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# Transgenic Pesticidal Crops and the Environment: The Case of Bt Maize and Natural Enemies

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## 1. Introduction

Stem borers are the most destructive field insect pests of maize (see Plate 1) in sub-Saharan Africa (SSA) (Insect Resistant Maize for Africa [IRMA], 2001; Songa et al., 2001). Important stem borer species include *Busseola fusca* Fuller (Lepidoptera: Noctuidae), *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) (Overholt et al., 1994).

Plate 1. *Sesamia calamistis* larvae feeding on a maize leaf

Stem borer control approaches that have been used (with varied degrees of success) fall into four broad categories: chemical (application of insecticides); cultural (use of a range of farm practices to delay or reduce insect attack); biological (use of natural enemies of stem borers); and host plant resistance (the plant offers its own resistance to insects). The use of Bt maize (genetically modified maize expressing a δ-endotoxin from *Bacillus thuringiensis* and therefore having an in-built ability to produce pesticidal toxins) has been found to be effective in the management of stem borers in other parts of the world (Sharma & Rodomiro, 2000). However, this strategy has not been widely employed in Africa despite recent efforts to develop Bt maize suitable for different agro-ecological zones in the region (Muhammad & Underwood, 2004). Also, there is still significant debate regarding the possible risks posed by this technology (Obonyo et al., 2010). Fears that have been raised include; food safety and human health concerns, environmental concerns, possible impact on agricultural systems, and socio-economic issues. Regulatory decisions on whether or not to adopt genetically modified (GM) crops should therefore take all these concerns into consideration.

Because the Bt toxin is embodied in the plant itself, Bt crops are regulated as pesticides in some jurisdictions. For example, the US Environmental Protection Agency (EPA) has for a long time regulated Bt crops under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (Frisvold & Reeves, 2010). The Food and Agriculture Organization (FAO) defines a pesticide as "any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies". The term includes substances intended for use as plant growth regulators, defoliants, desiccants, agents for thinning fruit or preventing the premature fall of fruit, or any substances applied to crops either before or after harvest to protect crop produce from deterioration during storage and transport (Food and Agriculture Organization of the United Nations [FAO], 2002).

Used within the context of Integrated Pest Management (IPM), Bt crops offer a number of advantages. They are safe and easy to use, requiring only planting seeds of an adapted, resistant cultivar (Kennedy, 2008). In general, resistant cultivars have been compatible with other IPM tactics, including cultural, biological and chemical controls (Smith, 2005, as cited in Kennedy, 2008). However, it is well established that plant-borne factors that affect herbivores also interact with natural enemies and consequently with the biological control function they provide. Natural enemies such as predators and parasitoids fulfil an important ecological and economic function by helping to keep stem borer populations below the economic injury level and thus contributing to sustainable IPM systems (Romeis et al., 2008a). Most IPM systems aim to enhance biological control through conservation of existing natural enemies (Bale et. al., 2008 as cited in Romeis et al., 2008a). Thus it is important to minimize the non-target effects of other components of IPM such as pesticides or habitat manipulation (Romeis et al., 2008a).

Transgenic insecticidal plants can have impacts on natural enemies (Kennedy & Gould, 2007, as cited in Romeis et al., 2008a); this may stem from changes in either the plant structure, or primary/secondary metabolites. Adverse effects may occur, for example, if the natural enemy is exposed to, and is susceptible to the plant-borne insecticidal factor. These factors can cause population level effects which might lead to changes in the level of biological control that natural enemies provide (Kennedy & Gould, 2007, as cited in Romeis et al., 2008a). This chapter reviews published literature on impacts of Bt maize on stem borer natural enemies, with particular attention to stem borer parasitoids. This is aimed at consolidating information pertaining to the potential impacts of Bt maize on the development and behaviour of maize stem borers and their natural enemies, with special emphasis on stem borer parasitoids such as the larval parasitoids *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) and *Xanthopimpla stemmmator* Thunberg (Hymenoptera: Ichneumonidae). However, this is not an environmental risk assessment of Bt maize but an analysis of the possible impacts of Bt maize on one category of beneficial organisms in the ecosystem.

## 2. Bt maize

The Bt maize plant has a built-in system that consistently delivers the Bt toxins to the target pest throughout the growing season. Bt maize has been used to control a common maize stem borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), in the northern temperate region (Matilde et al., 2006). Indeed, Bt maize has been commercially grown in the U.S.A since 1995 and the area under its cultivation is increasing (Minorsky, 2001; Sakiko, 2006). In Africa, Bt maize is commercially grown only in South Africa, though its cultivation is expected to spread to other countries of SSA (James, 2001).

*Bacillus thuringiensis* toxins expressed in current commercially available Bt maize hybrids are selective in their mode of action (Swadener, 1994). Therefore some authors (e.g., Schuler et al., 1999a) claim that the effects of Bt maize on non-target arthropods associated with maize should be minimal. However, it was Bt maize that was involved in significant controversy (the "monarch butterfly controversy"), following the publication, in *Nature,* of a preliminary study by Losey et al. (1999) (Minorsky 2001). Indeed, Losey et al. (1999) raised serious concerns about the ecological safety of Bt maize cultivation to non-target lepidopterans, in particular the larvae of the monarch butterfly, *Danaus plexipus* L. (Lepidoptera: Danainae). On the basis of laboratory assays, the authors concluded that monarch larvae reared on milkweed (*Asclepias syriaca*) leaves dusted with pollen from Bt maize ate less, grew more slowly, and suffered higher mortality than those reared on leaves dusted with non-transformed maize or on leaves without pollen. The conclusions of Losey et al. (1999) were challenged by other scientists on three grounds. First, the pollen doses used by Losey et al. (1999) were not quantitatively measured but were gauged by eye to match pollen dustings on milkweed leaves collected in the field. This raised concerns about subconscious biases on the part of the researchers. Secondly, concerns were raised as to the validity of extrapolating from the results of Losey at al. (1999), which considered only one type of pollen, to all types of Bt maize pollen. Lastly, the soundness of extrapolating from laboratory assays to the field was uncertain, although a subsequent field study by Jesse & Obrycki (2000) did seem to confirm the fears raised by the Losey et al. (1999) study (Minorsky, 2001). However, it should be emphasised that the continuous expression of the Bt toxins in the plant tissues throughout the growing period (Baumgarte & Tebbe, 2005) increases the chances and degree of exposure to non-target insects of ecological and economic importance. Hence, there are concerns about the possible adverse impacts of this novel pest control technology on the higher trophic level non-target arthropods (such as pollinators, pollen feeders and natural enemies of pests) through crop plant-based food chains.

### 2.1 Bacillus thuringiensis and genetic engineering technology

*Bacillus thuringiensis* is a soil-dwelling bacterium that produces large amounts of insecticidal δ-endotoxin when it sporulates into a resting stage. This bacterium, abundantly found in grain dust from silos and other grain storage facilities, was discovered in Japan in 1901 by Ishawata (Baum et al., 1999). Bt is related to two other important spore-forming bacilli, *B. cereus* and *B. anthracis* and is differentiated largely on the basis of containing several plasmid-encoded protoxin genes (Aronson & Shai, 2001). There are hundreds of Bt subspecies and most produce, primarily during sporulation, one or more parasporal inclusions each comprised of either one or several related insecticidal protoxins, the so-called δ-endotoxins (Schnepf et al., 1998). These endotoxins are biologically inactive protein toxins that crystallize into characteristic shapes. In bacteria, the endotoxins are mixtures of several specific crystalline protein toxins (hence referred to as Cry proteins, Ostlie et al., 1997) that are divided into several numbered classes; these are in turn subdivided into subclasses (Andow & Hilbeck, 2004). The mode of action of *B. thuringiensis* toxins (each of which is active on a subset of insect larvae from at least three orders of insects - Coleoptera, Lepidoptera, and Diptera, Gould and Keeton, 1996) involves ingestion followed by crystal solubilisation and proteolytic activation of protoxin in the insect midgut. Activated toxin binds to receptors in the midgut epithelial membrane and inserts into the membrane, leading to cell lysis and death of the insect (Schnepf et al., 1998). Because of unique but overlapping specificity profiles, Bt subspecies are generally effective against a broad range of insects, usually within a particular order of insects (Aronson & Shai, 2001). Also, many produce, during growth, less well characterized insecticidal proteins, the so-called vegetative insecticidal proteins (Estruch et al., 1996) as well as other pathogenic factors (Agaisee et al., 1999).

Researchers have isolated the δ-endotoxin gene from different strains of Bt, and have expressed it in several crops in order to control lepidopteran and coleopteran pests (Groot & Dicke, 2002). Several of the isolated proteins have selective insecticidal properties against specific insect species (Andow & Hutchison, 1998). Therefore not all commercial Bt maize hybrids express the same insecticidal protein. Moreover, Bt maize plants may not express the protein uniformly throughout the plant, nor continuously throughout the crop season. Bt maize hybrids containing and expressing one of four proteins *Cry1Ab*, *Cry1Ac* or *Cry9C*, and *Cry1F* have been developed and made available since 1996. Cry genes from *B. thuringiensis* are randomly inserted into plant chromosomes at different insertion sites via microprojectile bombardment using a particle gun technique (Bohorova et al., 1999). A promoter, a DNA sequence that regulates where, when, and to what degree an associated Cry gene is expressed (Ostlie et al., 1997), is attached to a Cry gene before it is inserted into a maize chromosome. A successful insertion of the new genetic package containing the modified Bt gene into a plant is called a transformation event (Rice & Pilcher, 1998). Different transformation events (in maize) provide varying levels of resistance to insect pest targets (Williams et al., 1997).

### 2.2 Plant-Insect tritrophic systems and Bt crops

Natural enemies have an important role to play in the co-evolution of plants and insects (Romeis et al., 2008a). “The third trophic level must be considered part of a plant’s battery of defences against herbivores” (Price et al., 1980 as cited in Romeis et al., 2008a). Plant protection by natural enemies is well documented and has been manipulated in the development of biological control strategies in many crops (Dicke & Sabelis, 1988; Whitman, 1994). Plants are well placed to influence the efficiency of parasitism and predation and they mediate numerous interactions between entomophagous arthropods and herbivores. Their structures and products often supply essential resources for parasitoids and predators. In addition, chemical and morphological plant attributes may affect the efficacy of biological control agents by influencing their abundance, survival, development time, fecundity and rate of attack (De Moraes et al., 2000). Moreover, plants influence the quality of parasitoids' herbivorous hosts by determining the quality of the host's nutrient intake (Vinson & Barbosa, 1987). Several studies show that secondary compounds ingested by the host affect parasitoids, either negatively or positively (De Moraes et al., 2000). Toxins and low nutritional quality may weaken the herbivore's immune system thus affecting its capacity to defend itself against parasitoid eggs (Benrey & Denno, 1997; Van den Berg & Van Wyk, 2007; Vinson & Barbosa, 1987).

In order to exploit arthropod herbivores, natural enemies must be able to locate small, highly dispersed targets within a complex spatial and chemical environment (De Moraes et al., 2000). Besides, herbivores have evolved numerous adaptations to avoid being discovered and attacked (Vet & Dicke, 1992). Plants provide both olfactory and visual signals used as foraging cues by parasitic and predaceous arthropods (Ma et al., 1992; Powell & Wright, 1991; Turlings et al., 1995). Some parasitoids use volatiles emitted by undamaged plants to locate the habitat and possibly microhabitat of their host (Ma et al., 1992; Ngi-Song et al., 1996). Plant volatiles released in response to mechanical damage by herbivores are known to be attractive to various parasitoids (Mattiaci et al., 1994; Steinberg et al., 1993). Volatiles released in response to herbivore feeding are generally reliable indicators of herbivore presence and can potentially bring parasitoids in close proximity to their hosts (De Moraes et al., 2000). Indeed, plants are actively involved in the production and release of chemical cues that guide foraging parasitoids (Turlings et al., 1995). Therefore Bt maize may affect, negatively or otherwise, host finding through the volatile emissions.

Extensive research has been published on the impacts of Bt plants on natural enemies within the context of agro-ecosystems (O' Callaghan et al., 2005; Romeis et al., 2006). Long-term, large scale field studies have indicated no meaningful impacts of Bt maize on predator populations even when the predator has acquired the toxin by feeding on intoxicated prey (Hellmich et al., 2005, as cited in Shelton et al., 2008). In addition, studies in which Bt crops were compared to conventional crops treated with insecticides have demonstrated the latter to be far more harmful to predators (Shelton et al., 2008). The situation, however, appears to be more complex for parasitoids. While an insect predator is characterised by feeding on multiple and various hosts during its lifetime, a parasitoid usually completes its entire lifetime within a single host and derives all its nutritional requirements by feeding on the host tissues. This intimate relationship between a parasitoid and its host would put the parasitoid at greater risk to any hazard its host encounters (Shelton et al., 2008). Parasitoids inside dead lepidopteran larvae that are exposed to *B. thuringiensis* usually suffer the same fate as the larvae. Thus, death of herbivore larvae caused by *B. thuringiensis* toxins may be detrimental to populations of parasitoids. Indeed, studies have found that herbivore larvae that were exposed to *B. thuringiensis*, but were themselves resistant to its effects, supported the normal development of parasitoids (Chilcutt & Tabashnik, 1999; Schuler et al., 1999a). Because the strains of *B. thuringiensis* currently in use are largely specific to Lepidoptera, there may be no direct consequences of *B. thuringiensis* on predators and parasites of herbivores (Agrawal, 2000). However, *B. thuringiensis* may have indirect negative effects on the populations of natural enemies of herbivores through the consumption of sick, dead, or dying herbivores (Agrawal, 2000). Critical questions that need to be considered in assessing the effect of Bt on natural enemies include: Do predators and parasitoids of herbivores avoid Bt exposed prey? Could behavioural mechanisms in parasitoids potentially reduce the indirect negative effect of Bt? Because the feeding of susceptible caterpillars on Bt plants is severely reduced, and plant damage attracts parasitoids, parasitoids may preferentially be attracted to either resistant larvae or susceptible larvae on Bt plants (Schuler et al., 1999b). Thus, a potential tri-trophic benefit of employing *B. thuringiensis* in agriculture is that parasitoids may act as agents for minimizing the evolution of resistance to *B. thuringiensis* in pests (Agrawal, 2000).

Bt toxins may have indirect effects on beneficial insects such as parasitoids either by killing the intoxicated host (Schuler et al., 1999a), or rendering the host nutritionally unsuitable (Down et al., 2000). In turn, parasitoids' host quality may be influenced by host plants, giving rise to tri-trophic interactions (Price et al., 1980). For example, Ashouri et al. (2001) reported reduced weight of adult *Aphidius nigripes* Ashmead (Hymenoptera: Braconidae) developing on *Macrosiphum euphorbiae* Thomas (Homoptera: Aphididae) that was feeding on Bt potato. Other studies (e.g. Atwood et al., 1997a,b; Liu et al., 2004) showed that when host larvae were fed on a diet containing Bt protein, larval duration, pupal weight, body weight of the newly emerged adult, parasitoid emergence rates and adult longevity were negatively affected. *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) developing inside *Pseudoplusia includens* Walker (Lepidoptera: Noctuidae) larvae that was feeding on Bt cotton suffered reduced longevity, and females had fewer ova (Baur and Boethel, 2003). *Cotesia flavipes* larval emergence was lower in Bt fed *C. partellus* larvae (23%), compared with non-Bt fed *C. partellus*, (83%) (Prütz & Dettner, 2004). Cocoon numbers and cocoon weight of parasitoids were decreased when *Helicoverpa armirgera* Hubner (Lepidoptera: Noctuidae) larvae fed on diet containing transgenic cotton leaf powder containing *Cry1A* plus *CpT* (Ren et al., 2004). Liu et al. (2005a), in studies on the effects of transgenic cotton on *Campoketis chloridae* Uchida (Hymenoptera: Ichneumonidae) observed that the body weight of larvae of the parasitoids were significantly reduced when parasitized hosts fed on transgenic cotton leaves compared to those fed on traditional cotton. Duration of egg and larvae stage were significantly prolonged while pupal and adult weight of *C. chloridae* was decreased when the host larvae fed on transgenic cotton leaves longer than 48 hours. Development of the larval parasitoid, *Microplitis mediator* Haliday (Hymenoptera: Braconidae) was negatively affected when the host, *H. armirgera*, larvae were reared on a diet containing *Cry1Ac* toxin (Liu et al., 2005b).

Nonetheless, a number of investigations show that Bt toxins are not pathogenic to parasitoids developing in infected hosts. For example, Orr & Landis (1997) observed that parasitism of European corn borer larvae by *Eriborus terebrans* Gravenhorst and *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae) was not significantly different in transgenic and non-transgenic plots. Schuler et al. (2004) observed that *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) eggs laid in Bt resistant *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae), fed on Bt oilseed rape leaves, developed to maturity and there was no effect of Bt plants on percentage parasitism, time to emergence from hosts, time to adult emergence, and percentage adult emergence from cocoons. Parasitoids reared on Bt susceptible hosts hatched, although premature host mortality did not allow the *C. plutellae* larvae to complete their development. This may support the thesis that the Bt toxin has no direct impacts on parasitoids, but rather that the impacts may be due to reduced host quality. Data so far indicate that parasitoids, in general, may be more susceptible to host quality and host-mediated impacts of GM crops compared with to predators (Lovei & Arpaia, 2005).

From the foregoing, it is apparent that Bt plants may affect natural enemies either directly or indirectly. For the insecticidal proteins of insect resistant GM plants to directly affect an individual natural enemy, the organism has to not only be exposed to the toxin but also be susceptible to it. Thus, an organism is not affected by the GM plant when either exposure or sensitivity (hazard) does not occur. However, for an effect to be of ecological relevance it must result in changes in population or community processes. Similarly, direct or indirect effects of the GM plant on individual natural enemies, natural enemy species or groups/guilds of natural enemies might not lead to a decreased biological control function (Romeis et al., 2008a). Moreover, natural enemies may be affected indirectly by the GM plant when they feed on sublethally impaired herbivores (sick prey). Such effects appear to be caused by declines in nutritional quality of the host/prey organism. These prey/host quality mediated effects appear to account for most (if not all) of the Bt plants' effects on natural enemies that have been reported from laboratory and glasshouse studies (Romeis et al., 2006). It is well established that parasitoids are especially vulnerable to changes in their hosts' quality, since they usually complete their development in a single host (Godfray, 1994). Therefore this review lays particular emphasis on the potential impacts of Bt maize on stem borer parasitoids. Bt maize are deployed to control Lepidoptera, which implies that lepidopteran parasitoid hosts would (as a direct consequence of being affected by the Bt toxin) invariably be less suitable for parasitoid development. Thus it is not surprising that parasitoid life-table parameters are significantly affected when the host suffers (Romeis et al., 2006). In extreme cases, parasitoids attack sublethally affected hosts that die before the parasitoid offspring completes development (Davidson et al., 2006; Schuler et al., 2004). Sections 2.2.1 to 2.2.5 provides a review of the potential impacts of Bt maize on stem borers and their natural enemies (specifically parasitoids).

### 2.2.1 Effect of Bt maize on stem borer oviposition preference

One of the major risks associated with the use of transgenic pesticidal crops is that pests can develop resistance which could reduce the efficacy of such crops as plant protection tools (Wolfenbarger & Phifer, 2000). Furthermore, if larvae developed resistance to the Bt toxin, there could be greater chances of natural enemies getting host-mediated exposure to the toxin (Obonyo et al., 2008a). When the US EPA reviewed the first registration for Bt plants, there was considerable concern in some sectors that resistance to the plants would rapidly occur and that not only would this be a concern to growers of Bt crops but also to organic farmers who relied on Bt as a foliar spray (Shelton et al, 2008). The high dose/refuge strategy (the use of high doses of one or more toxins, combined with a refuge of non-Bt plants) has been proposed as a likely means to delay the development of resistance by insects against transgenic plants (Bates et al., 2005). This strategy emphasises the presence of susceptible insect populations; these may slow down the evolution of resistance (Bentur et al., 2000; Shelton et al., 2000; Tang et al., 2001). The premise is that susceptible insects, if present in sufficient numbers, would mate with resistant insects and dilute resistance genes. However, several biological factors that influence the number of insects exposed to Bt toxin may substantially affect the success of the high dose/refuge strategy (Ives & Andow, 2002). One such factor is oviposition preference. Preference for Bt maize would require more refuge plants to counter an increased selection pressure. However, preference for refuge plants could have the opposite effect. From a resistance management perspective, an ideal plant, in addition to killing larvae, should repel adult oviposition (Hellmich et al., 1999). This would reduce selection for resistance because fewer larvae would be exposed to plant toxins. Potential effects of Bt transgenic maize on stem borer natural enemies could therefore partly depend on the oviposition preferences of stem borers, either for Bt or non-Bt maize.

Various studies have been conducted on effects of Bt maize on stem borer oviposition behaviour. In field tests, the number of eggs laid by susceptible European corn borer females did not differ between Bt corn (containing *Cry1Ab*) and non-Bt corn (Orr & Landis, 1997). Pilcher & Rice (2001) observed that *O. nubilalis* females did not show any oviposition preference towards non-Bt or Bt maize (using Event 176 and Bt11). Van den Berg & van Wyk (2007) reported that *S. calamistis* adults did not differentiate between Bt and non-Bt maize plants in oviposition choice experiments. More recently, Obonyo et al. (2008a) observed that *C. partellus* and *S. calamistis* moths did not discriminate between Bt and non-Bt maize plants for egg laying. This non-discriminatory oviposition behaviour could be due to the fact that the ratios of caterpillar-induced odour emissions of Bt maize plants are identical to those of non-Bt plants (Turlings et al., 2005) since genetic modification does not alter the volatile profile of undamaged maize plants (Dean & De Moraes, 2006). These results have important implications for pest resistance management and monitoring. Because oviposition is not affected by the Bt toxin, and females are exposed equally to Bt maize and non-Bt maize refuges, it can be assumed that eggs will be distributed equally between Bt and non-Bt maize hence there will always be a pool of insects on susceptible crops, which is necessary for resistance management and hence ensuring that the development of resistance is delayed as much as possible. Furthermore, since the development of resistance against Bt toxins requires the survival and development of at least two exposed larvae into a male and a female (Kumar, 2004) and since Bt maize causes up to 100% mortality (Obonyo et al., 2008a) the possibility of resistance development would be further restricted.

### 2.2.2 Effect of Bt maize on stem borer development and mortality

An understanding of the effect of Bt toxins on development of herbivorous insects is important because host development time could have a direct effect on natural enemies by influencing the ‘window of vulnerability’, the period during which the host is exposed to natural enemies (Schoenmaker et al., 2001; Schuler et al., 1999a; Wallner et al,. 1983). Also, the combined effects of developmental delays may result in temporal asynchrony of moths emerging from Bt and non-Bt maize- resulting in susceptible individuals mating before resistant adults emerge (Horner et al., 2003). Since the success of the refuge strategy requires that any resistant individuals mate with susceptible ones, such asynchrony in emergence from Bt and non-Bt maize plants could compromise the strategy and hence weaken the potential of Bt maize as an option for stem borer control. Furthermore, effects of Bt plants on host development could impact on the biology of a natural enemy developing in such a host (Walker et al., 2007; Weseloh, 1984).

Obonyo et al. (2008b) observed that Bt maize had significant effects on stem borer development time. Feeding of stem borer larvae on Bt plant tissue at the 3rd and 4th instars significantly lengthened the duration of the respective instars (but not the subsequent ones) while overall larval development time was not affected probably because the larvae were exposed to Bt for a relatively short duration. Schoenmaker et al. (2001) suggested that ingestion (by lepidopteran larvae) of sublethal doses of Bt toxin prolonged development time by temporarily inhibiting feeding. Continuous exposure to Bt toxin prolonged development of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) while exposure to toxin for shortened durations had no significant effects on larval development time (Dutton et al., 2005a). Therefore it seems that larvae may recover from the effects of the Bt-toxin, following transient exposure. Other lepidopteran larvae that ingest sublethal doses of Bt also resume normal development after a few days (Moreau and Bauce, 2003; Siegfried et al., 2001). Dutton et al. (2005a) reported that there were no significant effects on overall larval development when 3rd instars of *S. littoralis* larvae were exposed to Bt sprayed plants because the effect of the toxin did not persist for long due to rapid degradation of the Bt spray (Haddad et al., 2005). In contrast, significant effects, attributed to long toxin persistence, were reported when larvae were reared for four days on Bt maize (Haddad et al., 2005). Huang et al. (2006) observed larval development inhibition of *O. nubilalis*, *D. grandiosella* and *Diatraea saccharalis* F. (Lepidoptera: Pyralidae) fed on a diet prepared from *Cry1Ab* protein (extracted from Bt corn leaves). Similarly, transgenic maize containing *Cry1Ab* delayed larval development of *H. zeae* (Horner et al., 2003; Stewart et al., 2001) and *D. plexippus* (Dively et al., 2004). Development time of the 5th instar of *C. partellus* larvae subjected to transient feeding on Bt maize at the same growth stage was not affected (Obonyo et al., 2008b), possibly because pupation follows shortly after the 5th larval stage in this species at which time the larvae are relatively inactive and do not feed much (Tettamanti et al., 2007); and their large sizes enable them to tolerate more toxin (Huang et al., 1999). Overall, larval development time in these larvae was significantly longer as a consequence of Bt exposure (Obonyo et al., 2008b). This indicates a disturbance to the "normal" development cycle, from which the larvae may eventually recover. The increase in larval development time therefore increases the window of vulnerability during which stem borer parasitoids can get host mediated exposure to the Bt toxin. This in itself may not be cause for concern but rather the consequences of such exposure. Possible consequences of host-mediated exposure to Bt toxins are discussed in subsequent sections of this chapter.

### 2.2.3 Effect of Bt maize on the ability of parasitoids to locate hosts

The success of biological control agents depends on their efficiency to search for, and locate target hosts (Nordlund et al., 1988). Parasitoid host finding behaviour is complex and influenced by many factors (Ngi-Song et al., 1996; van Leerdam et al., 1985). One important factor is volatiles emitted by the host plant. There are significant quantitative (Turlings et al., 2005) and qualitative (Dean and De Moraes, 2006) differences in volatile emissions between Bt and non-Bt plants. Both the quantity and composition of emitted volatiles influence host finding by *Cotesia* species (Steinberg et al., 1993). Host species odours are also used by parasitoids for host location and hence any change in host physiology may alter parasitoids' host location behaviour (Takasu & Lewis, 2003).

Bt maize may influence host species odours and thus parasitoid host finding behaviour. Obonyo (2009) showed that damaged but uninfested Bt and non-Bt maize were similarly attractive to females of the larval parasitoids *C. flavipes* and *C. sesamiae*, and that both were also more attractive than the control air flow from a plantless cage. Females of *C. flavipes* and *C. sesamiae* were equally attracted to stem borer infested maize plants (irrespective of Bt status). This suggests that females of *C. flavipes* and *C. sesamiae* do not distinguish among plant- and host-derived cues from Bt and non-Bt maize when searching for stem borer hosts. Therefore the presence of Bt toxin in maize plants apparently did not affect the host location process of these parasitoids. Similar findings have been reported elsewhere (Ngi-Song and Overholt, 1997; Potting et al., 1997). Also, *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) and Microplitis rufiventris Kokujev (Hymenoptera: Braconidae), which are important larval parasitoids, were not able to distinguish between the odours of a Bt maize event and its near-isogenic line (Turlings et al., 2005). This indicates that growing Bt maize is not likely to affect host finding by stem borer larval parasitoids.

Furthermore, plant volatiles may act as cues for host location by pupal parasitoids (Obonyo 2009). Chemical analyses of collected odours between Bt and non-Bt maize revealed significant quantitative differences (Turlings et al., 2005); this could possibly affect host location by pupal parasitoids such as *X. stemmator*. Indeed, Obonyo (2009) found that *X. stemmator* parasitoids preferred host plant odours compared to odours from a blank control. However, volatiles from Bt plants were deterrent to *X. stemmator*. Oviposition preference of insects has been predicted to correlate with host suitability for offspring development (preference – performance hypothesis) (Jaenike, 1978). This hypothesis (known as the 'mother knows best' principle) (Johnson et al., 2006) was developed for herbivorous insects but is assumed to play an important role in parasitic Hymenoptera as well (Vinson and Iwantsch, 1980). According to this preference – performance hypothesis, *X. stemmator* avoids the Bt plants because potential hosts have a lower quality when feeding on Bt maize. In parasitoids, host organisms are the only source of nutrients for the immature stages (Sequeria & Mackauer, 1992), and thus parental fitness depends on the accurate assessment of host sites for their potential to sustain the development of their larvae (Meyling & Pell, 2006). Therefore adaptation to reliable cues, enabling the evaluation of the quality of potential hosts, is a selective advantage for ovipositing females. Chemical cues may not only attract but also deter parasitoids from entering host sites. Naive females of the solitary ectoparasitoid, *Lariophagus distinguendus* Forster (Hymenoptera: Pteromalidae) which parasitizes immature stages of several stored-product infesting beetle species avoid odours from mouldy grains which are unsuitable for the development of their larvae (Steiner et al., 2007).

Considering that Bt maize has an adverse effect on the host location behaviour of *X. stemmator*, it could possibly compromise the biocontrol potential of this parasitoid, hence impacting negatively on the use of Bt maize as part of IPM strategies.

### 2.2.4 Effect of Bt maize on parasitoid biology

Changes in host plant chemistry may negatively affect natural enemy fitness through reducing survivorship, clutch size, body size and/or fecundity (Ode, 2006). Such negative impacts may occur either directly (when the natural enemy encounters the toxin in its host or prey) or indirectly (when natural enemy fitness is reduced due to lower prey/host size or quality). A number of studies have found variable results on effects of Bt toxins on parasitoid life history parameters; these include no apparent negative effect (Obonyo, 2009; Schuler et al., 1999a), synergism between the transgenic plants and parasitoids (Tounou et al., 2007), lower parasitoid survival (Blumberg et al., 1997), or emergence rates (Atwood et al., 1997b; Liu et al., 2005a), increased parasitoid development times (Liu et al., 2005a; Liu et al., 2005b; Vojtech et al., 2005), reduced longevity (Baur & Boethel, 2003), reduced body mass (Ashouri et al., 2001; Liu et al., 2005a), and altered parasitoid sex ratios (Wallner et al., 1983).

Changes in host plant chemistry may also affect acceptance of the plants by their hosts, with consequences on associated natural enemies. Obonyo (2009), however, showed that there were no significant differences in host acceptance ratio between Bt exposed and non-Bt reared larvae. Turlings et al. (2005) observed that braconid parasitoids did not distinguish between odours of Bt and non-Bt maize plants in olfactometer experiments. Although a number of studies have found no significant differences in oviposition choice between hosts fed on transgenic and non-transgenic diets (Bell et al., 1999, Schoenmaker et al., 2001; Schuler et al., 1999b), there are cases where parasitoids seem to distinguish by host quality (reviewed in Steidle and van Loon, 2003; Overholt et al., 1994; Sallam et al., 1999). More recently, Obonyo (2009) reported a higher host acceptance ratio of *C. flavipes* for *C. partellus* compared with *S. calamistis*. These contrasting results could be due to the different dietary material (plant material and microbial formulations) used in the various studies. However, it is more likely that the lack of significant effects of Bt was due to the transient feeding of the host on Bt maize (Obonyo, 2009). Negative effects of Bt toxins on parasitoids are often indirect, occurring via reduced host quality (Chen et al., 2008; Vojtech et al., 2005; Walker et al., 2007) but larvae exposed to the Bt toxin and subsequently transferred to a Bt-free diet may recover by replacing damaged mid-gut cells and excreting the toxin (Tounou et al., 2007).

As already mentioned, host quality directly impacts on parasitoid development. Ingestion of Bt toxins by stem borer larvae could therefore affect parasitoid developing within these larvae. Temerak (1980), Salama et al. (1991) and Atwood et al. (1997b) observed that incorporation of microbial Bt formulations in host food decreased the emergence of parasitoids larvae. Wanyama (2004) found that Bt contaminated diets significantly increased *C. partellus* cocoon development time. Bernal et al. (2002) found a longer development time of *Parallorghas pyralophagus* Marsh (Hymenoptera: Braconidae) on Bt fed hosts. In contrast, Obonyo (2009) and Prutz & Dettner (2004) observed no effects of host ingested Bt toxins on the mortality of *C. flavipes* inside cocoons. Also, Prutz & Dettner (2004) found no significant effects of Bt-contaminated diets on *C. flavipes* pre-cocoon development time.

Besides, the proportion of female parasitoids produced in each generation is an important factor in the success and survival of parasitoid populations (Godfray, 1994). A female biased sex ratio is an important characteristic of a biocontrol agent, especially parasitoids, because only females contribute to pest mortality upon release (Waage, 1982). In fact, the failures of numerous biological control projects have been attributed to male biased sex ratios (Stouthamer et al., 1992). A number of studies (e.g., Bernal et al., 2002; Obonyo, 2009, Prutz & Dettner, 2004; Walker et al., 2007) have reported no effect of Bt toxin on sex ratios of parasitoid progeny. Therefore adoption of Bt maize is not likely to impact on larval parasitoid sex ratios and/or parasitoid populations. It is clear though that Bt maize may have variable effects on the biology (and hence the effectiveness) of stem borer parasitoids.

### 2.2.5 Effect of Bt maize on fluctuating asymmetry of parasitoids and parasitoid size

Environmental or genetic stress can cause an increase in the fluctuating asymmetry (FA) of bilaterally morphological traits (Parsons, 1990) and hence may be used as a measure of the ability of individuals to cope with different kinds of environmental stresses (Jones, 1987; Leary and Allendorf, 1989; Parsons, 1990). FA refers to random deviations from symmetry of otherwise bilaterally symmetric traits; it occurs when an individual is unable to undergo identical development on both sides of a bilaterally symmetrical trait (Liu et al., 2005c). Environmental stress, including extreme temperatures (Mpho et al., 2002; Sciulli et al., 1979), pesticides (Hoffmann & Parsons, 1990), and qualitative and/or quantitative food deficiency (Liu et al., 2005c; Parsons, 1990) may cause FA in morphological traits during development. For example, the FA value of the third segment of antenna in aphids was significantly higher on Bt cotton compared to control cotton (Liu et al., 2005c). Generally, there is a negative correlation between the degree of FA and the fitness of populations (Moller, 1997). For instance, the lifespan of *Malacosoma disstria* Hubner (Lepidoptera: Lasiocampidae) shortened as the degree of FA of the first segment of foreleg tarsi increased (Naugler and Leech, 1994). Wing asymmetry, in contrast, may influence flight ability and hence the ability of parasitoids to reach hosts (Bennet & Hoffmann, 1998).

There have been variable results on effects of exposure to Bt toxins on FA values. Obonyo (2009) observed that transient feeding of hosts on Bt maize had a number of effects on FA depending on the trait under consideration as well as the host and parasitoids species. Transient feeding of *C. partellus* and *S. calamistis* hosts on Bt maize adversely affected *C. sesamiae* and *C. flavipes* parasitoids, respectively; this was reflected in the higher FA values for antennal length and wing length in *C. sesamiae* and *C. flavipes*, respectively that were developing on hosts subjected to transient feeding on Bt maize. In contrast, *C. sesamiae* exposed to *S. calamistis* hosts that had been subjected to transient feeding on Bt maize showed lower FA values. The lower FA values could be due to the improved performance of *C. sesamiae* on Bt exposed *S. calamistis* thus indicating that there could be instances where Bt maize could actually enhance parasitoid performance. Similarly, Tounou et al. (2007) observed positive effects of Bt intoxicated *S. calamistis* larvae on *C. sesamiae*. Such positive effects (on exposure to Bt) could be attributed to the weakening of the immune system of the host, resulting in lower encapsulation rate of the parasitoids eggs by the host larva (Tounou et al., 2007). Encapsulation of *C. sesamiae* eggs has been reported in *S. calamistis* (Gitau et al., 2007; Hailemichael, 1998). It is possible that this encapsulation reaction could affect parasitoid development. The success of the encapsulation reaction depends on the vigour of the herbivore (Siva-Jothy & Thompson, 2002) which may be reduced by host plant induced stresses (Blumberg, 1997, Souissi & Le-Ru, 1998, Turlings & Benrey, 1998). The parasitoid *Cotesia kazak* Telenga (Hymenoptera: Braconidae) has more success on its host *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), fed on less toxic Bt-amended diets (Walker et al., 2007) compared with *Tranosema rostrale rostrale* Brishke (Hymenoptera: Ichneumonidae) developing on Bt fed spruce budworm *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae) (Schoenmaker et al., 2001). Transient feeding of hosts on Bt maize apparently had no significant effects on FA of antennae and wing lengths in the other host parasitoid combinations (Obonyo, 2009). This was possibly because the levels of toxin ingested were not high enough to affect these traits in these host-parasitoid combinations or alternatively because these traits are not sensitive to the effects of the Bt toxins. Transient feeding of *S. calamistis* on Bt maize did not adversely affect *C. flavipes* development. However, *C. sesamiae* developing on *C. partellus* subjected to transient feeding on Bt maize had greater FA values compared with those developing on *C. partellus* exclusively reared on non-Bt maize. Similarly, Wanyama (2004) did not detect any significant effects of transient feeding of host on Bt toxins on parasitoid development time. Even though a significant difference was not detected in the percentage of dissected pupae that contained immature parasitoids, they were much fewer on pupae from larvae exposed to transient feeding on Bt maize. Wanyama (2004) found a significantly higher proportion of dead parasitoids in pupae of Bt fed hosts.

Female parasitoids are usually able to control the proportion of fertilized eggs, which they oviposit, depending on host size and quality (Charnov and Stevens, 1988). The strong male bias in Bt fed hosts could indicate inferior host quality. Obonyo (2009) and Liu et al., (2005d) showed that longevity of adult parasitoid wasps was unaffected by transient feeding on Bt maize, indicating that adoption of Bt maize may not have significant effects on parasitoid longevity and may therefore not adversely impact on parasitoid biocontrol potential.

Therefore it is clear that where transient feeding of hosts on Bt maize increases FA, it does so in a trait-specific way. The lack of consistency in FA responses across traits may reflect variation between trait types in their susceptibility to environmental stress (Woods et al, 1999). We conclude that the FA of some traits (and consequently parasitoid fitness) in the parasitoids may be affected by transient feeding of their hosts on Bt maize. Thus in assessing the risk posed to non-target organisms by transgenic plants it may be necessary to ascertain the relationship between fitness parameters and the FA values of the non-target organisms.

In addition to FA, parasitoid size may also have an impact on parasitoid fitness. Many studies have established positive associations between adult body size and standard laboratory and field fitness measures (e.g., Godfray, 1994; Bennet & Hoffmann, 1998). Increase in hind tibia length increased the fitness of *Trichogramma carverae* Oatman and Pinto (Hymenoptera: Trichogrammatidae) (Bennet and Hoffmann, 1998). Parasitoid size may be determined indirectly by measuring hind tibia length (Bennet & Hoffmann, 1998; Kazmer & Luck, 1995; West et al., 1996), antennal length and wing length. Studies (e.g., Obonyo, 2009) show that transient feeding of hosts on Bt maize has various effects on trait sizes depending on the trait, host and parasitoid species. For example, transient feeding of *C. partellus* on Bt maize adversely affected *C. flavipes* by reducing their antennal length. In contrast, antennal length was significantly increased in *C. sesamiae* developing on *S. calamistis* hosts subjected to transient feeding on Bt maize. The longer antenna lengths in *C. sesamiae* on *S. calamistis* subjected to transient feeding on Bt maize could be due to the improved performance of *C. sesamiae* on Bt exposed *S. calamistis*.

The effect of Bt toxins on wing length and hind tibia length of parasitoids have been reported in a number of studies. Obonyo (2009) showed that wing length of some parasitoid species were affected by feeding of hosts on Bt maize. He also found that hind tibia length were significantly reduced on *C. flavipes* developing on *S. calamistis* hosts subjected to transient feeding on Bt maize, and *C. sesamiae* developing on *C. partellus* subjected to transient feeding on Bt maize. In contrast, hind tibia lengths of *C. sesamiae* were not significantly affected following transient feeding of their *S. calamistis* hosts on Bt maize (Obonyo, 2009). Similarly, Obonyo (2009) did not observe significant effects on parasitoid hind tibia lengths following exposure of *X. stemmator* to hosts that had been subjected to transient feeding on Bt maize. This indicates that Bt toxins probably had no significant effect on parasitoid size. Therefore it is expected that *X. stemmator* emerging from hosts subjected to transient feeding on Bt maize would perform equally well in the field as those emerging from non-Bt fed hosts. Also, the lack of significant differences in FA values between parasitoids reared on Bt fed and non-Bt maize fed hosts may suggest that the Bt toxin did not provide a stressful environment for the developing parasitoids. Although exposure of *X. stemmator* to hosts subjected to transient feeding on Bt maize did not affect the biology of *X. stemmator*, it significantly reduced the proportion of female progeny (Obonyo, 2009). A female biased sex ratio is important for biocontrol agents, especially parasitoids because only females contribute to pest mortality upon release (Waage, 1982).

It is clear from the foregoing that Bt maize may impact on parasitoid fitness as well as sex ratios and hence could possibly affect parasitoid biocontrol potential.

## 3. Conclusion

It is difficult to determine, from existing literature, whether the observed host mediated effects of Bt maize on parasitoids are direct or indirect. Parasitoid performance can be affected as a result of the Bt toxin reducing host's biomass (Farrar and Ridgeway, 1995; Deml et al., 1998), or changing the host hemolymph-pH, hemolymph ion concentration, and nutrient concentration (Tanada and Kaya, 1993), which in turn can affect the parasitoid larvae living in the hemolymph. Alternatively, parasitoid larvae may be affected by ingesting the Bt toxin present in the hemolymph. Whatever the case, the most important consideration should be whether Bt maize can cause significant harm to stem borer natural enemies and most significantly the overall environmental consequences of such impacts. In order to determine the overall impact of growing Bt maize on stem borer natural enemies, the issues raised in this chapter should be placed in the context of real-life scenarios, taking into account *inter alia*, local agricultural practices, agro-ecological conditions, trade policies etc.

This review has confined itself to potential impacts of Bt maize on stem borer natural enemies, specifically parasitoids and it is therefore not possible to make generalizations. Any judgment on the potential impact (s) of Bt maize, or any transgenic crop for that matter, should be made on a case-by-case basis using a rational, evidence-based scientific approach. In contrast to application of chemical insecticides with contact toxicity, insecticidal proteins expressed by GM plants have to be ingested to affect parasitoids. Consequently, when assessing the potential impacts of growing Bt maize on natural enemies it may be necessary to assess which organism (s) may be exposed under actual field conditions, and at what level. Indeed, the level at which an organism can be exposed to a plant expressed insecticidal protein may vary depending on the concentration of the toxin in the plant or environment, the plant tissue in which the protein is expressed, and the feeding behaviour of the non-target organism (Dutton et al., 2005b; Romeis et al, 2008a,b,c). Therefore exposure pathways can be predicted only if the relevant information for the GM plant, the environment and the natural enemy is available. Also, it may be necessary to make comparisons between the potential consequences of Bt maize on natural enemies and the use of conventional insecticides. Evidently, most studies have focussed mainly on making comparisons between Bt maize and non-Bt maize, without looking at the alternatives. It is only when a comparative approach is adopted that it may be possible to make prudent judgements regarding this novel method of insecticide delivery. Therefore in assessing the potential impact of Bt maize on the environment, it would be useful to pose the question: how would the use of Bt maize compare to the alternative (s)?" When assessment is not comparative decision making becomes non-objective. For example, it may be known that Bt maize reduces wing lengths of some stem borer parasitoids. However, with this information alone it may not be possible to reach useful conclusions. In contrast, if comparisons were made between the effects of Bt maize and the alternative (s) e.g., broad spectrum insecticides (effect on natural enemies), it would be easier to make more informed and objective conclusions (and hence decisions) regarding this novel technology.

## 4. Disclaimer

The views expressed in this article are those of the individual authors and do not necessarily reflect the views and policies of the International Centre for Genetic Engineering and Biotechnology or the University of Venda.

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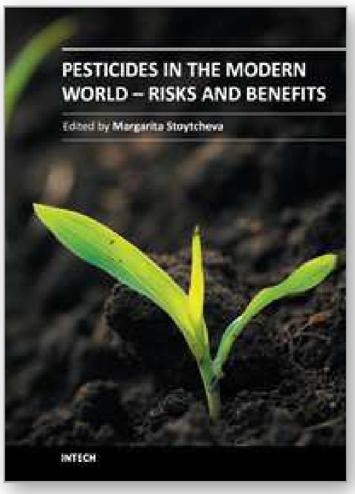

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