipel spray maize, the toxin is present on the leaf surface by spraying, which could give an uneven amount of the toxin, and thus it is not activated until it can be ingested by the insect larva. Then the protoxin is cleaved in the insect midgut which has a high enough pH to produce the fully activated toxin (see Chapter 1).

The present study showed that the transgenic maize (MON 810) expression Cry1Ab toxin and Dipel spray contains Cry1Ab toxin from the *B. thuringiensis*, Berliner, did not affect the survival and development time of *S. littoralis.* The survival of larvae and the development time of *S. littoralis* represent a period of the time scale in which *C. marginiventris* larvae are developing within the host. In contrast to this finding, Vojtech et al. (2005) showed that there was a negative effect by Bt maize on the survival and development time of *S. littoralis*. The higher survival was in the larvae maintained on the Control (Monumental cultivar) when compared with the MON810 group. Several studies on the transgenic maize cultivar showed negative effects on the survival and development of *Spodoptera* larvae (Dutton et al., 2002, Dutton et al., 2005). *S. littoralis* larvae reared on transgenic maize

(N4046Bt, Syngenta, formerly Northurp King) had lower survival compared with larvae reared on control maize (N4046). Thus, surviving larvae fed on this transgenic maize had a prolonged time to pupate. A study by Bokonon-Ganta et al. (2003) on *S. frugiperda* reared on transgenic maize (MON810), showed that the lowest survival and prolonged development time were observed in the larvae reared on this transgenic maize. Dutton et al. (2003a) tested the effect of Dipel spray (at the recommended field concentration) on *S. littoralis* larvae. They found that there was a significant increase in time needed to reach the second larval stage when larvae were reared on Dipel spray maize. Similarly, Dutton et al. (2005) also observed that *S. littoralis* larvae reared on Dipel spray plants took a significantly longer time to complete the second instar when compared to the time required for larvae maintained on control plants.

These present findings showed that such variability in larval performance might have been due to the inbreeding of the caterpillars, food stress of the caterpillars, or variation in biotic or abiotic factors. More replications in the same condition need to be undertaken, as the effect of the Bt toxin can be variable due conditions and times. Moreover, these will give a strong baseline data for further experiments in Chapter 3.

2.6.2 Effect of Bt toxin on *Cotesia marginiventris*

This study showed that the Cry1Ab toxins from the transgenic maize and Dipel spray maize negatively affected the survival and development of *C. marginiventris* developing within *S. littoralis* larvae. The lowest survival of the parasitoid was observed in the group exposed to transgenic maize. Moreover, the weight of the parasitized *S. littoralis* larvae at 2 and 5 days after parasitization was lowest in the larvae reared on transgenic maize when compared with other maize treatments. The change in weight of *S. littoralis* transgenic maize-fed larvae from day 2 to day 5 after parasitization was lowest compared to other maize-fed larvae. This could be an indirect effect of the Cry1Ab toxin on the quality of the hosts and this will have potential

consequences for the higher trophic levels. Moreover, this trend was reflected in the lighter weight of the parasitoid cocoons in both the transgenic and Dipel spray treatments when compared with the non-transgenic and control spray treatments. This is in line with the study by Vojtech et al. (2005), where the parasitoid *C. marginiventris* suffered greater mortality when parasitizing *S. littoralis* larvae fed on transgenic maize. The parasitized *S. littoralis* larvae had reduced weight when fed on MON810 maize in both 3 and 6 days after parasitization. As *C. marginiventris* parasitoids partly consume the host body, these insects are unlikely to be directly affected by Cry1Ab toxin. As this toxin binds to the specific receptors in the midgut epithelium of the lepidopteran hosts, the immature parasitoids may be influenced indirectly through lethal or sublethal impacts on the health and development of the hosts.

There are several studies on the effect of Bt toxin on the survival and development of the parasitoids (Hilbeck et al., 1999, Liu et al., 2005, Sanders et al., 2007). These authors observed the decreased weight of parasitized hosts and parasitoid cocoons when the hosts were fed on Bt toxin. For example, Liu et al. (2005) showed that the offspring of the parasitoid, *Microplitis mediator*, developed more slowly and pupal weight was reduced significantly when the parasitized host larvae (*Helicoverpa armigera*) fed on the Bt cotton powder leaf diet compared with non-Bt treatment. This finding supports that size and the quality of the hosts are important for the growth and development of the parasitoids (Godfray, 1994).

To conclude, the use of Bt maize and Bt-spray expressing Cry toxin affects the growth of *S. littoralis* as well as the survival and the growth of *C. marginiventris* developing within *S. littoralis* hosts. The host reared on the transgenic maize weighed the lowest when compared to other maize-fed larvae from day 6 to 20. The parasitized hosts which fed on transgenic maize and Dipel spray maize weighed significantly lower in relative to control treatments. Moreover, the weights of *C. marginiventris* cocoon in the transgenic and Dipel

spray maize were lighter when compared to those in the non-transgenic and control spray maize. These findings of reduced host size when fed on Cry1Ab toxin could be suggested an investigation into the quality of hosts as food resources on the higher trophic level (e.g. parasitoid). Changes in the quality of the host may influence the numbers of parasitoids, therefore parasitoids should parasitize healthy hosts to maintain their fitness measured by size, development time, and survival (Hemerik and Harvey, 1999). In addition to studies of parasitoid larval survival and development, full evaluation of the impacts of the Cry1Ab toxin requires other examinations such as oviposition preferences, parasitism rate, number of successfully emerged parasitoid and development time. These examinations will be undertaken in the next experiments in Chapter 3.

Chapter 3

Behaviour of *Cotesia marginiventris* with *Spodoptera littoralis* hosts in no-choice tests

3.1 Introduction

A parasitoid is an insect species whose immature life stage develops within a single insect host, feeds on the body fluids and organs of the host and ultimately kills the host (Godfray, 1994). This makes parasitoids important in the control of herbivorous insect pests (Prutz et al., 2004), as parasitoids reduce the numbers of the hosts and decrease the damage caused by agricultural pests (Morales et al., 2007). For example, *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) is a polyphagous, solitary endoparasitoid and is considered as a generalist because it attacks many lepidopteran species. Therefore, this parasitoid is a good model non-target insect for assessing the impacts of Bt toxin from transgenic crops.

The successful development of the parasitoid depends on the host providing nutrients to the developing immature parasitoid (Godfray, 1994, Thompson, 1999). Host quality correlates with fitness in parasitoids on three levels; (i) adult size, (ii) development time, and (iii) survival (Godfray, 1994, Hemerik and Harvey, 1999, Dutton et al., 2002, Vojtech et al., 2005, Urrutia C et al., 2007). If a host is unable to survive, then the developing parasitoid follows the same fate (Sanders et al., 2007). The size of the hosts is also important for the survival, growth and development of parasitoids (Godfray, 1994, Thompson, 1999). As the size of the parasitoid, especially the female parasitoid, covaries with the size of host, the larger female parasitoids live longer and have higher fecundity (Couty et al., 2001a, Sagarra et al., 2001, King and Napoleon, 2006). Insect resistant plants could affect the behaviour of parasitoids. Therefore, changes in these signals in transgenic plants may prevent the parasitoids locating hosts effectively. Moreover, the parasitoid could be affected by Bt toxin used to control pest species, as parasitoids should avoid unhealthy hosts which would provide them with less successful larval development (Godfray, 1994). It is well documented in the literature that when the larvae fed on transgenic maize they have reduced weight and prolonged development of *S. littoralis* hosts (Dutton et al., 2002, Dutton et al., 2005, Vojtech et al., 2005).

These effects lead to decreased cocoon weight and prolonged development of *C. marginiventris* developing within *S. littoralis* host (Vojtech et al., 2005). Moreover, in Chapter 2 results are presented which show *S. littoralis* hosts had less weight when fed on the transgenic (MON810) and Dipel spray maize leaf. These effects could make *C. marginiventris* parasitoids suffer in terms of fitness, leading to a reduction in their population.

This chapter describes the parasitoid behaviour on hosts fed on different maize-leaf treatments. Parasitoid behaviour, such as attack rate and oviposition rate, under no-choice hosts were tested. The hosts were fed on four maize treatments (non-transgenic, transgenic, control spray-, and Dipel spray leaves). Hosts exposed to the Cry1Ab toxin, having reduced size and prolonged development, were offered to parasitoids. Attack and oviposition rate, time to attack, number of parasitoid cocoon and number of emerged parasitoid were compared among these treatments.

3.2 Aims

The aim was to investigate the performance of the parasitoid in terms of the behaviour such as attack rate and oviposition, on hosts maintained on Bt toxin maize leaves under the no-choice host condition, and to examine how quality of the hosts related to indirect effects of the Bt toxin on natural enemies.

3.3 Hypotheses

1. Parasitoids attack fewer hosts which have fed on maize leaves containing Bt (GM or Bt spray).
2. Parasitoids develop slowly in the hosts which have fed on maize leaves containing Bt (GM or Bt spray).

3.4 Materials and Methods

3.4.1 Maize

Transgenic maize (*Zea mays*) plants (event MON810, Monsanto) expressing Cry1Ab protein and the conventional cultivar (DK315, Monsanto) were used in all experiments. Genetically modified maize line MON810 (trade name YieldGard®) was developed to be resistant to attack by the European corn borer (ECB, *Ostrinia nubilalis*). MON810 contains the cauliflower mosaic virus (CaMV) 35S promoter which expresses the toxin throughout the season in leaves, stalk, roots, and kernels. The plants were cultivated in 15 cm plastic pots (3 grains per pot), using vapogro soil (Vapogro Ltd, Glastonbury, Somerset, UK), and were kept in a greenhouse. New plants were sown weekly and were used for experiments when 4-5 weeks old. No fertilizers were applied.

The plants were grouped as follows:

- transgenic maize plants (Bt maize):
- non-transgenic plants (non Bt maize):
- non-transgenic plants which had been sprayed with Dipel (Bt spray maize) as follows:
    a) Prepare 20 mg Dipel in 20 mL Tween solution (0.5 mL in 1 L distilled water) for spraying the non-transgenic maize plants;
    b) Spray the plants with a manual sprayer (amount: 0.8 mL per leaf);
    c) Spray only once and allow the plants to dry before being infested by *S. littoralis* larvae;
- non-transgenic plants which were sprayed with Tween solution (Control spray maize) as follows:
    a) Prepare the Tween solution (0.5 mL in 1 L distilled water);
    b) Spray the plants with a manual sprayer (amount: 0.8 mL per leaf);
    c) Spray only once and allow the plants to dry before being infested by *S. littoralis* larvae.

3.4.2 *Spodoptera littoralis*

Eggs of *S. littoralis* were supplied on netting by Syngenta, Bracknell, UK, each week. The eggs were left on the netting and placed in 250mL pots (Roundstone, Catering Equipment Ltd, Melksham, Wiltshire, UK) with ventilation. The eggs were maintained in a Thermo Electron A/013 cooled incubator at 25 ^{o}C ± 1.0 ^{o}C, photoperiod 14 hrs light/ 10 hrs dark.

In the experimental trials, the eggs were maintained in a 1.5 L plastic box and were allowed to develop into first instar larvae. The plant material (Non-Bt (DK315), Bt (MON810), control-spray and Bt-spray maize leaves) were changed every day.

3.4.3 *Cotesia marginiventris*

Cocoons of *C. marginiventris* were obtained from the Laboratoire d'Entomologie Evolutive, Institut de Zoologie, Université de Neuchâtel, Switszerland. The insect adults were maintained in 30x30x30 cm Perspex cages at standardized conditions. Adults were reared on a 20% honey/water solution. This was provided on soaked cotton wool which was changed every 48 hours. For general culturing, 35 second instar *S. littoralis* were removed from large emergence groups and placed in 250mL pots (Roundstone, Catering Equipment Ltd, Melksham, Wiltshire, UK) with ventilation, containing an excess of artificial diet (Beet Armyworm diet F9220B Bio-Serve, Frenchtown, NJ 08825). *S. littoralis* larvae were offered to two mated *C. marginiventris* females (2-6 days old); these pots also contained a small ball of damp cotton wool dipped in honey/water solution. Pots were observed until at least one female attacked a host. The *C. marginiventris/S. littoralis* groups were then left to parasitize for 24 hours. After 24 hours, the adult *C. marginiventris* and honey cotton ball were removed. *C. marginiventris* larvae took between 8-12 days to emerge during which time the pots were monitored and any large

unparasitized *S. littoralis* were removed and fresh artificial diet added as required.

3.4.4 *C. marginiventris* behaviour under no-choice *S. littoralis* host;

In the experimental trial, fifteen *S. littoralis* larvae were offered to one mated *C. marginiventris* female. These insects were kept in 250 mL pots (Roundstone, Catering Equipment Ltd, Melksham, Wiltshire, UK) with ventilation. The stop watch was started and left for 15 minutes upon introduction of the parasitoid, in which the time to the first attack, the time until the 15th attack and the number of total attacks were recorded. Once the 15 minute-period was completed the parasitoid was removed from the container to prevent any further attacks. The parasitized larvae were kept until cocoon formation and adult emergence. During this time the plant materials were changed daily.

The experimental protocols are as follows;

3.4.4.1 Parasitization: same age S. littoralis larvae (different size);

On the fourth day of incubation, fifteen non-transgenic maize-fed *S. littoralis* larvae were offered to one mated female *C. marginiventris* until parasitization was observed. The number of attacks was recorded for 15 minutes. The time taken until the 15th attack was also recorded. The numbers of cocoon formation, number of adult emergence were recorded as well as the proportion of all attacks resulting in cocoon formation and adult emergence. The time until cocoon formation and the time until adult emergence of the parasitoids were recorded. The same method was repeated for the Bt, Control spray, and Bt spray maize-fed *S. littoralis* larvae. The experiment was replicated 20 times for each treatment.

3.4.4.2 Parasitization: same size S. littoralis larvae (different age);

Fifteen non-transgenic maize-fed and 15 control-spray maize-fed *S. littoralis* larvae aged 4 days old were offered to one mated female *C. marginiventris* until parasitization was observed. The number of attacks was recorded for 15 minutes. The time taken until the 15^{th} attack was also recorded. The numbers of cocoon formation, number of adult emergence were recorded as well as the proportion of all attacks resulting in cocoon formation and adult emergence. The time until cocoon formation and the time until adult emergence of the parasitoids were recorded. The same method was repeated for the Bt, and Bt spray maize-fed *S. littoralis* larvae. Bt and Bt spray maize-fed *S. littoralis* larvae aged 5 days old had the same size as non-transgenic and control spray *S. littoralis* aged 4 days old. The experiment was replicated 20 times for each treatment.

3.4.4.3 Parasitization of one individual same age S. littoralis larvae by one mated female C. marginiventris

In this experimental trial, one same age *S. littoralis* larva was offered to one mated *C. marginiventris* female. These insects were kept in 250 mL pots (Roundstone, Catering Equipment Ltd, Melksham, Wiltshire, UK) with ventilation. After parasitism, parasitoid was removed from the container. The parasitized larva was kept until cocoon formation and adult emergence. During this time the plants were changed daily. The time to cocoon formation and adult emergence were recorded. The method was repeated for the Bt, Control spray, and Bt spray maize-fed *S. littoralis* larvae. The experiment was replicated 30 times for each treatment.

3.4.5 Statistical analysis

All data were analysed using SPSS for Windows version 14.0 (SPSS Inc., Chicago, USA). All data were tested by the Kolmogorov-Smirnov test for a background to check whether the population had a normal distribution.

One-way analysis of variance (ANOVA) was used in all data (time taken to the first attack of parasitoid, mean of time taken to the 15^{th} attack of parasitoid, time to parasitoid cocoon formation, time to parasitoid adult emergence, number of parasitoid attack over 15 minutes, number of parasitoid cocoon, number of parasitoid adult emergence, proportion of all attacks resulting in parasitoid cocoons, and proportion of all cocoons resulting parasitoid adults). Mean values between treatments were compared with Tukey HSD tests. The data on the proportions of all attacks resulting in parasitoid cocoons, and proportion of all cocoons resulting in parasitoid adults were transformed using Arcsine transformation prior to analysis in order to conform to a normal distribution.

3.5 Results

3.5.1 Parasitization: same age *S. littoralis* larvae (different size) by 1 mated female *C. marginiventris*;

Parasitism of the same age maize-fed *S. littoralis* hosts was observed at the time taken to the first attack, time taken until the 15th attack, time to parasitoid cocoon formation, time to parasitoid emerged adult, number of parasitoid attacks over 15 minutes, number of parasitoid cocoons, number of parasitoid emerged adults, and the proportions of all attacks resulting in cocoon formation and adult emergence (Fig. 3.1- 3.9).

Using the mean of time taken to first attack by one mated female *C. marginiventris* on fifteen same aged *S. littoralis* larvae, one-way ANOVA revealed that there was a significant difference between the non-transgenic and the transgenic treatments (Fig. 3.1, $F_{3,76} = 3.143$, $p = 0.030$). The mated female *C. marginiventris* had a significantly shorter time taken to the first attack on larvae reared on the non-transgenic maize (0.61 ± 0.14 min.) when compared to those reared on transgenic maize (1.85 ± 0.40 min.) ($p = 0.028$). However, there was no significant differences between the time taken to the first attack by the female parasitoid in the control spray and Dipel spray treatments ($p = 0.947$). No significant differences were observed between the time taken to the first attack in the non-transgenic and the control spray ($p = 0.255$) and Dipel treatments ($p = 0.085$). There were no significant differences in the time taken to the first attack between the transgenic, control spray and Dipel spray treatments ($p = 0.753$ and $p = 0.970$, respectively).

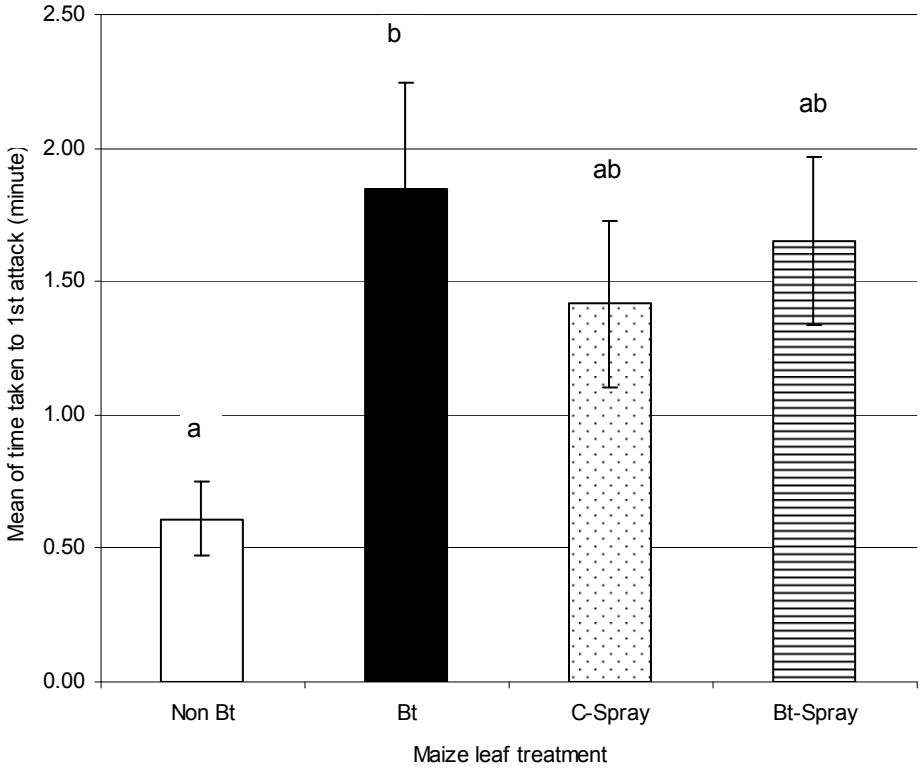


Figure 3.1. Mean of time taken to the first attack by *C. marginiventris* on the same age *S. littoralis* larvae fed either non-transgenic maize (Non Bt), transgenic maize (Bt), control spray maize (C spray) or Dipel spray maize (Bt maize). Columns with different letters represent treatment means that are significantly different at $p = 0.030$.

One-way ANOVA showed that the mean of time taken to the 15th attack by the parasitoid on the host in these treatments had no significant difference (Fig. 3.2, $F_{3,76} = 0.630$, $p = 0.598$).

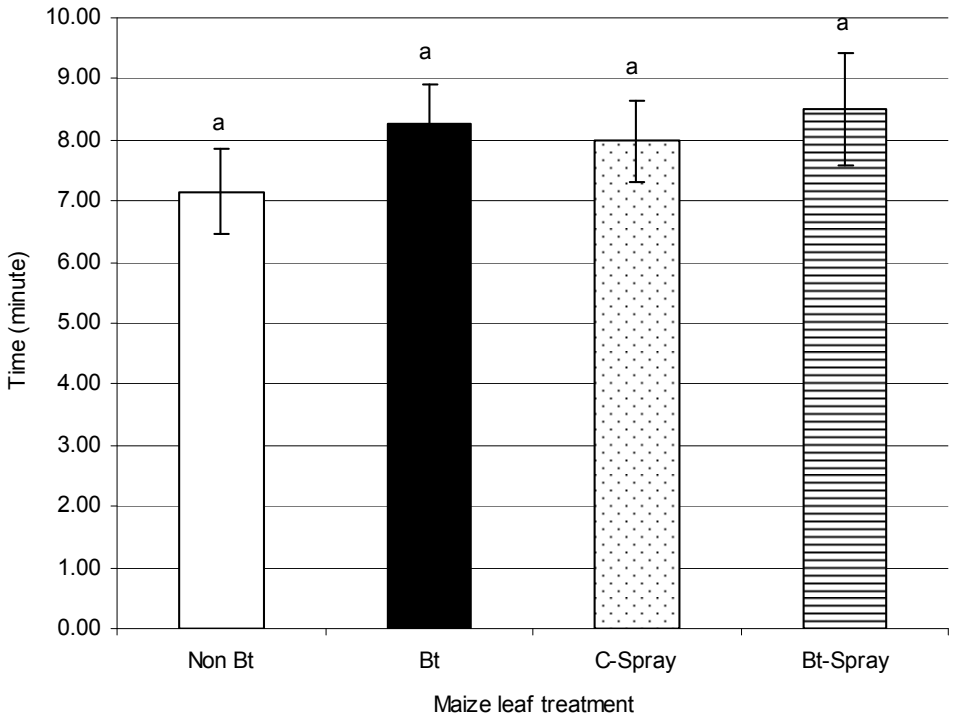


Figure 3.2. Mean of time taken to the 15th attack by *C. marginiventris* on the same age *S. littoralis* larvae. Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different (p=0.598).

One-way ANOVA showed that there was significantly different in the time to cocoon formation between these treatments (Fig 3.3, $F_{3,71} = 15.216$, $p < 0.001$). *C. marginiventris* developed within non-transgenic hosts took on average 10.05 days to pupate when compared to 13.22 days in the transgenic ($p < 0.001$) and 12.29 days in the Dipel spray treatments ($p < 0.001$). The time to pupation in the transgenic treatment was significantly longer than in the non-transgenic and control spray treatments ($p < 0.001$ and $p < 0.001$, respectively). However this was not significant when comparing the Dipel spray treatment ($p = 0.296$).

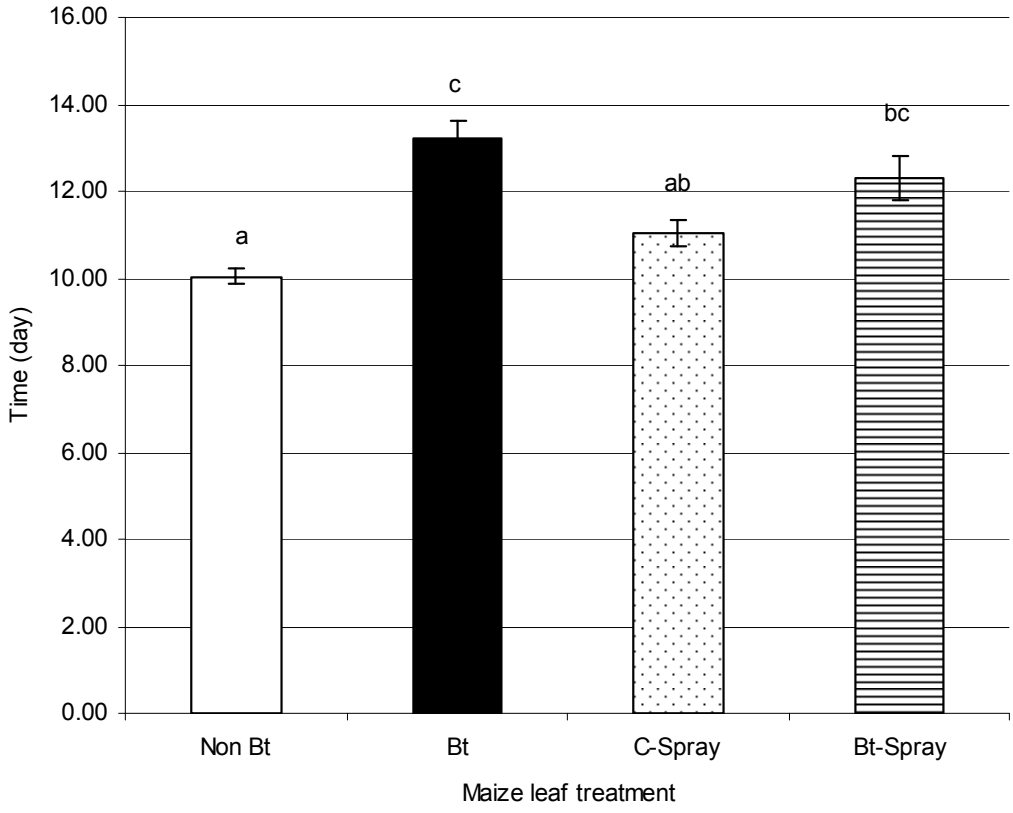


Figure 3.3. Mean of time to *C. marginiventris* cocoon formation. Columns with different letters represent treatment means that are significantly different at $p < 0.001$.

There were significant differences in the time to parasitoid adult emergence between these treatments (Fig. 3.4, $F_{3,69} = 5.817$, $p = 0.001$). In the non-transgenic treatment, the time to parasitoid adult emergence was the shortest (14.95 ± 0.49 days) when compared to the transgenic (17.94 ± 0.57 days; $p = 0.006$) and the Dipel spray treatments (17.88 ± 0.91 days; $p = 0.009$). No significant difference in the time to parasitoid adult emergence was observed between the parasitoid developing within the non-transgenic and control spray hosts ($p = 0.749$).

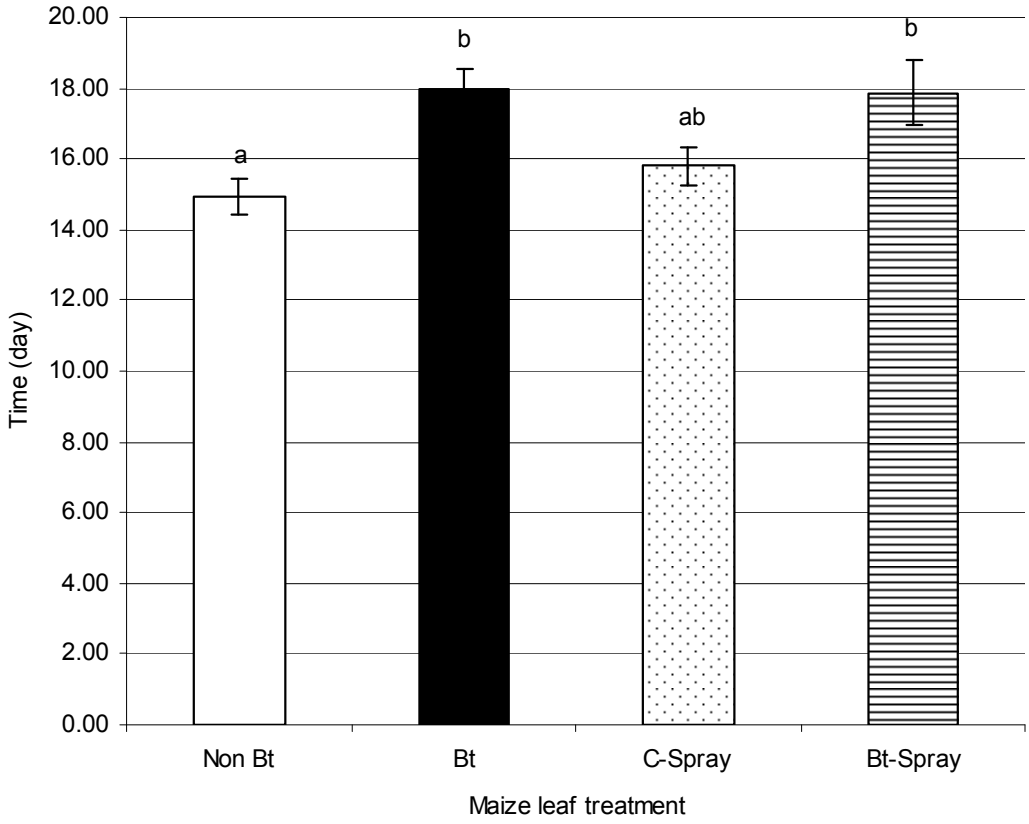


Figure 3.4. Mean of time to *C. marginiventris* adult emergence. Columns with different letters represent treatment means that are significantly different at $p = 0.001$.

A one-way ANOVA showed that there were significant differences in the mean number of attacks over 15 minutes among these treatments (Fig. 3.5, $F_{3,76}$ = 4.019, p = 0.010). The highest number of attacks over 15 minutes by *C. marginiventris* female was in *S. littoralis* larvae fed the non-transgenic maize (27.60 ± 1.79) compared to those fed transgenic maize (21.05 ± 1.44; p = 0.025) and Dipel spray maize (20.75 ± 1.67; p = 0.017), respectively. No significant differences were observed on the mean number of attack over 15 minutes either between transgenic and control spray treatments (p = 0.520) or transgenic and Dipel spray treatments (p = 0.999). Similarly, there was no significant difference in the number of attacks over 15 minutes between the control spray and Dipel spray treatments (p = 0.439).

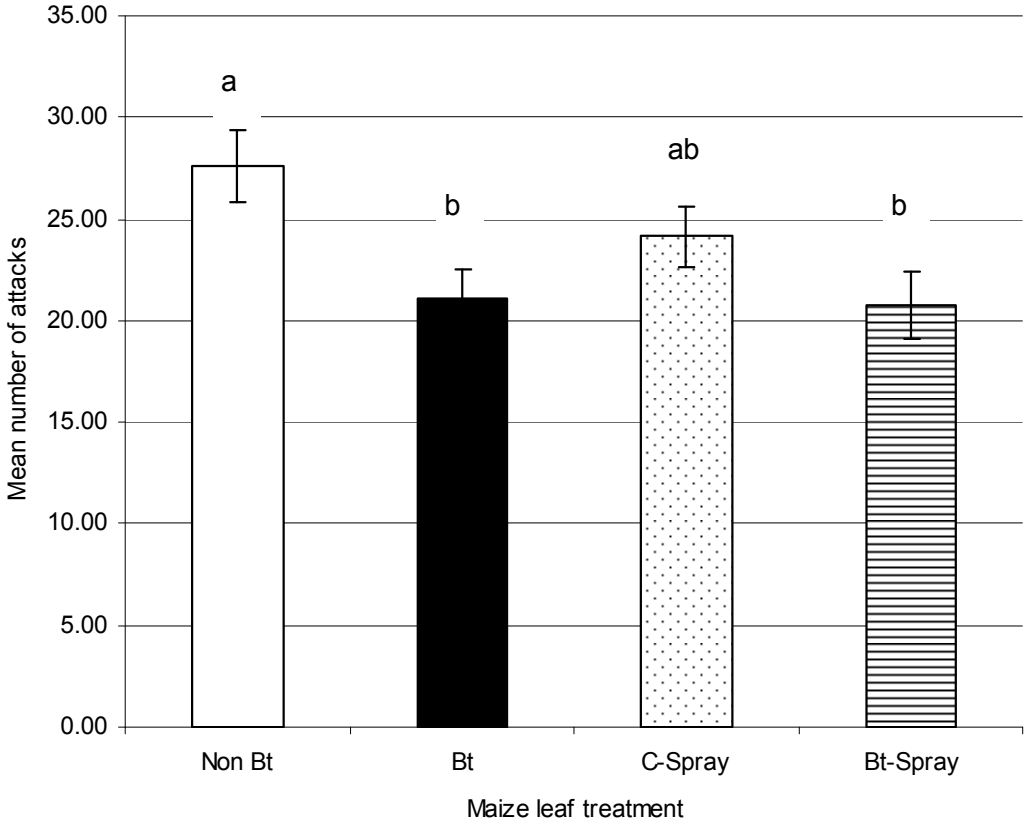


Figure 3.5. Mean number of attack over 15 minutes by *C. marginiventris* on the same age *S. littoralis* larvae. Columns with different letters represent treatment means that are significantly different at p = 0.010.

There were significant differences in the mean number of *C. marginiventris* cocoon formation among these treatments (Fig. 3.6, $F_{3,71} = 17.804$, $p < 0.001$). The average number of cocoons was significantly highest in the non-transgenic treatment (9.40 ± 0.65) when compared to the control spray (6.00 ± 0.74; $p = 0.002$), Dipel spray (4.69 ± 0.77; $p < 0.001$), and transgenic treatments (3.06 ± 0.44; $p < 0.001$), respectively. There was no significant difference in the number of cocoons between the transgenic treatment and Dipel spray treatment ($p = 0.283$). No significant difference was observed in the number of cocoons between the control spray and Dipel spray treatments ($p = 0.521$).

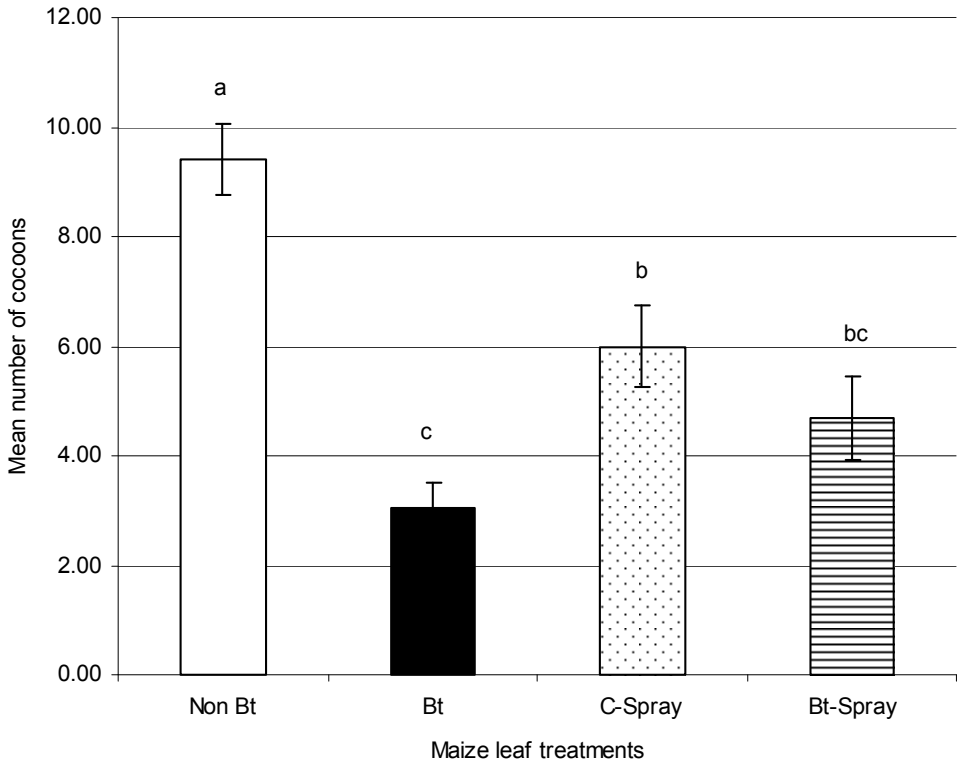


Figure 3.6. Mean number of *C. marginiventris* cocoon. Columns with different letters represent treatment means that are significantly different at $p < 0.001$.

A one-way ANOVA showed that there were significant differences in the number of emerged parasitoids between these treatments (Fig. 3.7, $F_{3,69}$ = 19.378, $p < 0.001$). According to the highest number of parasitoid cocoon in the non-transgenic group, as expected, the number of parasitoid adults in this group was the highest (7.89 ± 0.56) compared to those in the control spray (4.75 ± 0.58; $p < 0.001$), Dipel spray (3.31 ± 0.53; $p < 0.001$), and transgenic groups (2.67 ± 0.41; $p < 0.001$). The lowest number of adult parasitoids was observed in the group exposed to transgenic maize. There were no significant differences in the number of adult parasitoid either between the transgenic and Dipel spray treatments ($p = 0.841$) or control spray and Dipel spray groups ($p = 0.242$).

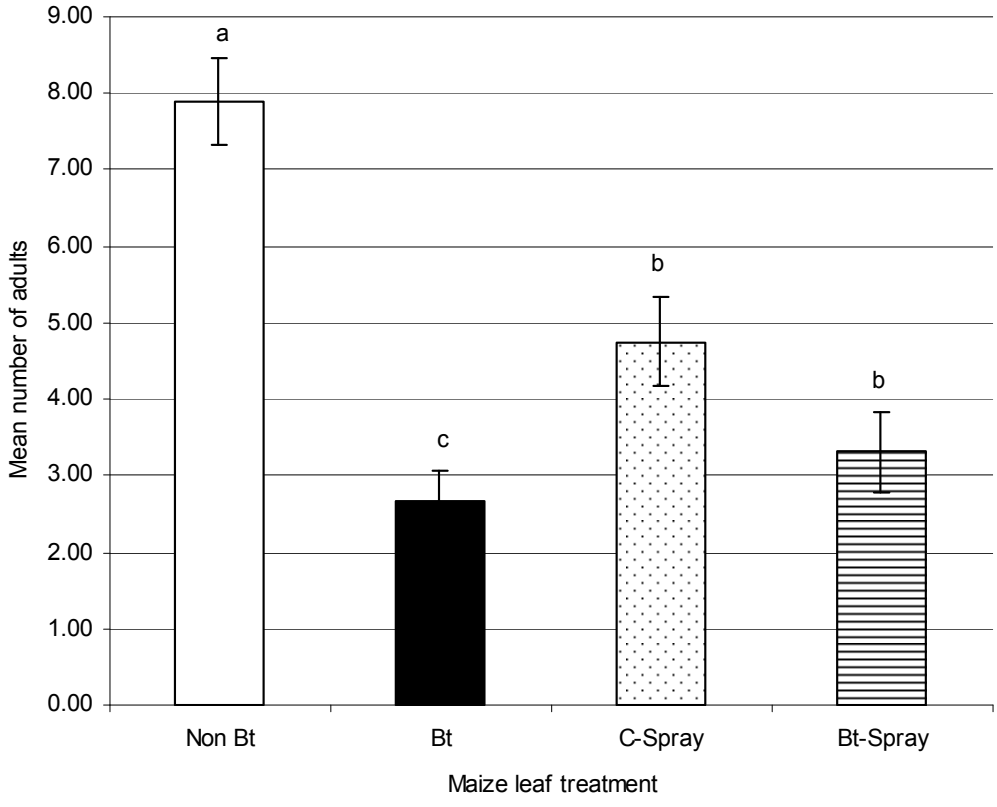


Figure 3.7. Mean number of *C. marginiventris* adult. Columns with different letters represent treatment means that are significantly different at $p < 0.001$.

The results of the proportion of all attacks resulting in cocoon formation and the proportion of all cocoons resulting in the emerged adult parasitoids are shown in Figure 3.8 and 3.9, respectively.

There were significant differences in the proportion of all attacks resulting in cocoon formation among these treatments (Fig. 3.8, $F_{3,70} = 10.700$, $p < 0.001$). The proportion of all attacks resulting in cocoon formation in the non-transgenic was the highest (35%) compared to 24% in the control spray ($p = 0.021$), 24% in the Dipel spray ($p = 0.034$) and 14% in the transgenic treatments ($p < 0.001$), respectively. Hosts reared on transgenic maize had a significantly different proportion forming cocoons compared to those reared on Dipel spray maize ($p = 0.050$).

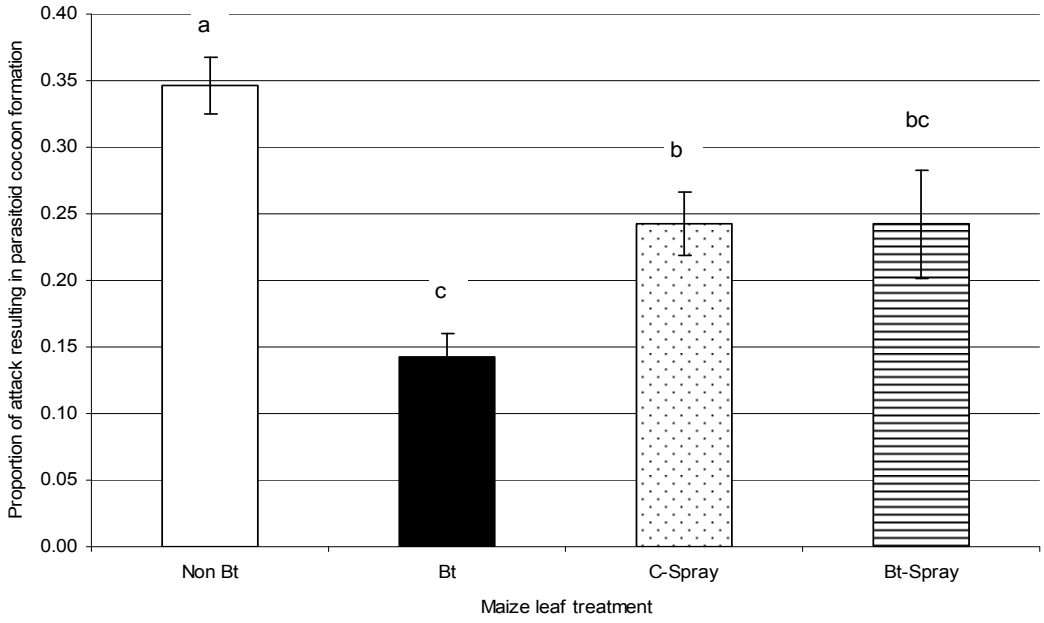


Figure 3.8. Mean of proportions of all attacks resulting in parasitoid cocoon formation. Columns with different letters represent treatment means that are significantly different at $p < 0.001$.

However, one-way ANOVA revealed that there were no significant differences in the proportion of all cocoons resulting in emerged parasitoids among these treatments (Fig. 3.9, $F_{3,69} = 0.558$, $p = 0.644$).

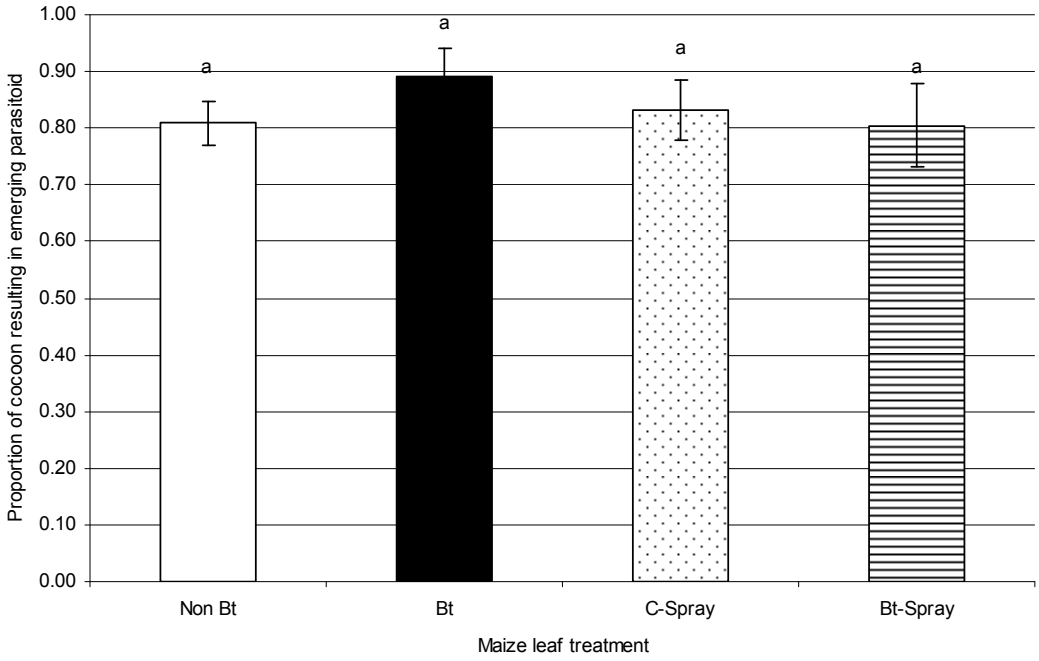


Figure 3.9. Mean of proportion of all cocoons resulting in parasitoid adult emergence. Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different (p=0.644).

3.5.2 Parasitization: same size *S. littoralis* larvae (different age) by 1 mated female *C. marginiventris*;

Parasitism of same size maize-fed *S. littoralis* hosts was observed as the time taken to the first attack, time taken to the 15th attack, time to cocoon formation, time to adult emergence, number of attacks over 15 minutes, number of cocoons, number of adults, proportions of all attacks resulting in cocoon formation and adult emergence. Figure 3.10 – 3.18 show the result from the non-choice test of parasitoids when provided with the same-size hosts.

A one-way ANOVA showed that there were no significant differences in the time taken to the first attack among these treatments (Fig. 3.10, $F_{3,76} = 1.587$, $p = 0.200$).

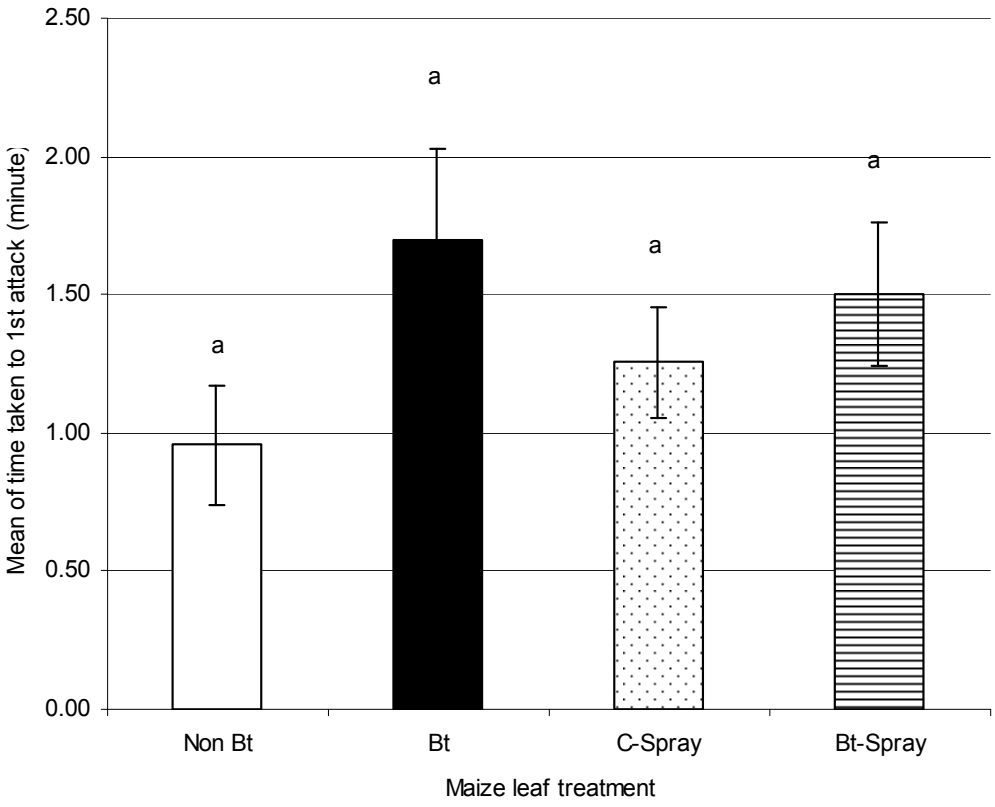


Figure 3.10. Mean of time taken to the first attack by *C. marginiventris* on *S. littoralis* larvae. Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different (p=0.200).

Significant differences in the mean of time to 15th attack were found among these treatments (Fig. 3.11, $F_{3,76} = 6.386$, $p = 0.001$). In the non-transgenic group, the time taken until the 15th attack was faster (5.26 ± 0.62 min.) compared with the transgenic group (8.08 ± 0.55 min.; $p = 0.003$), and the Dipel spray group (8.30 ± 0.48 min.; $p = 0.001$) which showed the longest time to attack. No significant differences were observed in the time taken until the 15th attack between either the non-transgenic and control spray treatments ($p = 0.284$) or transgenic and Dipel spray treatments ($p = 0.993$).

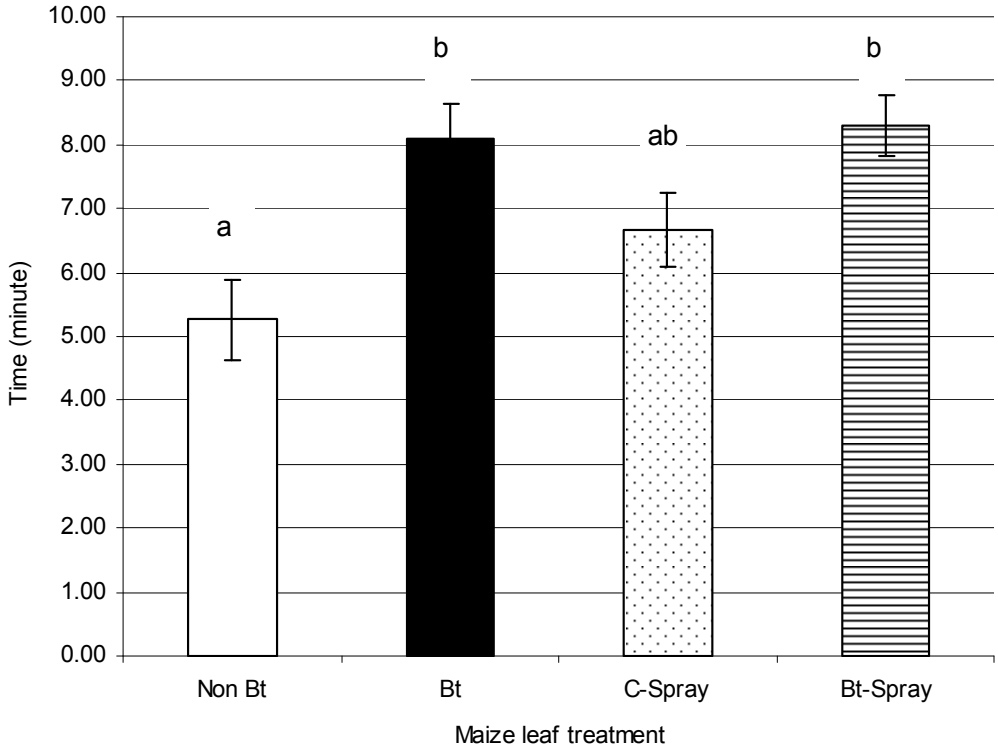


Figure 3.11. Mean of time taken to the 15th attack by *C. marginiventris* on *S. littoralis* larvae. Columns with different letters represent treatment means that are significantly different at $p = 0.001$.

There are no significant difference in the time to parasitoid cocoon formation between these treatments (Fig. 3.12, $F_{3,71} = 1.230$, $p = 0.305$).

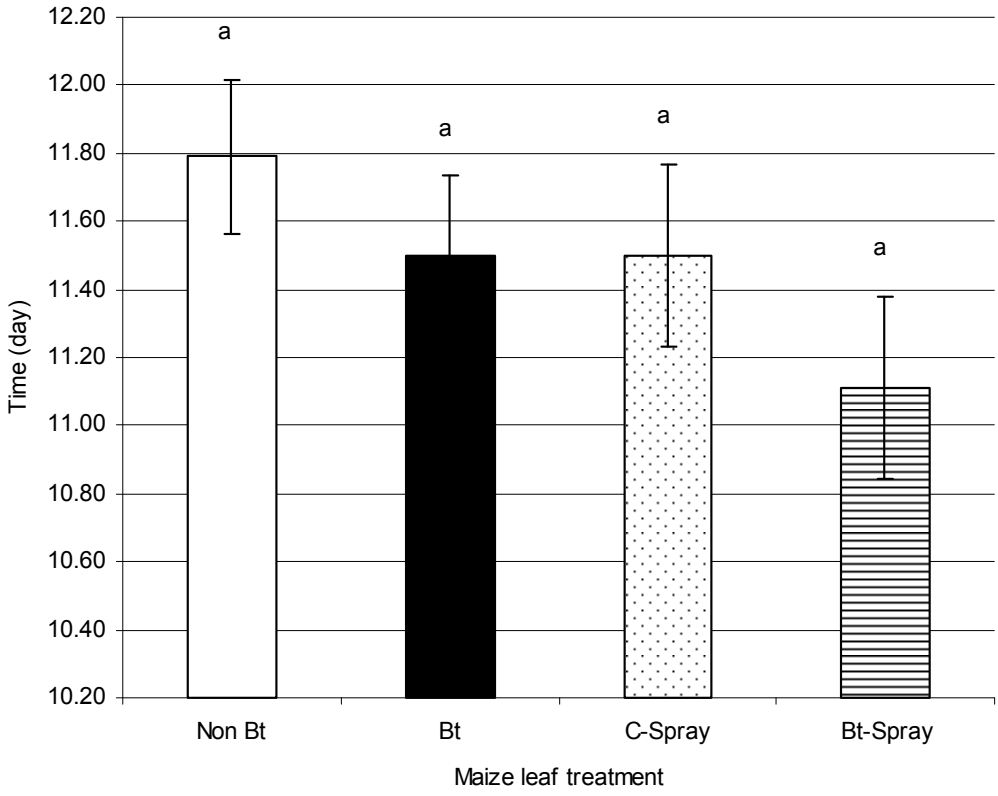


Figure 3.12. Mean of time to *C. marginiventris* cocoon formation. Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different (p=0.305).

No significant differences in the time of parasitoid adult emergence were observed among these treatments (Fig. 3.13, $F_{3,71} = 1.481$, $p = 0.227$).

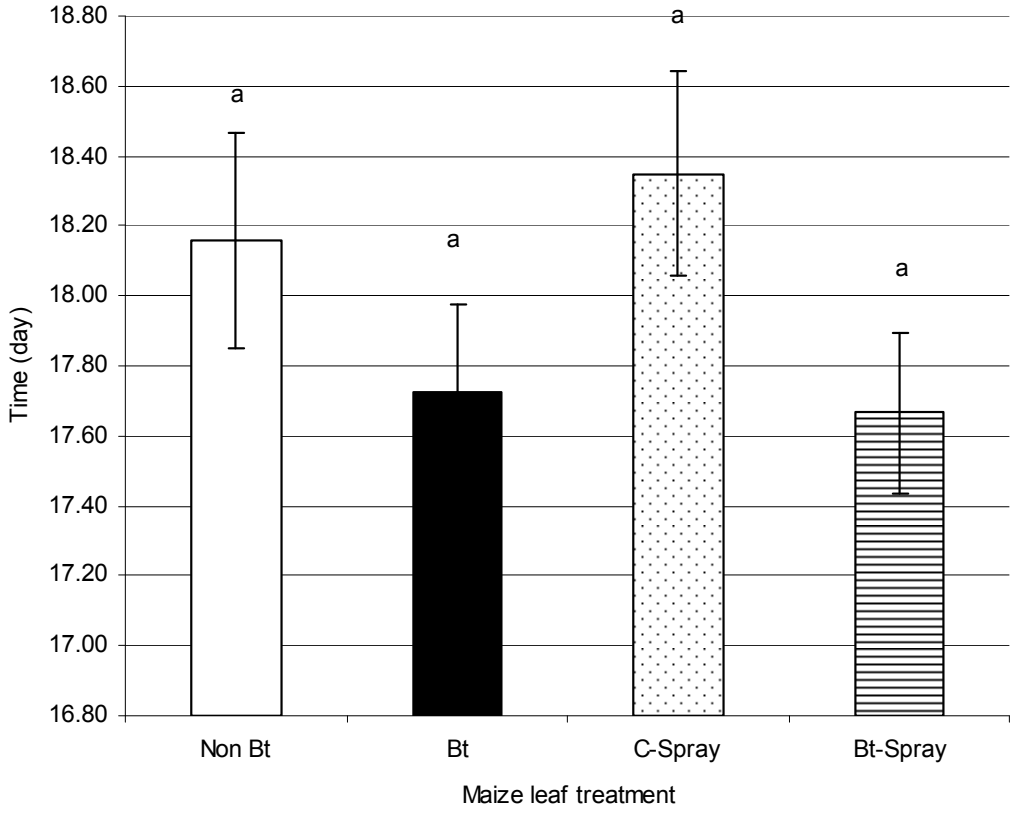


Figure 3.13. Mean of time to *C. marginiventris* adult emergence. Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different (p=0.227).

A one-way ANOVA showed that there were significant differences in the number of attacks over 15 minutes among these treatments (Fig. 3.14, $F_{3,76}$ = 9.054, $p < 0.001$). Larvae reared on non-transgenic leaves suffered more attacks on average (29.65 ± 2.17) than those reared on control spray leaves (22.05 ± 0.94; $p = 0.002$), transgenic leaves (21.75 ± 1.35; $p = 0.001$) and Dipel spray leaves (19.90 ± 0.89; $p < 0.001$) respectively. There were no significant differences in the number of parasitoid attacks between either the transgenic and control spray treatments ($p = 0.999$) on the transgenic and Dipel spray treatments ($p = 0.798$). No significant differences in the numbers of attacks over 15 minutes were found between the control spray and the Dipel spray treatments ($p = 0.715$).

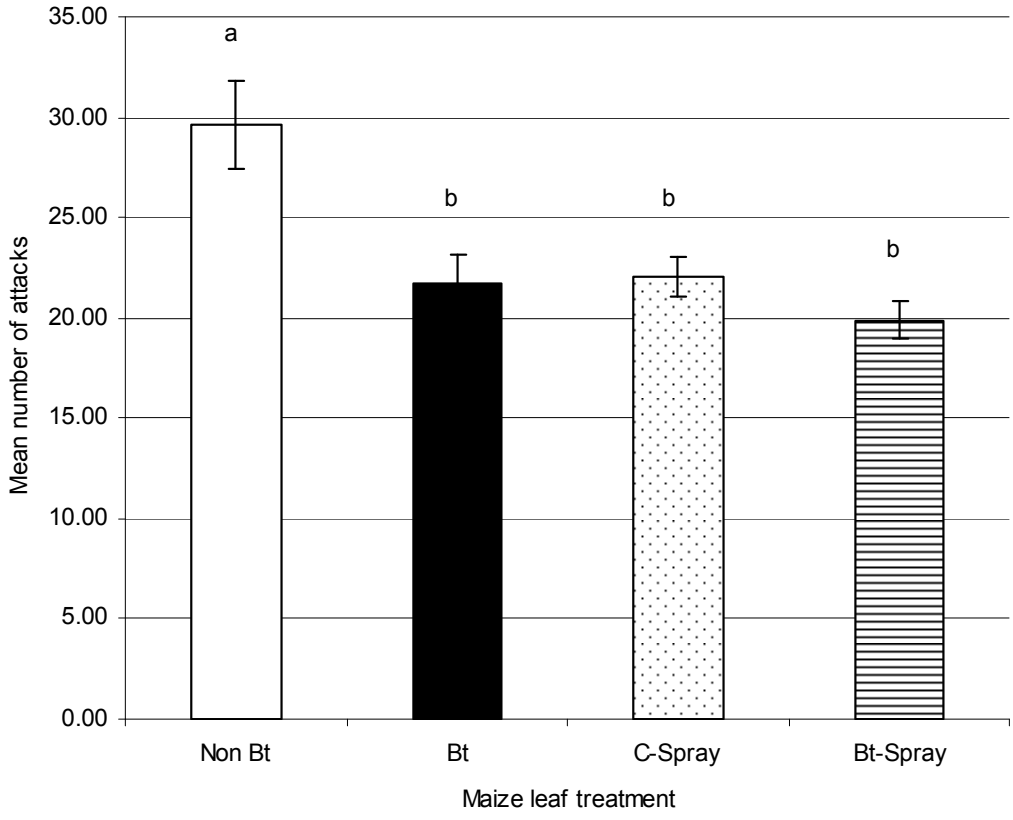


Figure 3.14. Mean number of attacks over 15 minutes by *C. marginiventris* on *S. littoralis* larvae. Columns with different letters represent treatment means that are significantly different $p < 0.001$.

No significant differences in the mean number of parasitoid cocoon were observed among these treatments (Fig. 3.15, $F_{3,71} = 1.988$, $p = 0.123$).

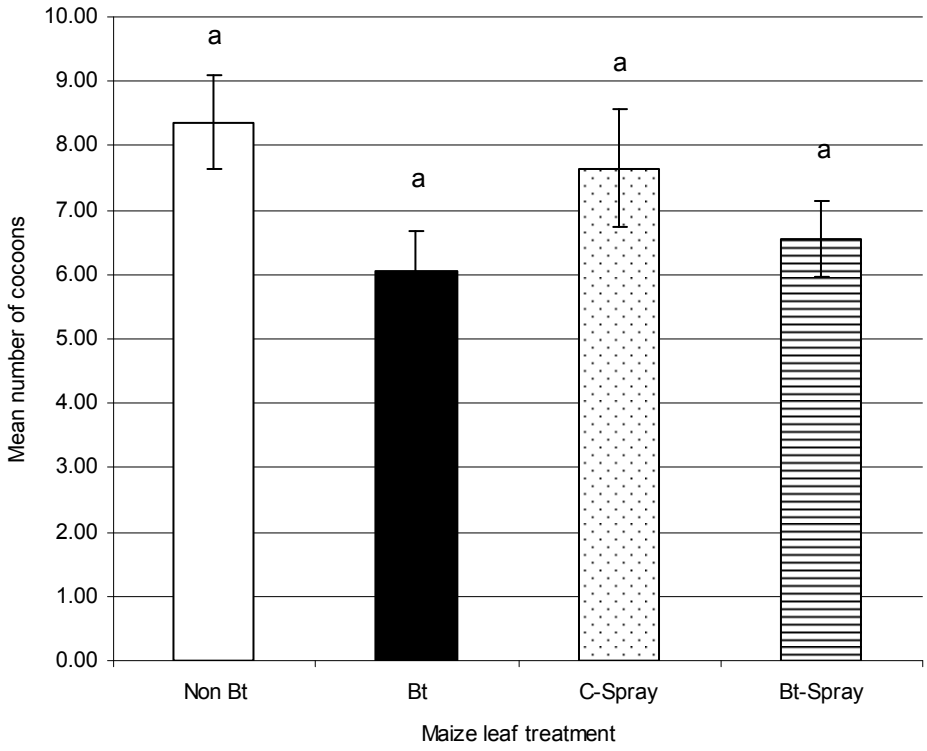


Figure 3.15. Mean number of *C. marginiventris* cocoons. Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different (p=0.123).

According to the above results; there were also no significant differences in the number of parasitoid adult emergence between these treatments (Fig. 3.16, $F_{3,70} = 2.209$, $p = 0.095$).

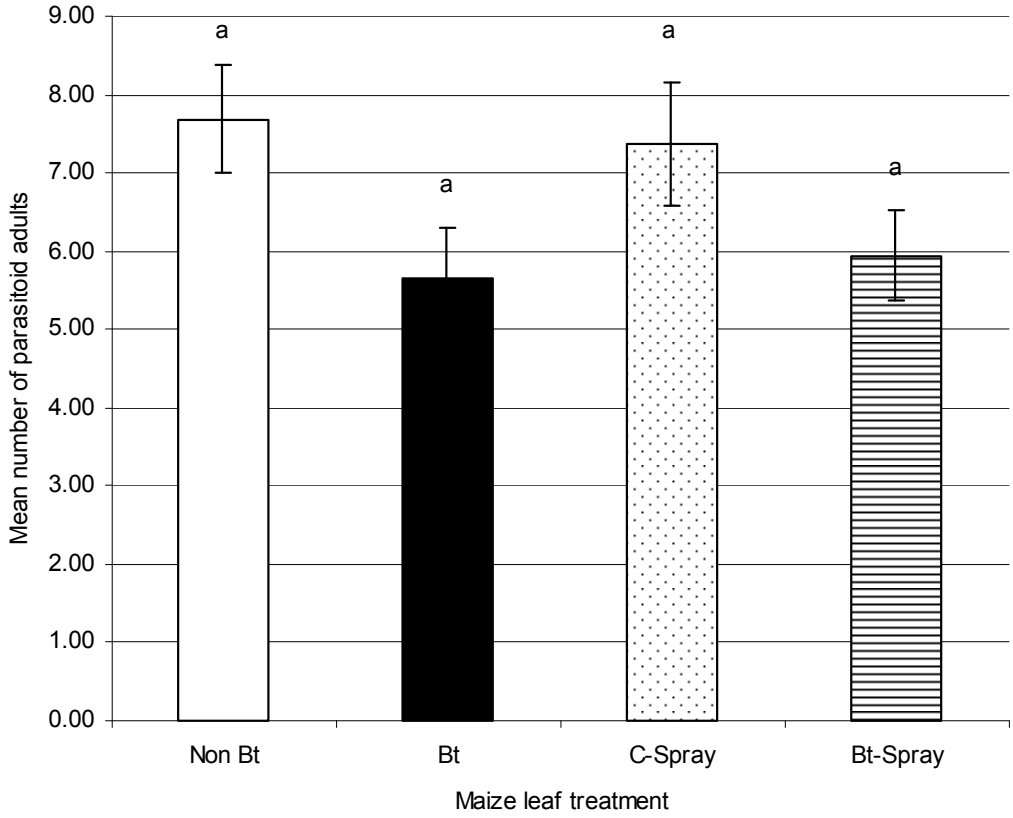


Figure 3.16. Mean number of *C. marginiventris* adult. Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different (p=0.095).

The proportion of all attacks resulting in parasitoid cocoon formation was not significantly different between these treatments (Fig. 3.17, $F_{3,71} = 0.506$, $p = 0.680$).

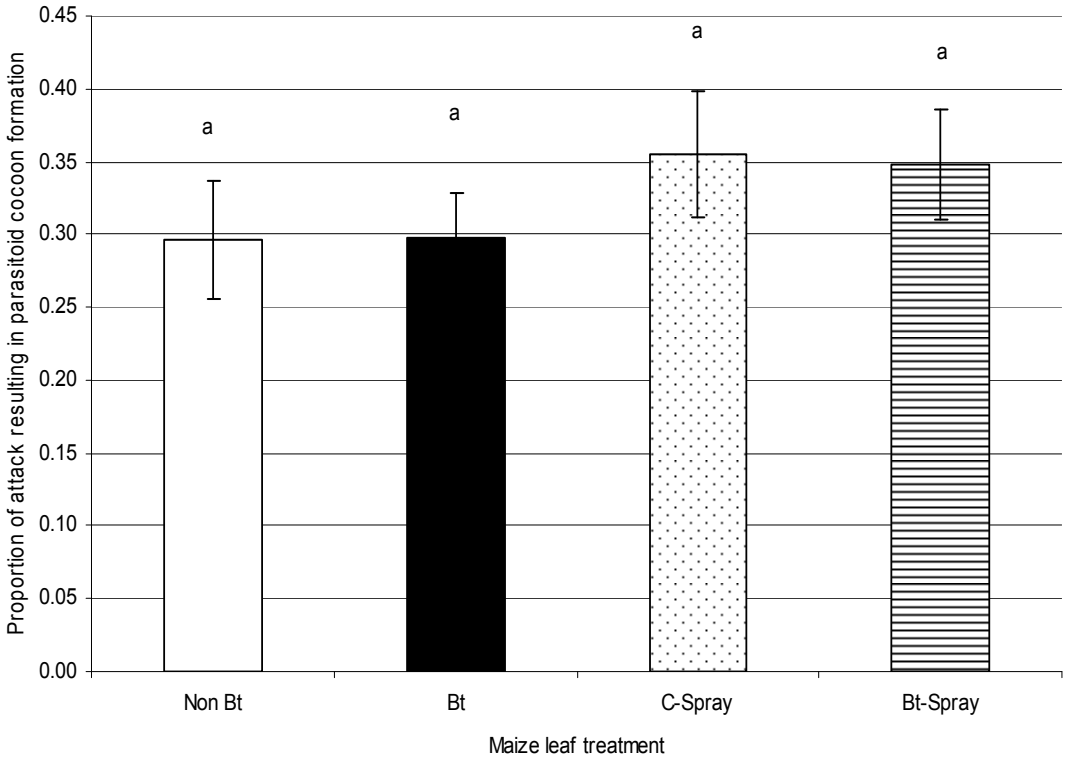


Figure 3.17. Mean of proportion of all attacks resulting in parasitoid cocoon formation. Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different (p=0.680).

Moreover, one-way ANOVA showed that there were also no significant differences in the proportion of all cocoons resulting in emerged parasitoid adult between these maize treatments (Fig.3.18, $F_{3,70} = 0.479$, $p = 0.698$).

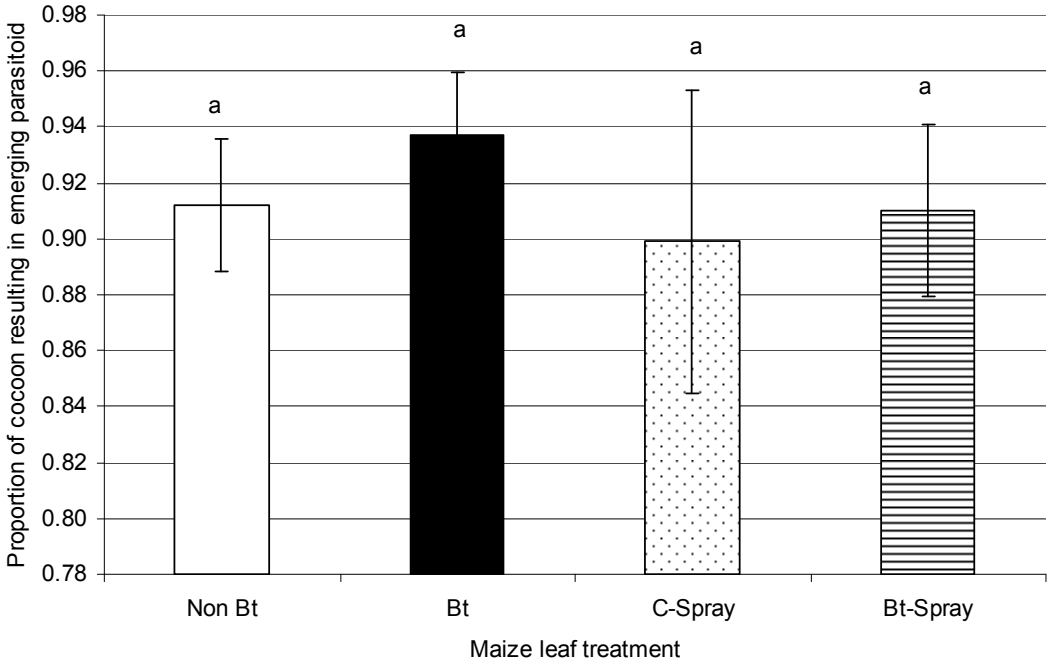


Figure 3.18. Mean of proportion of all cocoons resulting in parasitoid adult emergence. Columns with different letters represent treatment means that are significantly different. Treatments are not significantly different (p=0.698).

3.5.3 Parasitization of one individual same age *S. littoralis* larva by one mated female *C. marginiventris*

The same age larvae were chosen for this experiment. One mated female *C. marginiventris* and one *S. littoralis* host were used. Mean time to cocoon formation and adult emergence were recorded. The results are displayed in figure 3.19 and 3.20.

The results of the time to parasitoid cocoon formation are shown in Figure 3.19. A one-way ANOVA revealed that there were significant differences in the time to cocoon formation between these treatments ($F_{3,47} = 8.485$, $p < 0.001$). The non-transgenic group showed the shortest time to cocoon formation (16.53 ± 0.48 days) compared with the group maintained on Dipel spray (19.60 ± 0.68 days; $p = 0.004$), transgenic (19.25 ± 0.58 days; $p < 0.001$), and control spray maize (18.33 ± 0.16 days; $p = 0.019$), respectively. No significant differences were observed in the larvae maintained either on the transgenic and control spray ($p = 0.516$), or the control spray and the Dipel spray maize ($p = 0.485$).

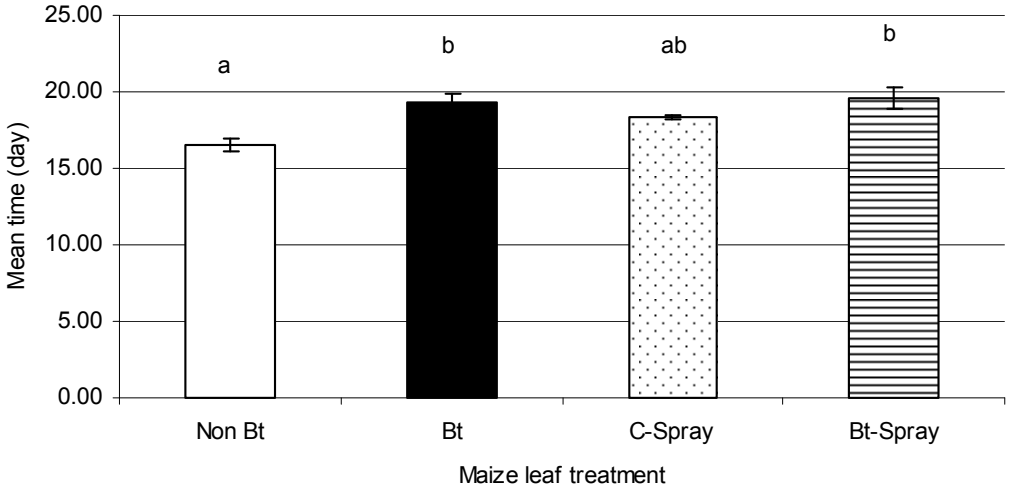


Figure 3.19. Mean of time to *C. marginiventris* cocoon formation. Columns with different letters represent treatment means that are significantly different at $p < 0.001$.

There were significant differences in the time to parasitoid emergence between these groups (Fig. 3.20, $F_{3,45} = 7.571$, $p < 0.001$). The result showed that the non-transgenic group took 22.89 days to emerge compared with 26.45 days in the transgenic group ($p < 0.001$). There was no significant difference in the mean time to the adult emergence between non-transgenic and control spray maize ($p = 0.320$) and Dipel spray maize ($p = 0.073$).

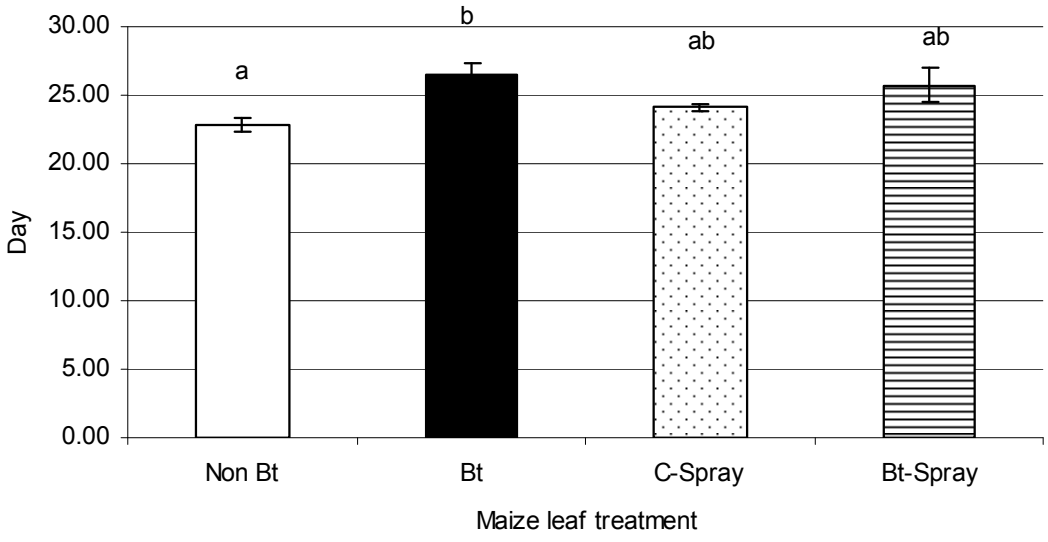


Figure 3.20. Mean of time to *C. marginiventris* adult emergence. Columns with different letters represent treatment means that are significantly different at $p < 0.001$.

3.6 Discussion

3.6.1 Parasitization on the same age host;

This study investigated the effect of Bt toxin including transgenic maize expressing Cry1Ab and Dipel spray containing Cry1Ab with regards to the parasitoid *C. marginiventris* in terms of the attack rate, parasitism and development. This was carried out using a non-choice test by offering the same age *S. littoralis* larvae (4 days old) to *C. marginiventris* mated females. The time taken to the first attack by the parasitoid was significantly longer in the hosts fed transgenic maize when compared to the hosts fed on non-transgenic maize. However, there were no significant differences in the times taken to the 15^{th} attack by the parasitoids among these maize treatments. On the non-transgenic treatment, parasitoids took a shorter time to attack hosts as well as a shorter time to pupation and time to adult emergence, when compared with other maize treatments. The number of attacks over 15 minutes in the transgenic treatment was 24% less than in the non-transgenic treatment. Thus, the parasitoid attacked only 25% of the host reared on Dipel spray maize compared to those reared on non-transgenic maize. The number of cocoons and number of emerged adults were highest in the non-transgenic treatment whereas the transgenic treatment resulted in the lowest number of cocoons and emerged adults. There was a significant difference in the proportion of all attacks resulting in cocoon formation. In the non-transgenic group, 35% of all attacks resulted in cocoon formation compared to 14% in the transgenic group. However, there was no significant difference in the proportion of all cocoons resulting in adult emergence in these treatments.

These results suggested that the parasitoids preferred to attack larvae fed on non-transgenic maize than larvae fed Bt maize. Parasitoids were significantly more successful developed within the host fed on non-transgenic and control spray leaves. This is probably linked to the fact that the same age *S. littoralis* hosts reared on transgenic maize developed slower and had a smaller size when

compared to hosts reared on non-transgenic maize. This supports the findings of Vojtech et al. (2005) that the parasitoid, *C. marginiventris*, had higher mortality, smaller cocoons and prolonged development when developing in 2-5-day-old 2nd instar *S. littoralis* larvae fed on transgenic maize. Hence the survival of *C. marginiventris* until cocoon formation was significantly higher when it had developed within hosts fed on non-transgenic maize than in hosts fed on transgenic maize (Vojtech et al., 2005). Schuler et al. (1999) showed that in the non-choice situation, the parasitoids oviposited into Bt-treated hosts, but parasitoid larvae failed to complete their development. Moreover, Meissle et al. (2004) also showed that development time of the parasitoid, *Camploletis sonorensis*, was significantly longer when developed within caterpillars fed on transgenic maize. In contrast to the study by Sanders et al. (2007), they showed that the development time of *C. sonorensis* was not affected by host maize-fed hybrid and there were no significant differences in the number of hosts attacked from the different maize treatments. Whereas the study by Couty et al. (2001a) showed that parasitoids which had developed within aphids fed on transgenic GNA-expressing potato plants had poor development and delayed emergence. According to the previous results (see chapter 2), the maize consumption was higher in the host fed on non-transgenic maize than other maize. This is in agreement with the study by Couty et al. (Couty et al., 2001b) that GNA aphids had lighter weight than control aphids sp. (Couty et al., 2001b). Host sizes and smaller quantities of resources may affect the growth and development of the parasitoid (Urrutia C et al., 2007). Nourishment and development of parasitoids is highly integrated with the biology of their hosts (Thompson et al., 2005). This could be interpreted to be an indirect effect of Cry1Ab toxin on the parasitoids via the poor quality hosts as hosts provided fewer nutrients for the parasitoid to develop and survive. The nutritional quality of the host available could influence the population dynamics and community structure of the parasitoids.

3.6.2 Parasitization on the same size host;

This was carried out using a non-choice test, offering the same-size *S. littoralis* larvae to *C. marginiventris* mated females. In order to eliminate the size differences of host, *S. littoralis* larvae used in this study were different in age; 4 days old in the non-transgenic and control spray maize-fed groups, and 5 days old in the transgenic and Dipel spray maize-fed groups. There were no significant differences in the times taken to the first attack by the parasitoid in these treatments. However, the time taken until 15th attack in the Dipel spray and transgenic treatments took longer than in the non-transgenic treatment. There were no significant differences in either the time to cocoon formation or the time to adult emergence. The highest numbers of attacks over 15 minutes were observed in the host reared on non-transgenic maize leaves. There were no significant differences in the number of cocoons and emerged adults in these treatments. The proportion of all attacks resulting in cocoon formation and the proportion of all cocoons resulting in adult emergence were not significantly different among these treatments. Couty et al. (2001b) showed that no significant differences in the percentage of overall parasitism success, mummies formed, and emergence success of *Aphelinus abdominalis* parasitoid were observed in the similar size aphid fed on GNA. This can be suggested that Bt toxin has an indirect effect on parasitoid fitness parameters.

Godfray (1994) pointed out that host age can be a determinant in host acceptance of parasitoids, and there is a correlation observed between the host age and attack rate. Moreover, Khafagi and Hegazi (2004) suggested that the development of parasitoids was affected by host age at the time of parasitism. They showed that *Microplitis rufiventris* preferred to oviposit the 3rd instar *S. littoralis* larvae. These indicated that older hosts have more effective defenses against parasitoid eggs or newly hatched larvae (Khafagi and Hegazi, 2004).

In conclusions, parasitoids were negatively affected when they had to choose the host fed Bt toxin. Dutton et al. (2005) showed that 3rd instar *S. littoralis* larvae reared on transgenic maize had significant lower weight when compared to larvae reared on non-transgenic maize. The low parasitism rate and non-emergence of parasitoids may be due to the decreased quality of hosts, which can not provide sufficient nutrients for the development of the parasitoid larvae (Vinson and Iwantsch, 1980a, Godfray, 1994). Walker et al. (2007) also suggested that poor survival of parasitoids had more affected by reduced host nutrition than by Bt toxins. As the mode of action of Bt toxins requires the specific receptors in midgut epithelium of host larvae for binding and forming pores in the gut (Swadener, 1994, Joung and Cote, 2000, Avisar et al., 2004), this makes the insect stops eating and gain less nutrients when compared to the larvae fed on non Bt toxin maize. Hemerik and Harvey (1999) found that in small, nutritionally insufficient hosts, parasitoid might consume the host before it has grown sufficiently to support parasitoid development, and both will die.

3.7 Suggestion for further experiments

According to these results, further optimization is required such as host quality in terms of nutritional content. Since host quality represents variation in the quality of nutrients for the developing parasitoid (Godfray, 1994), then host quality influences the fitness of parasitoids; (i) adult size, (ii) development time, and (iii) survival (Hemerik and Harvey, 1999, Dutton et al., 2002, Vojtech et al., 2005, Urrutia C et al., 2007). The successful development of the parasitoid depends on the host providing adequate nourishment to the developing immature parasitoid (Thompson, 1999). Female parasitoids should find the best quality hosts and ignore unhealthy hosts to maximize their fitness. From the literature, reduced weight and prolonged development time of *S littoralis* were investigated when the larvae fed on the transgenic maize (Dutton et al., 2002, Dutton et al., 2005, Vojtech et al., 2005). These effects lead to decreased cocoon weight and increased development time of *C.*

marginiventris developing within the host (Vojtech et al., 2005). This low host quality cannot provide enough nutrients for the developing parasitoid larvae.

The results in Chapter 2 showed that the parasitized *S. littoralis* larvae reared on Bt toxin plants had reduced weight when compared with non-Bt toxin plants. Moreover, these Bt toxin maize-fed larvae consumed more maize than the others but gave smaller parasitoid cocoons. This describes the nutrition in terms of energy content of the hosts and then the influential of the host quality has on the parasitoid fitness such as growth, survival and fecundity. It is suggested that a bomb calorimeter is used to measure energy content of maize leaf and maize-fed *S. littoralis* larvae (See appendix II).

Chapter 4

General Discussion

4.1 Summary of key findings

4.1.1 Effect of Cry1Ab toxin on second and third trophic levels (Chapter 2)

- When fed transgenic maize (MON810) or Dipel sprayed maize, *Spodoptera littoralis* larvae had significant lower weight compared to the non-transgenic and control spray maize.
- No significant effect of Cry1Ab toxin was observed on the survival and development time of *S. littoralis* larvae.
- *S. littoralis* larvae raised on transgenic maize had a higher relative growth rates than those raised on either maize over their juvenile life cycle. However, the larvae fed on transgenic maize consumed more than the control spray or Dipel spray maize.
- The lowest weight of parasitized *S. littoralis* larvae at 2 and 5 days after parasitism was found in the group exposed to transgenic maize (MON810).
- The change in weight of parasitized *S. littoralis* transgenic maize-fed larvae from day 2 to day 5 after parasitization was lowest among other maize-fed larvae.
- When *Cotesia marginiventris* developed within host fed transgenic maize expressing Cry1Ab, parasitoids showed the lowest survival.
- Weight of *C. marginiventris* cocoons when developed within the larvae fed with transgenic and Dipel spray maize were lighter when compared with the non-transgenic and control spray groups.

4.1.2 *Cotesia marginiventris* behaviour under no-choice *Spodoptera littoralis* host (Chapter 3)

4.1.2.1 Parasitization: same-age host

- Time taken to the first attack by *C. marginiventris* was significantly longer on hosts fed on transgenic maize when compared to other maize-fed hosts.
- The time taken to the 15th attack by the parasitoid did not differ for the four maize treatments.
- Development times of parasitoids such as time to pupate and time to adult emerged, were shorter in the non-transgenic maize group when compared to others.
- Numbers of parasitoid cocoons and adults emerged were affected by the host fed on transgenic maize.

4.1.2.2 Parasitization: same-size host

- No significant differences in the time taken to the first attack by the parasitoid were observed in these four maize treatments.
- The time taken to the 15th attack of the parasitoid from the Dipel spray and transgenic maize took significantly longer than the non-transgenic and control spray maize.
- No significant effect of Cry1Ab on the time to cocoon formation and the time to adult emergence were observed.
- Number of parasitoid cocoons and adults emerged did not differ on the four maize treatments.

The aim of this study was to investigate the potential effects of the *Bacillus thuringiensis* toxin (Cry1Ab) from both transgenic maize and microbial Bt spray on the non-target parasitoids, *Cotesia marginiventris*. The present study attempted to link the risk assessment of the Bt toxin (Cry1Ab) on the non-target insects and the ecology of the multi-trophic interactions. Experiments were separated into two sections; which are 1) ecological risk assessment of Cry1Ab on a tri-trophic system (Chapter 2), and 2) the effects of Cry1Ab on the ecology of the non-target parasitoid, *C. marginiventris* (Chapter 3). The first trophic level was either transgenic maize expressing Cry1Ab toxin (MON810) or the microbial Bt spray (Dipel) which contains Cry1Ab toxin. The second trophic level was *S. littoralis*, a non-target pest herbivore; and the third trophic level was *C. marginiventris*, one of the natural enemies of the Noctuid pests.

4.2 Relevance to risk assessment

Bt toxin used in insect pest management is an alternative practice from using hazardous synthetic chemical insecticides (Swadener, 1994, Chattopadhyay et al., 2004, Federici, 2005, Sharma et al., 2008). It has been widely used in the sprayable formulations and recently this toxin has been integrated into the plant cells (Schuler et al., 1998). There are concerns about widespread use of Bt and the effects it may have on non-target insects other than the insect pest targeted (Swadener, 1994) such as herbivores, natural enemies or pollinators (Romeis et al., 2006, Lovei, 2001, Dutton et al., 2005). Bt toxin negatively affected the non-target insects in many ways including prolonged development, high mortality and decreasing weight (Losey et al., 1999, Jesse and Obrycki, 2000, Hellmich et al., 2001, Dutton et al., 2003b, Dutton et al., 2005, Vojtech et al., 2005).

S. littoralis is partially susceptible to Cry1C and Cry1E toxins, and has been widely used as an organism to indicate the effects of Cry1Ab toxin (Hilbeck et

al., 1999, Dutton et al., 2002, Dutton et al., 2003a, Dutton et al., 2005, Meissle et al., 2005, Vojtech et al., 2005). In the present study, *S. littoralis* had been used in Cry1Ab risk assessment as the non-target herbivore or host for natural enemies – *C. marginiventris*. Also, continuous maize feeding provided the chronic exposure of Cry1Ab toxin to the herbivores, may reflect a more realistic demonstration of the effects of toxin on the natural enemies, since in the maize field the toxin is also present throughout the life of the herbivores and natural enemies (Andow and Hilbeck, 2004, Vojtech et al., 2005). As observed in the present study (Chapter 2), when neonate *S. littoralis* larvae were fed maize containing Cry1Ab toxin (transgenic maize and Dipel spray maize), it had a significant negative effect on their growth and development, such as reduced weight and prolonged development time. *S. littoralis* larvae fed on transgenic maize, as observed in this study, had a higher relative growth rates, however, these larvae consumed more than those raised on other maize. This could be the mode of delivery of the Cry1Ab toxin that the insects can not digest the plant leaf materials. The results confirm those of previous studies (see reviews - Bokonon-Ganta et al., 2003, Dutton et al., 2005, Vojtech et al., 2005, Sanders et al., 2007), which showed that Cry1Ab toxins expressed in the transgenic maize and in the microbial Bt spray (Dipel) had negative effects on the development of the insects at the second trophic level. It can be concluded that *S. littoralis* is directly affected by Bt toxin from transgenic maize (MON810). No significant effect of Cry1Ab toxin observed on the survival and development time of *S. littoralis* larvae. This is in contrast with other studies (see reviews - Dutton et al., 2002, Bokonon-Ganta et al., 2003, Dutton et al., 2003a, Dutton et al., 2005, Vojtech et al., 2005). The present study had shown several measurement endpoints such as growth and development could indicate the negative effects of Cry1Ab toxin on the second trophic level, the herbivores. Thus, this negative effect of the insect herbivores could obtain biologically relevant data when assessing the risk to the parasitoids.

Cry1Ab toxin negatively affected the parasitoid *C. marginiventris* in terms of survival and development. Parasitized *S. littoralis* larvae exposed to Bt toxin

from transgenic maize had the lowest weight and this reflects in the lighter weight of the parasitoid cocoons. The lowest survival of parasitoid was found when parasitoid developed within the host fed transgenic maize. A combination of several factors could explain the negative effects of Cry1Ab toxin observed on the survival and development of *C. marginiventris* when fed *S. littoralis* reared either transgenic or Bt spray maize such as the chronic exposing of Cry1Ab toxin to *S. littoralis* hosts made the host decreased in weight; and changes in the amino acid composition of the haemolymph of *S. littoralis* larvae. This first tier test laboratory study indicates that the observed negative effects on the parasitoid provided with Cry1Ab-fed *S. littoralis* was due to a reduction in the host quality and not to the direct toxic effect. The ecological risk assessment of Bt toxin (plant and spray), concluded that the long-term exposure of Bt toxin has a low risk the parasitoids.

4.3 Relevance to ecological theory

As the immature parasitoids develops within the hosts and feeds on the body tissues/fluids of the hosts, the successful development of parasitoid depends on the host nutrients (Godfray, 1994, Thompson, 1999). The quality of host influences three correlates of the parasitoid fitness: 1) adult size, 2) development time and 3) survival (Godfray, 1994, Hemerik and Harvey, 1999, Couty et al., 2001a, Dutton et al., 2002, Vojtech et al., 2005, Urrutia C et al., 2007). The size of host also has a major impact on parasitoid fitness as it provides the amount of food available for the developing parasitoid (Godfray, 1994).

Studies of the multi-trophic interactions have demonstrated the effects of Bt toxin on the ecology of the parasitoids used in this study (Chapter 3). As parasitoids complete their development on a single host individual (Godfray, 1994) and are likely to be adversely affected if their Bt susceptible hosts are treated with Bt toxin, this is referred to the host-quality mediated effects

(Vojtech et al., 2005, Walker et al., 2007). For example, Vojtech et al. (2005) stated that the negative effect of Bt maize on the parasitoid *C. marginiventris* was host-quality mediated when it developed inside susceptible *S. littoralis* larvae that fed on transgenic Bt corn. In the present study, when offered Bt or Bt spray maize-fed *S. littoralis* hosts, low parasitism rates and higher rates non-emerged *C. marginiventris* parasitoids were observed in the same-age host fed either on transgenic or Bt spray maize. *C. marginiventris* had significantly low survival rates and decreased cocoon weight when developed within a host reared on Bt or Bt spray maize. This has shown that the unhealthy host had an adverse effect on the growth and development of the immature parasitoids (Vinson and Iwantsch, 1980a, Godfray, 1994). Chen et al. (2008) indicated that the negative impact of transgenic broccoli expressing Cry1C toxin on the parasitoid *Diadegma insulae* was due to the poor host quality as the result of ingestion and susceptibility to Bt toxin, rather than direct toxicity to the parasitoid. A reduced host size when fed on maize containing Bt (GM maize or Bt spray), could imply an indirect effect of Bt toxin, as the sick host provides poor nutrient resources for the developing parasitoid and could lead to the changes in host-parasitoid population dynamics in the ecosystem. Thus it can be suggested that the host-quality mediated effects have adverse effect on the parasitoid fitness and the number of emerged parasitoid is a more important measure of success since the parasitoids may suppress the next generation of the pest. Host fed with Bt toxin provided poor nutrients to the parasitoids and this could be affected the parasitoid population over the next generation. Thus parasitoids are sensitive to changes in nutritional quality host by the Bt toxin which align with theory and confirm this is suitable model for developing ecological theories.

4.4 Integrating the risk assessment of transgenic plants and the ecological theory of the non-target insects

Risk assessment and ecological methods may reflect different philosophies of science (Raybould, 2007, Filser, 2008). Risk assessment acknowledges the importance of non-scientific criteria and this research provides data that can predict whether harm (Chapman, 2002) will result from the cultivation of transgenic plants (Raybould, 2007). These data are useful to decision-makers because they allow general predictions of impacts on environmental components of value. The ecological method is based on scientific criteria and tends to answer the questions about systems such as biodiversity, biotic interactions and ecosystem functions (Chapman, 2002) rather than testing hypotheses. The ecological data produced are little use in making a risk decision making (Raybould, 2007). In the present study an attempt is made to link these two approaches and this can help more readily assessable data on which decisions about risk can be based. The irrelevant data for decision-makings could be removed. However, the ecological method should meet several key criteria so that the results are sound and ecologically interpretable. These criteria combine the strengths of ecotoxicological methods with other criteria specific to transgenic crops (Andow and Hilbeck, 2004).

The present study has shown the direct effect of Cry1Ab toxin on the growth and development of the *S. littoralis* herbivores. This is reflecting in smaller host, which provided fewer nutrients available for the developing immature parasitoids. Cry1Ab toxin does not have a direct effect on the parasitoid but this demonstrated the host-mediated effects of Bt toxin on the parasitoids. In this study, integrating measurement endpoints such as host body weight and development time and ecological theory such as parasitism rate and fitness of the parasitoid can help to assess the possible impacts of the Cry1Ab toxin at the population level. In assessing potential Cry1Ab toxin effects on the non-target organisms, it is essential to consider components such as generational relative fitness. This is a particularly relevant experimental endpoint for risk

assessment tests of GM plants (Andow and Hilbeck, 2004), because adverse effects of transgenic plants on non-target species would occur through some component of relative fitness. For example, Lang and Vojtech (2006) studied the effect of Bt pollen on the swallowtail, *Papilio machaon* L. They observed that body weight of the adult female swallowtails which were fed Bt maize pollen as first instar larvae had a lower body weight. The body mass of newly eclosed butterflies is strongly correlated with fat body containing nutrients and energy reserves for female egg production, therefore, lower body weight of swallowtail butterflies is likely to be associated with lower reproductive fitness (Lang and Vojtech, 2006).

4.5 Further study

In the parasitoid, the provision of optimal nutrition is accomplished by a tri-trophic ecological interaction; the host or second trophic level, and the first trophic level, a plant (Thompson, 1999). The nutritional of the host could influence the population and community structure of the parasitoids (Thompson, 1999). This suggests that quality of hosts when fed on maize containing Bt (GM or Bt spray) could be the concern. The energy content (kilocalories) and sufficient nutrients such as proteins, carbohydrates and lipids, should be studied to provide a baseline data for host-parasitoid interaction in the ecosystem (Appendix II and unpublished study).

From the previous results of non-choice test of *C. marginiventris* parasitoid in Chapter 3, this suggests that the quality of the host affect the fitness of the parasitoid such as parasitism, survival rate and development time. Long-term study such as generational study of the parasitoid should be investigated as they can predict the changes in the population level. This experiment will be tested on parasitoid which developed in four different types of maize leaves. Then the parasitoid in the next generation will be used to parasitize the hosts fed on different maize leaves. This experiment is summarized in Figure 4.1.

Another suggestion of the study is the field study or the laboratory accomplished under similar to those in the field as possible i.e. pests should be reared on actual plants, then subjected to attack by the relevant parasitoid.

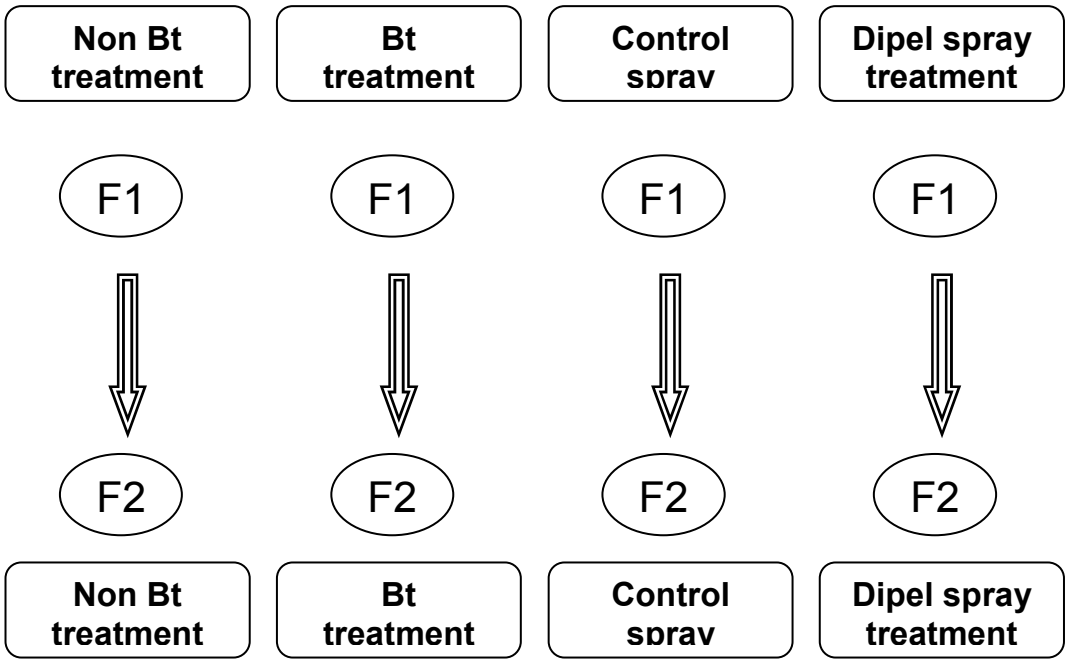


Figure 4.1. Cumulative exposure of Bt toxin on *C. marginiventris* progeny

Appendices

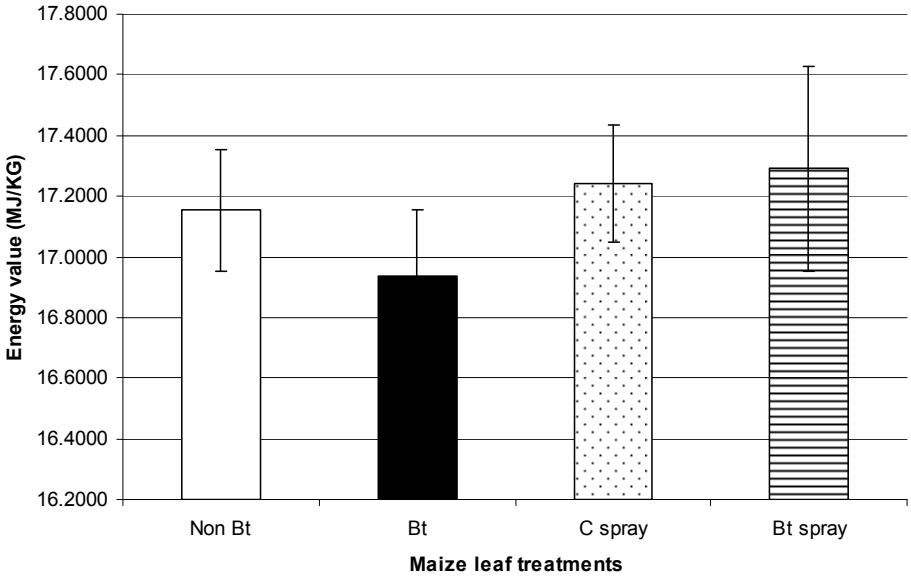
Appendix I : ELISA results

The mean concentrations of Cry1Ab in maize leaves are as followed;

	Non-transgenic maize	Transgenic maize	Control-spray maize	Dipel-spray maize
Cry1Ab concentration (μg/g)	NA	1.7 ± 0.21	NA	1.4 ± 0.32

Appendix II : Preliminary Result of Energy measurement:

To investigate the effect of the quality of hosts influenced on the parasitoid development and success when develop within hosts rear on Bt toxin maize leaves, a bomb calorimeter (PARR 1351, Parr Instrument Company, Moline, IL, USA) is used to measure energy content of maize leaf and maize-fed *S. littoralis* larvae. The results of the energy content in maize leaves are shown in this figure.



Energy content of maize leaf; Non-transgenic (Non Bt), transgenic (Bt), control spray (C spray) and Dipel spray (Bt spray) leaves.

Photo of a bomb calorimeter

Appendix III : Statistical tables

A repeated measures one-way ANOVA of the mean weight of *S. littoralis* larvae from day 6 until day 20;

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
factor1	Sphericity Assumed	.002	3	.001	6.732	.002	.490
	Greenhouse-Geisser	.002	1.760	.001	6.732	.012	.490
	Huynh-Feldt	.002	2.306	.001	6.732	.006	.490
	Lower-bound	.002	1.000	.002	6.732	.036	.490
Error(factor1)	Sphericity Assumed	.002	21	.000			
	Greenhouse-Geisser	.002	12.321	.000			
	Huynh-Feldt	.002	16.139	.000			
	Lower-bound	.002	7.000	.000			

Pairwise Comparisons

Measure: MEASURE_1

(I) factor1	(J) factor1	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	.021*	.006	.008	.007	.034
	3	-.001	.004	.900	-.011	.010
	4	.009*	.004	.043	.000	.018
2	1	-.021*	.006	.008	-.034	-.007
	3	-.021*	.008	.031	-.040	-.003
	4	-.011*	.004	.038	-.022	-.001
3	1	.001	.004	.900	-.010	.011
	2	.021*	.008	.031	.003	.040
	4	.010	.005	.104	-.003	.023
4	1	-.009*	.004	.043	-.018	.000
	2	.011*	.004	.038	.001	.022
	3	-.010	.005	.104	-.023	.003

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

One-way repeated measures ANOVA of the maize consumption of *S. littoralis* from day 6 until day 20;

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Day_Consumed	Sphericity Assumed	.009	3	.003	3.417	.036	.328
	Greenhouse-Geisser	.009	1.842	.005	3.417	.067	.328
	Huynh-Feldt	.009	2.470	.004	3.417	.048	.328
	Lower-bound	.009	1.000	.009	3.417	.107	.328
Error(Day_Consumed)	Sphericity Assumed	.019	21	.001			
	Greenhouse-Geisser	.019	12.895	.001			
	Huynh-Feldt	.019	17.287	.001			
	Lower-bound	.019	7.000	.003			

Pairwise Comparisons

Measure: MEASURE_1

(I) Day_Consumed	(J) Day_Consumed	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	.015	.013	.305	-.017	.047
	3	.041	.018	.055	-.001	.083
	4	.038*	.010	.007	.014	.062
2	1	-.015	.013	.305	-.047	.017
	3	.026	.021	.253	-.023	.076
	4	.023	.011	.085	-.004	.050
3	1	-.041	.018	.055	-.083	.001
	2	-.026	.021	.253	-.076	.023
	4	-.003	.013	.816	-.033	.027
4	1	-.038*	.010	.007	-.062	-.014
	2	-.023	.011	.085	-.050	.004
	3	.003	.013	.816	-.027	.033

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

One-way ANOVA of the relative growth rate (RG) of *S. littoralis* larvae ;

ANOVA

RG

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	62720.600	3	20906.867	12.554	.000
Within Groups	126564.5	76	1665.323		
Total	189285.1	79			

Multiple Comparisons

Dependent Variable: RG

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	-72.72448*	12.90474	.000	-106.6226	-38.8263
	C spray	-23.09478	12.90474	.286	-56.9929	10.8033
	Bt spray	-54.27821*	12.90474	.000	-88.1763	-20.3801
Bt	Non Bt	72.72448*	12.90474	.000	38.8263	106.6226
	C spray	49.62969*	12.90474	.001	15.7316	83.5278
	Bt spray	18.44627	12.90474	.485	-15.4519	52.3444
C spray	Non Bt	23.09478	12.90474	.286	-10.8033	56.9929
	Bt	-49.62969*	12.90474	.001	-83.5278	-15.7316
	Bt spray	-31.18343	12.90474	.083	-65.0816	2.7147
Bt spray	Non Bt	54.27821*	12.90474	.000	20.3801	88.1763
	Bt	-18.44627	12.90474	.485	-52.3444	15.4519
	C spray	31.18343	12.90474	.083	-2.7147	65.0816

*. The mean difference is significant at the .05 level.

One-way ANOVA of the relative food consumption (RC) of *S. littoralis* larvae;

ANOVA

RC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5025998	3	1675332.709	25.868	.000
Within Groups	4922191	76	64765.677		
Total	9948190	79			

Multiple Comparisons

Dependent Variable: RC

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	-614.71870*	80.47713	.000	-826.1158	-403.3217
	C spray	-20.59242	80.47713	.994	-231.9895	190.8046
	Bt spray	-111.52501	80.47713	.512	-322.9221	99.8720
Bt	Non Bt	614.71870*	80.47713	.000	403.3217	826.1158
	C spray	594.12628*	80.47713	.000	382.7292	805.5233
	Bt spray	503.19370*	80.47713	.000	291.7966	714.5907
C spray	Non Bt	20.59242	80.47713	.994	-190.8046	231.9895
	Bt	-594.12628*	80.47713	.000	-805.5233	-382.7292
	Bt spray	-90.93258	80.47713	.672	-302.3296	120.4645
Bt spray	Non Bt	111.52501	80.47713	.512	-99.8720	322.9221
	Bt	-503.19370*	80.47713	.000	-714.5907	-291.7966
	C spray	90.93258	80.47713	.672	-120.4645	302.3296

*. The mean difference is significant at the .05 level.

Two-way ANOVA (treatments and day of larval weighing with repeated measures of day of larval weighing) of mean weight of parasitized *S. littoralis* larvae at 2 and 5 days after parasitization;

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
DayofWeighing	Sphericity Assumed	.015	1	.015	30541.686	.000
	Greenhouse-Geisser	.015	1.000	.015	30541.686	.000
	Huynh-Feldt	.015	1.000	.015	30541.686	.000
	Lower-bound	.015	1.000	.015	30541.686	.000
DayofWeighing * Treatment	Sphericity Assumed	.001	3	.000	538.423	.000
	Greenhouse-Geisser	.001	3.000	.000	538.423	.000
	Huynh-Feldt	.001	3.000	.000	538.423	.000
	Lower-bound	.001	3.000	.000	538.423	.000
Error(DayofWeighing)	Sphericity Assumed	.000	397	4.88E-007		
	Greenhouse-Geisser	.000	397.000	4.88E-007		
	Huynh-Feldt	.000	397.000	4.88E-007		
	Lower-bound	.000	397.000	4.88E-007		

One-way ANOVA of changes in weight of parasitized *S. littoralis* from day 2 to day 5 after parasitization;

ANOVA

Diff_5D_2D

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	3	.001	538.423	.000
Within Groups	.000	397	.000		
Total	.002	400			

Multiple Comparisons

Dependent Variable: Diff_5D_2D

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	.00630233*	.00015885	.000	.0058925	.0067122
	C spray	.00138109*	.00013115	.000	.0010427	.0017195
	Bt spray	.00157155*	.00012970	.000	.0012369	.0019062
Bt	Non Bt	-.00630233*	.00015885	.000	-.0067122	-.0058925
	C spray	-.00492124*	.00016004	.000	-.0053341	-.0045083
	Bt spray	-.00473078*	.00015885	.000	-.0051406	-.0043209
C spray	Non Bt	-.00138109*	.00013115	.000	-.0017195	-.0010427
	Bt	.00492124*	.00016004	.000	.0045083	.0053341
	Bt spray	.00019047	.00013115	.468	-.0001479	.0005288
Bt spray	Non Bt	-.00157155*	.00012970	.000	-.0019062	-.0012369
	Bt	.00473078*	.00015885	.000	.0043209	.0051406
	C spray	-.00019047	.00013115	.468	-.0005288	.0001479

*. The mean difference is significant at the .05 level.

One-way ANOVA of mean weight of parasitoid cocoon;

ANOVA

Weight_Cocoon

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	221.316	.000
Within Groups	.000	289	.000		
Total	.000	292			

Multiple Comparisons

Dependent Variable: Weight_Cocoon

Tukey HSD

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	.00055175*	.00002906	.000	.0004767	.0006268
	Control Spray	-.00000002	.00002121	1.000	-.0000548	.0000548
	Bt Spray	.00035160*	.00002152	.000	.0002960	.0004072
Bt	Non Bt	-.00055175*	.00002906	.000	-.0006268	-.0004767
	Control Spray	-.00055177*	.00002809	.000	-.0006244	-.0004792
	Bt Spray	-.00020015*	.00002832	.000	-.0002733	-.0001270
Control Spray	Non Bt	.00000002	.00002121	1.000	-.0000548	.0000548
	Bt	.00055177*	.00002809	.000	.0004792	.0006244
	Bt Spray	.00035163*	.00002019	.000	.0002995	.0004038
Bt Spray	Non Bt	-.00035160*	.00002152	.000	-.0004072	-.0002960
	Bt	.00020015*	.00002832	.000	.0001270	.0002733
	Control Spray	-.00035163*	.00002019	.000	-.0004038	-.0002995

*. The mean difference is significant at the .05 level.

Parasitism in the same age hosts;

One-way ANOVA of mean of time taken to the first attack by *C. marginiventris*;

ANOVA

Time_1stAttack

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.684	3	5.895	3.143	.030
Within Groups	142.540	76	1.876		
Total	160.225	79			

Multiple Comparisons

Dependent Variable: Time_1stAttack

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	-1.23550*	.43307	.028	-2.3731	-.0979
	C Spray	-.80450	.43307	.255	-1.9421	.3331
	Bt Spray	-1.04100	.43307	.085	-2.1786	.0966
Bt	Non Bt	1.23550*	.43307	.028	.0979	2.3731
	C Spray	.43100	.43307	.753	-.7066	1.5686
	Bt Spray	.19450	.43307	.970	-.9431	1.3321
C Spray	Non Bt	.80450	.43307	.255	-.3331	1.9421
	Bt	-.43100	.43307	.753	-1.5686	.7066
	Bt Spray	-.23650	.43307	.947	-1.3741	.9011
Bt Spray	Non Bt	1.04100	.43307	.085	-.0966	2.1786
	Bt	-.19450	.43307	.970	-1.3321	.9431
	C Spray	.23650	.43307	.947	-.9011	1.3741

*. The mean difference is significant at the .05 level.

One-way ANOVA of mean of time taken to parasitoid cocoon formed;

ANOVA

Day_to_Cocoon

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	109.646	3	36.549	15.216	.000
Within Groups	170.541	71	2.402		
Total	280.187	74			

Multiple Comparisons

Dependent Variable: Day_to_Cocoon

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	-3.17222*	.50353	.000	-4.4970	-1.8475
	C Spray	-1.00000	.49010	.183	-2.2894	.2894
	Bt Spray	-2.24412*	.51126	.000	-3.5892	-.8990
Bt	Non Bt	3.17222*	.50353	.000	1.8475	4.4970
	C Spray	2.17222*	.50353	.000	.8475	3.4970
	Bt Spray	.92810	.52415	.296	-.4509	2.3071
C Spray	Non Bt	1.00000	.49010	.183	-.2894	2.2894
	Bt	-2.17222*	.50353	.000	-3.4970	-.8475
	Bt Spray	-1.24412	.51126	.080	-2.5892	.1010
Bt Spray	Non Bt	2.24412*	.51126	.000	.8990	3.5892
	Bt	-.92810	.52415	.296	-2.3071	.4509
	C Spray	1.24412	.51126	.080	-.1010	2.5892

*. The mean difference is significant at the .05 level.

One-way ANOVA of mean of time taken to parasitoid emergent;

ANOVA

Day_to_Adult_emerge

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	123.131	3	41.044	5.817	.001
Within Groups	486.842	69	7.056		
Total	609.973	72			

Multiple Comparisons

Dependent Variable: Day_to_Adult_emerge

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	-2.99708*	.87369	.006	-5.2973	-.6969
	C Spray	-.85263	.85096	.749	-3.0930	1.3877
	Bt Spray	-2.92763*	.90129	.009	-5.3005	-.5547
Bt	Non Bt	2.99708*	.87369	.006	.6969	5.2973
	C Spray	2.14444	.86300	.071	-.1276	4.4165
	Bt Spray	.06944	.91267	1.000	-2.3334	2.4723
C Spray	Non Bt	.85263	.85096	.749	-1.3877	3.0930
	Bt	-2.14444	.86300	.071	-4.4165	.1276
	Bt Spray	-2.07500	.89093	.101	-4.4206	.2706
Bt Spray	Non Bt	2.92763*	.90129	.009	.5547	5.3005
	Bt	-.06944	.91267	1.000	-2.4723	2.3334
	C Spray	2.07500	.89093	.101	-.2706	4.4206

*. The mean difference is significant at the .05 level.

One-way ANOVA of mean number of attack over 15 minutes by *C. marginiventris*;

ANOVA

NO_15thAttack

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	614.938	3	204.979	4.019	.010
Within Groups	3876.050	76	51.001		
Total	4490.988	79			

Multiple Comparisons

Dependent Variable: NO_15thAttack

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	6.55000*	2.25833	.025	.6178	12.4822
	C Spray	3.45000	2.25833	.426	-2.4822	9.3822
	Bt Spray	6.85000*	2.25833	.017	.9178	12.7822
Bt	Non Bt	-6.55000*	2.25833	.025	-12.4822	-.6178
	C Spray	-3.10000	2.25833	.520	-9.0322	2.8322
	Bt Spray	.30000	2.25833	.999	-5.6322	6.2322
C Spray	Non Bt	-3.45000	2.25833	.426	-9.3822	2.4822
	Bt	3.10000	2.25833	.520	-2.8322	9.0322
	Bt Spray	3.40000	2.25833	.439	-2.5322	9.3322
Bt Spray	Non Bt	-6.85000*	2.25833	.017	-12.7822	-.9178
	Bt	-.30000	2.25833	.999	-6.2322	5.6322
	C Spray	-3.40000	2.25833	.439	-9.3322	2.5322

*. The mean difference is significant at the .05 level.

One-way ANOVA of mean number of parasitoid cocoons;

ANOVA

Total_Cocoon

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	434.202	3	144.734	17.804	.000
Within Groups	577.185	71	8.129		
Total	1011.387	74			

Multiple Comparisons

Dependent Variable: Total_Cocoon

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	6.45263*	.91342	.000	4.0495	8.8558
	C Spray	3.40000*	.90163	.002	1.0279	5.7721
	Bt Spray	4.71250*	.95632	.000	2.1965	7.2285
Bt	Non Bt	-6.45263*	.91342	.000	-8.8558	-4.0495
	C Spray	-3.05263*	.91342	.007	-5.4558	-.6495
	Bt Spray	-1.74013	.96744	.283	-4.2854	.8051
C Spray	Non Bt	-3.40000*	.90163	.002	-5.7721	-1.0279
	Bt	3.05263*	.91342	.007	.6495	5.4558
	Bt Spray	1.31250	.95632	.521	-1.2035	3.8285
Bt Spray	Non Bt	-4.71250*	.95632	.000	-7.2285	-2.1965
	Bt	1.74013	.96744	.283	-.8051	4.2854
	C Spray	-1.31250	.95632	.521	-3.8285	1.2035

*. The mean difference is significant at the .05 level.

One-way ANOVA of mean number of parasitoid adult emergent;

ANOVA

Total_Emerg

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	299.078	3	99.693	19.378	.000
Within Groups	354.977	69	5.145		
Total	654.055	72			

Multiple Comparisons

Dependent Variable: Total_Emerg

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	5.22807*	.74604	.000	3.2639	7.1922
	C Spray	3.14474*	.72663	.000	1.2317	5.0578
	Bt Spray	4.58224*	.76961	.000	2.5560	6.6084
Bt	Non Bt	-5.22807*	.74604	.000	-7.1922	-3.2639
	C Spray	-2.08333*	.73691	.031	-4.0234	-.1432
	Bt Spray	-.64583	.77933	.841	-2.6976	1.4059
C Spray	Non Bt	-3.14474*	.72663	.000	-5.0578	-1.2317
	Bt	2.08333*	.73691	.031	.1432	4.0234
	Bt Spray	1.43750	.76077	.242	-.5654	3.4404
Bt Spray	Non Bt	-4.58224*	.76961	.000	-6.6084	-2.5560
	Bt	.64583	.77933	.841	-1.4059	2.6976
	C Spray	-1.43750	.76077	.242	-3.4404	.5654

*. The mean difference is significant at the .05 level.

One-way ANOVA of mean of proportion of all attacks resulting parasitoid cocoon formation;

ANOVA

Prop_All_Attack_cocoon_Formation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.394	3	.131	10.700	.000
Within Groups	.860	70	.012		
Total	1.254	73			

Multiple Comparisons

Dependent Variable: Prop_All_Attack_cocoon_Formation

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	.20372*	.03600	.000	.1090	.2985
	C Spray	.10400*	.03504	.021	.0118	.1962
	Bt Spray	.10338*	.03717	.034	.0056	.2012
Bt	Non Bt	-.20372*	.03600	.000	-.2985	-.1090
	C Spray	-.09972*	.03600	.035	-.1945	-.0050
	Bt Spray	-.10035*	.03807	.050	-.2006	-.0001
C Spray	Non Bt	-.10400*	.03504	.021	-.1962	-.0118
	Bt	.09972*	.03600	.035	.0050	.1945
	Bt Spray	-.00063	.03717	1.000	-.0984	.0972
Bt Spray	Non Bt	-.10338*	.03717	.034	-.2012	-.0056
	Bt	.10035*	.03807	.050	.0001	.2006
	C Spray	.00063	.03717	1.000	-.0972	.0984

*. The mean difference is significant at the .05 level.

Parasitism on the same size hosts;

One-way ANOVA of time taken to the 15th attack by *C. marginiventris*;

ANOVA

Time_15Attack

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	119.845	3	39.948	6.386	.001
Within Groups	475.400	76	6.255		
Total	595.245	79			

Multiple Comparisons

Dependent Variable: Time_15Attack

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	-2.82900*	.79090	.003	-4.9065	-.7515
	C Spray	-1.41850	.79090	.284	-3.4960	.6590
	Bt Spray	-3.04500*	.79090	.001	-5.1225	-.9675
Bt	Non Bt	2.82900*	.79090	.003	.7515	4.9065
	C Spray	1.41050	.79090	.289	-.6670	3.4880
	Bt Spray	-.21600	.79090	.993	-2.2935	1.8615
C Spray	Non Bt	1.41850	.79090	.284	-.6590	3.4960
	Bt	-1.41050	.79090	.289	-3.4880	.6670
	Bt Spray	-1.62650	.79090	.177	-3.7040	.4510
Bt Spray	Non Bt	3.04500*	.79090	.001	.9675	5.1225
	Bt	.21600	.79090	.993	-1.8615	2.2935
	C Spray	1.62650	.79090	.177	-.4510	3.7040

*. The mean difference is significant at the .05 level.

One-way ANOVA of mean number of attack over 15 minutes by *C. marginiventris*;

ANOVA

NO_15thAttack

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1116.838	3	372.279	9.054	.000
Within Groups	3125.050	76	41.119		
Total	4241.888	79			

Multiple Comparisons

Dependent Variable: NO_15thAttack

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non Bt	Bt	7.90000*	2.02778	.001	2.5734	13.2266
	C Spray	7.60000*	2.02778	.002	2.2734	12.9266
	Bt Spray	9.75000*	2.02778	.000	4.4234	15.0766
Bt	Non Bt	-7.90000*	2.02778	.001	-13.2266	-2.5734
	C Spray	-.30000	2.02778	.999	-5.6266	5.0266
	Bt Spray	1.85000	2.02778	.798	-3.4766	7.1766
C Spray	Non Bt	-7.60000*	2.02778	.002	-12.9266	-2.2734
	Bt	.30000	2.02778	.999	-5.0266	5.6266
	Bt Spray	2.15000	2.02778	.715	-3.1766	7.4766
Bt Spray	Non Bt	-9.75000*	2.02778	.000	-15.0766	-4.4234
	Bt	-1.85000	2.02778	.798	-7.1766	3.4766
	C Spray	-2.15000	2.02778	.715	-7.4766	3.1766

*. The mean difference is significant at the .05 level.

References

ANDOW, D. A. & HILBECK, A. (2004) Science-based risk assessment for nontarget effects of transgenic crops. *Bioscience,* 54, 637-649.

AVISAR, D., KELLER, M., GAZIT, E., PRUDOVSKY, E., SNEH, B. & ZILBERSTEIN, A. (2004) The role of Bacillus thuringiensis Cry1C and Cry1E separate structural domains in the interaction with Spodoptera littoralis gut epithelial cells. *Journal of Biological Chemistry,* 279, 15779-15786.

BEDE, J. C., MCNEIL, J. N. & TOBE, S. S. (2007) The role of neuropeptides in caterpillar nutritional ecology. *Peptides,* 28, 185-196.

BOKONON-GANTA, A. H., BERNAL, J. S., PIETRANTONIO, P. V. & SETAMOU, M. (2003) Survivorship and development of fall armyworm, Spodoptera frugiperda (J. E. Smith) (Lepidoptera : Noctuidae), on conventional and transgenic maize cultivars expressing Bacillus thuringiensis Cry9C and Cry1A(b) endotoxins. *International Journal of Pest Management,* 49, 169-175.

CHAPMAN, P. M. (2002) Integrating toxicology and ecology: putting the "eco" into ecotoxicology. *Marine Pollution Bulletin,* 44, 7-15.

CHATTOPADHYAY, A., BHATNAGAR, N. B. & BHATNAGAR, R. (2004) Bacterial insecticidal toxins. *Critical Reviews in Microbiology,* 30, 33-54.

CHEN, M., ZHAO, J.-Z., COLLINS, H. L., EARLE, E. D., CAO, J. & SHELTON, A. M. (2008) A Critical Assessment of the Effects of Bt Transgenic Plants on Parasitoids. *PLoS ONE,* 3, e2284.

COLLIER, T. R., MURDOCH, W. W. & NISBET, R. M. (1994) Egg Load and the Decision to Host-Feed in the Parasitoid, Aphytis-Melinus. *Journal of Animal Ecology,* 63, 299-306.

CONNER, A. J., GLARE, T. R. & NAP, J. P. (2003) The release of genetically modified crops into the environment - Part II. Overview of ecological risk assessment. *Plant Journal,* 33, 19-46.

COUTY, A., CLARK, S. J. & POPPY, G. M. (2001a) Are fecundity and longevity of female Aphelinus abdominalis affected by development in GNA-dosed Macrosiphum euphorbiae? *Physiological Entomology,* 26, 287-293.

COUTY, A., DE LA VINA, G., CLARK, S. J., KAISER, L., PHAM-DELEGUE, M. H. & POPPY, G. M. (2001b) Direct and indirect sublethal effects of Galanthus nivalis agglutinin (GNA) on the development of a potato-aphid parasitoid, Aphelinus abdominalis (Hymenoptera : Aphelinidae). *Journal of Insect Physiology,* 47, 553-561.

DALE, P. (2003) Genetically modified crops and risk assessment in the UK. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science,* 53, 19-21.

DE MAAGD, R. A., BRAVO, A. & CRICKMORE, N. (2001) How Bacillus thuringiensis has evolved specific toxins to colonize the insect world. *Trends in Genetics,* 17, 193-199.

DEML, R., MEISE, T. & DETTNER, K. (1999) Effects of Bacillus thuringiensis delta-endotoxins on food utilization, growth, and survival of selected phytophagous insects. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie,* 123, 55-64.

DUTTON, A., KLEIN, H., ROMEIS, J. & BIGLER, F. (2002) Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator Chrysoperla carnea. *Ecological Entomology,* 27, 441-447.

DUTTON, A., KLEIN, H., ROMEIS, J. & BIGLER, F. (2003a) Prey-mediated effects of Bacillus thuringiensis spray on the predator Chrysoperla carnea in maize. *Biological Control,* 26, 209-215.

DUTTON, A., ROMEIS, J. & BIGLER, F. (2003b) Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. *Biocontrol,* 48, 611-636.

DUTTON, A., ROMEIS, J. & BIGLER, F. (2005) Effects of Bt maize expressing Cry1Ab and Bt spray on Spodoptera littoralis. ***Entomologia Experimentalis Et Applicata,*** **114, 161-169.**

FEDERICI, B. A. (2005) Insecticidal bacteria: An overwhelming success for invertebrate pathology. ***Journal of Invertebrate Pathology,*** **89, 30-38.**

FILSER, J. (2008) Ecotoxicology and ecosystems: Relevance, restrictions, research needs. ***Basic and Applied Ecology,*** **9, 333-336.**

GATEHOUSE, A. M. R., FERRY, N. & RAEMAEKERS, R. J. M. (2002) The case of the monarch butterfly: a verdict is returned. ***Trends in Genetics,*** **18, 249-251.**

GODFRAY, H. C. J. (1994) ***Parasitoids. Behavioral and Evolutionary Ecology*****, Princeton University Press.**

HAILS, R. S. (2000) Genetically modified plants - the debate continues. ***Trends in Ecology & Evolution,*** **15, 14-18.**

HARVEY, J. A., HARVEY, I. F. & THOMPSON, D. J. (1994) Flexible Larval Growth Allows Use of a Range of Host Sizes by a Parasitoid Wasp. ***Ecology,*** **75, 1420-1428.**

HELLMICH, R. L., SIEGFRIED, B. D., SEARS, M. K., STANLEY-HORN, D. E., DANIELS, M. J., MATTILA, H. R., SPENCER, T., BIDNE, K. G. & LEWIS, L. C. (2001) Monarch larvae sensitivity to Bacillus thuringiensis- purified proteins and pollen. ***PNAS*****, 211297698.**

HEMERIK, L. & HARVEY, J. A. (1999) Flexible larval development and the timing of destructive feeding by a solitary endoparasitoid: an optimal foraging problem in evolutionary perspective. ***Ecological Entomology,*** **24, 308-315.**

HEMERIK, L. & VAN DER HOEVEN, N. (2003) Egg distributions of solitary parasitoids revisited. ***Entomologia Experimentalis Et Applicata,*** **107, 81-86.**

HILBECK, A., BAUMGARTNER, M., FRIED, P. M. & BIGLER, F. (1998a) Effects of transgenic Bacillus thuringiensis corn-fed prey on

mortality and development time of immature Chrysoperla carnea (Neuroptera : Chrysopidae). ***Environmental Entomology,*** **27, 480-487.**

HILBECK, A., ECKEL, C. & KENNEDY, G. G. (1998b) Impact of Bacillus thuringiensis - insecticides on population dynamics and egg predation of the Colorado potato beetle in North Carolina potato plantings. ***Biocontrol,*** **43, 65-75.**

HILBECK, A., MOAR, W. J., PUSZTAI-CAREY, M., FILIPPINI, A. & BIGLER, F. (1998c) Toxicity of Bacillus thuringiensis Cry1Ab toxin to the predator Chrysoperla carnea (Neuroptera : Chrysopidae). ***Environmental Entomology,*** **27, 1255-1263.**

HILBECK, A., MOAR, W. J., PUSZTAI-CAREY, M., FILIPPINI, A. & BIGLER, F. (1999) Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator Chrysoperla carnea. ***Entomologia Experimentalis Et Applicata,*** **91, 305-316.**

HOFTE, H. & WHITELEY, H. R. (1989) Insecticidal Crystal Proteins of Bacillus-Thuringiensis. ***Microbiological Reviews,*** **53, 242-255.**

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

JOUNG, K. & COTE, C.-J. (2000) A review of the environmental impacts of the microbial insecticide *Bacillus thuringiensis*. *Technical Bulletin,* 29, 1-16.

KELLER, M., SNEH, B., STRIZHOV, N., PRUDOVSKY, E., REGEV, A., KONCZ, C., SCHELL, J. & ZILBERSTEIN, A. (1996) Digestion of delta-endotoxin by gut proteases may explain reduced sensitivity of advanced instar larvae of Spodoptera littoralis to CryIC. ***Insect Biochemistry and Molecular Biology,*** **26, 365-373.**

KHAFAGI, W. E. & HEGAZI, E. M. (2004) Is the host or the parasitoid in control?: effects of host age and temperature on pseudoparasitization by Microplitis rufiventris in Spodoptera littoralis. ***Journal of Insect Physiology,*** **50, 577-584.**

KING, B. H. (1998) Host age response in the parasitoid wasp Spalangia cameroni (Hymenoptera : Pteromalidae). ***Journal of Insect Behavior,*** **11, 103-117.**

KING, B. H. & NAPOLEON, M. E. (2006) Using effects of parasitoid size on fitness to test a host quality model assumption with the parasitoid wasp Spalangia endius. ***Canadian Journal of Zoology-Revue Canadienne De Zoologie,*** **84, 1678-1682.**

LANG, A. & VOJTECH, E. (2006) The effects of pollen consumption of transgenic Bt maize on the common swallowtail, Papilio machaon L. (Lepidoptera, Papilionidae). ***Basic and Applied Ecology,*** **7, 296-306.**

LIU, X. X., ZHANG, Q. W., ZHAO, J. Z., LI, H. C., XU, B. L. & MA, X. M. (2005) Effects of Bt transgenic cotton lines on the cotton bollworm parasitoid Microplitis mediator in the laboratory. ***Biological Control,*** **35, 134-141.**

LOSEY, J. E., RAYOR, L. S. & CARTER, M. E. (1999) Transgenic pollen harms monarch larvae. ***Nature,*** **399, 214-214.**

LOVEI, G. L. (2001) Ecological Risks and Benefits of Transgenic Plants. ***New Zealand Plant Protection,*** **54, 93-100.**

LOVEI, G. L. & ARPAIA, S. (2005) The impact of transgenic plants on natural enemies: a critical review of laboratory studies. ***Entomologia Experimentalis Et Applicata,*** **114, 1-14.**

MAGG, T., MELCHINGER, A. E., KLEIN, D. & BOHN, M. (2001) Comparison of Bt maize hybrids with their non-transgenic counterparts and commercial varieties for resistance to European corn borer and for agronomic traits. ***Plant Breeding,*** **120, 397-403.**

MANACHINI, B. & LOZZIA, G. C. (2004) Studies on the effects of Bt corn expressing Cry1Ab on two parasitoids of *Ostrinia nubilalis* Hb. (Lepidoptera: Crambidae). ***GMOs in Integrated Production. IOBC wprs Bulletin,*** **27, 109-116.**

MARTINEZ, C., PORCAR, M., LOPEZ, A., DE ESCUDERO, I. R., PEREZ-LLARENA, F. J. & CABALLERO, P. (2004) Characterization of a Bacillus thuringiensis strain with a broad

spectrum of activity against lepidopteran insects. *Entomologia Experimentalis Et Applicata,* 111, 71-77.

MEISSLE, M., VOJTECH, E. & POPPY, G. M. (2004) Implications for the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) when developing in Bt maize-fed *Spodoptera littoralis* larvae (Lepidoptera: Noctuidae). *GMOs in Integrated Production. IOBC wprs Bulletin,* 27, 117-123.

MEISSLE, M., VOJTECH, E. & POPPY, G. M. (2005) Effects of Bt maize-fed prey on the generalist predator Poecilus cupretis L. (Coleoptera : Carabidae). *Transgenic Research,* 14, 123-132.

MORALES, J., MEDINA, P. & VINUELA, E. (2007) The influence of two endoparasitic wasps, Hyposoter didymator and Chelonus inanitus, on the growth and food consumption of their host larva Spodoptera littoralis. *Biocontrol,* 52, 145-160.

MORSE, S., BENNETT, R. M. & ISMAEL, Y. (2005) Genetically modified insect resistance in cotton: some farm level economic impacts in India. *Crop Protection,* 24, 433-440.

POPPY, G. (2000) GM crops: environmental risks and non-target effects. *Trends in Plant Science,* 5, 4-6.

POPPY, G. M. (2004) Geneflow from GM plants - towards a more quantitative risk assessment. *Trends in Biotechnology,* 22, 436-438.

POPPY, G. M. & SUTHERLAND, J. P. (2004) Can biological control benefit from genetically-modified crops? Tritrophic interactions on insect-resistant transgenic plants. *Physiological Entomology,* 29, 257-268.

POPPY, G. M. & WILKINSON, J. M. (Eds.) (2005) *Gene flow from GM plants*, Blackwell Publishing Ltd.

PRUTZ, G., BRINK, A. & DETTNER, K. (2004) Transgenic insect-resistant corn affects the fourth trophic level: effects of Bacillus thuringiensis-corn on the facultative hyperparasitoid Tetrastichus howardi. *Naturwissenschaften,* 91, 451-454.

RAYBOULD, A. (2007) Ecological versus ecotoxicological methods for assessing the environmental risks of transgenic crops. *Plant Science,* 173, 589-602.

RAYBOUND, A. F. (Ed.) (2004) *Aspects of Applied Biology*, The Association of Applied Biologists.

REGEV, A., KELLER, M., STRIZHOV, N., SNEH, B., PRUDOVSKY, E., CHET, I., GINZBERG, I., KONCZKALMAN, Z., KONCZ, C., SCHELL, J. & ZILBERSTEIN, A. (1996) Synergistic activity of a Bacillus thuringiensis delta-endotoxin and a bacterial endochitinase against Spodoptera littoralis larvae. *Applied and Environmental Microbiology,* 62, 3581-3586.

RIDDICK, E. W. (2001) Effect of cold storage on emergence, longevity, fertility, and survival of Cotesia marginiventris (Hymenoptera : Braconidae). *Journal of Entomological Science,* 36, 366-379.

RIDDICK, E. W. (2006) Egg load and body size of lab-cultured Cotesia marginiventris. *Biocontrol,* 51, 603-610.

ROMEIS, J., MEISSLE, M. & BIGLER, F. (2006) Transgenic crops expressing Bacillus thuringiensis toxins and biological control. *Nature Biotechnology,* 24, 63-71.

RUBERSON, J. R., HERZOG, G. A., LAMBERT, W. R. & LEWIS, W. J. (1994) Management of the Beet Armyworm (Lepidoptera, Noctuidae) in Cotton - Role of Natural Enemies. *Florida Entomologist,* 77, 440-453.

SAGARRA, L. A., VINCENT, C. & STEWART, R. K. (2001) Body size as an indicator of parasitoid quality in male and female Anagyrus kamali (Hymenoptera : Encyrtidae). *Bulletin of Entomological Research,* 91, 363-367.

SALAMA, H. S., ZAKI, F. N., SALEM, S. A. & SHAMS EL-DIN, A. (1990) Comparative effectiveness of *Bacillus thuringiensis* and lannate against *Spodoptera littoralis*. *Journal of Islamic Academy of Sciences,* 3, 325-329.

SANDERS, C. J., PELL, J. K., POPPY, G. M., RAYBOULD, A., GARCIA-ALONSO, M. & SCHULER, T. H. (2007) Host-plant mediated effects of transgenic maize on the insect parasitoid Campoletis sonorensis (Hymenoptera : Ichneumonidae). ***Biological Control,*** **40, 362-369.**

SCHNEPF, E., CRICKMORE, N., VAN RIE, J., LERECLUS, D., BAUM, J., FEITELSON, J., ZEIGLER, D. R. & DEAN, D. H. (1998) Bacillus thuringiensis and its pesticidal crystal proteins. ***Microbiology and Molecular Biology Reviews,*** **62, 775-806.**

SCHULER, T. H., POPPY, G. M., KERRY, B. R. & DENHOLM, I. (1998) Insect-resistant transgenic plants. ***Trends in Biotechnology,*** **16, 168-175.**

SCHULER, T. H., POTTING, R. P. J., DENHOLM, I. & POPPY, G. M. (1999) Parasitoid behaviour and Bt plants (vol 400, pg 825, 1999). ***Nature,*** **402, 750-750.**

SHARMA, H. C., DHILLON, M. K. & ARORA, R. (2008) Effects of Bacillus thuringiensis delta-endotoxin-fed Helicoverpa armigera on the survival and development of the parasitoid Campoletis chlorideae. ***Entomologia Experimentalis Et Applicata,*** **126, 1-8.**

SNEH, B., SCHUSTER, S. & BROZA, M. (1981) Insecticidal Activity of Bacillus-Thuringiensis Strains against the Egyptian Cotton Leaf Worm Spodoptera-Littoralis [Lep, Noctuidae]. ***Entomophaga,*** **26, 179-190.**

SWADENER, C. (1994) Bacillus thuringiensis (B.T.). ***Journal of Pesticide Reform,*** **14, 13-20.**

THOMPSON, S. N. (1999) Nutrition and culture of entomophagous insects. ***Annual Review of Entomology,*** **44, 561-592.**

THOMPSON, S. N., REDAK, R. A. & WANG, L. W. (2005) Host nutrition determines blood nutrient composition and mediates parasite developmental success: Manduca sexta L. parasitized by Cotesia congregata (Say). ***Journal of Experimental Biology,*** **208, 625-635.**

URRUTIA C, M. A., WADE, M. R., PHILLIPS, C. B. & WRATTEN, S. D. (2007) Influence of host diet on parasitoid fitness: unravelling the complexity of a temperate pastoral agroecosystem.

USEPA (1992) Framework for Ecological Risk Assessment. ***Risk Assessment Forum,*** **EPA/630/R-92/001.**

VINSON, S. B. & IWANTSCH, G. F. (1980a) Host Regulation by Insect Parasitoids. ***Quarterly Review of Biology,*** **55, 143-165.**

VINSON, S. B. & IWANTSCH, G. F. (1980b) Host Suitability for Insect Parasitoids. ***Annual Review of Entomology,*** **25, 397-419.**

VOJTECH, E., MEISSLE, M. & POPPY, G. M. (2005) Effects of Bt maize on the herbivore Spodoptera littoralis (Lepidoptera : Noctuidae) and the parasitoid Cotesta marginiventris (Hymenoptera : Braconidae). ***Transgenic Research,*** **14, 133-144.**

WALKER, G. P., CAMERON, P. J., MACDONALD, F. M., MADHUSUDHAN, V. V. & WALLACE, A. R. (2007) Impacts of Bacillus thuringiensis toxins on parasitoids (Hymenoptera : Braconidae) of Spodoptera litura and Helicoverpa armigera (Lepidoptera : Noctuidae). ***Biological Control,*** **40, 142-151.**

WHALON, M. E. & WINGERD, B. A. (2003) Bt: Mode of action and use. ***Archives of Insect Biochemistry and Physiology,*** **54, 200-211.**

WILKINSON, M. J., SWEET, J. & POPPY, G. M. (2003) Risk assessment of GM plants: avoiding gridlock? ***Trends in Plant Science,*** **8, 208-212.**

ZANGERL, A. R., MCKENNA, D., WRAIGHT, C. L., CARROLL, M., FICARELLO, P., WARNER, R. & BERENBAUM, M. R. (2001) Effects of exposure to event 176 Bacillus thuringiensis corn pollen on monarch and black swallowtail caterpillars under field conditions. ***Proceedings of the National Academy of Sciences of the United States of America,*** **98, 11908-11912.**

ZHANG, J. H., WANG, C. Z., QIN, J. D. & GUO, S. D. (2004) Feeding behaviour of Helicoverpa armigera larvae on insect-resistant transgenic cotton and non-transgenic cotton. ***Journal of Applied Entomology,*** **128, 218-225.**