

**COMPARATIVE PHENOLOGY OF
LEPIDOPTERA ON GENETICALLY MODIFIED
BT- AND NON-BT MAIZE**

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ABSTRACT

The maize stem borers, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) are economically important pests of maize in South Africa. Genetically modified Bt maize (MON810) expressing Cry1Ab protein is used to control these pests on approximately 425 000 hectares in South Africa. Before this study no information was available on the diversity of Lepidoptera on maize in South Africa or the potential impact of Bt maize on non-target Lepidoptera species under field conditions. There was also no information on the susceptibility to Bt maize of another stem borer species, *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae), which is not a target species of Bt maize. The aims of this study were to determine which Lepidoptera species occur and feed on maize and could be directly exposed to Bt toxin as well as to assess the levels of infestation of target stem borer species and non-target Lepidoptera species on Bt- and non-Bt maize fields. Field collections of Lepidoptera that were directly exposed to Bt toxin through feeding on Bt maize plants were done between January 2004 and May 2006. Surveys were conducted in the North-West, Free State, Gauteng and Limpopo provinces. In order to quantify infestation levels and incidence of larvae on plants, sampling was done by inspecting between 300 – 900 plants per field. Studies were also done to compare the incidence of damaged plants and larvae on plants between Bt- and adjacent non-Bt maize fields. The susceptibility of *S. calamistis* to several Bt maize hybrids was evaluated under laboratory and greenhouse conditions. Fifteen species of Lepidoptera were recorded on maize plants. The following six species were recorded to feed on Bt maize and were reared on Bt maize until the adult stage: *Acantholeucania loreyi* (Noctuidae), *Agrotis segetum* (Noctuidae), *B. fusca* (Noctuidae), *Helicoverpa armigera* (Noctuidae), *Eublemma gayneri* (Noctuidae) and *Nola phaeocraspis* (Nolidae). Although Bt maize was damaged by several species of leaf, stem and ear feeding Lepidoptera in this study, the incidence of damage was always significantly lower on Bt maize fields than susceptible fields. This study provided base line data on Lepidoptera that feed on Bt maize in South Africa. Non-target Lepidoptera species that are directly exposed to Bt toxin was identified. An ecological model was

used to develop a preliminary risk assessment for Bt maize through which priority species for research and monitoring was identified as well as species that are at risk of resistance development.

Keywords: *Busseola fusca*, *Chilo partellus*, Lepidoptera, MON810, non-target species, resistance, stem borers, transgenic maize.

OPSOMMING

Titel: Vergelykende fenologie van Lepidoptera op Bt- en nie-Bt-mielies

Die mieliestamruspers, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) en *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) is ekonomies-belangrike plae van mielies in Suid-Afrika. Geneties gemanipuleerde Bt-mielies (MON810) wat die Cry1Ab-proteïen bevat, word gebruik om hierdie plae op ongeveer 425 000 hektaar in Suid-Afrika te beheer. Voordat hierdie studie uitgevoer is, was geen informasie oor Lepidoptera-diversiteit, of die potensiële impak van Bt-mielies op nie-teiken Lepidoptera-spesies, in Suid-Afrika beskikbaar nie. Daar was ook geen informasie beskikbaar rakende die vatbaarheid van die nie-teiken stamrusper, *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae), vir Bt-mielies nie. Die doel van die studie was om te bepaal watter Lepidoptera-spesies op mielies voed en direk blootgestel word aan Bt-toksien asook die vlakke van infestasië van teiken- en nie-teiken Lepidoptera wat in Bt- en nie-Bt-mielielande voorkom. Lepidoptera wat direk blootgestel is aan Bt-toksiene deur voeding op Bt-mielieplante, versamel oor 'n periode vanaf Januarie 2004 tot Mei 2006. Opnames is gedoen in die volgende provinsies: Noordwes, Vrystaat, Gauteng en Limpopo. Om infestasiëvlakke en teenwoordigheid van larwes op plante te kwantifiseer, is opnames gedoen deur tussen 300 en 900 plante per land te inspekteer. Studies is ook gedoen om die voorkoms van beskadigde plante en larwes op plante te vergelyk tussen Bt- en naburige nie-Bt-lande. Die vatbaarheid van *S. calamistis* teenoor verskillende Bt-variëteite is geëvalueer onder laboratorium- en kweekhuistoestande. Vyftien Lepidoptera-spesies is op mielies aangetref. Die volgende ses spesies wat voed op Bt-mielies is aangeteken en is suksesvol deurgeteel tot volwassenes: *Acantholeucania loreyi* (Noctuidae), *Agrotis segetum* (Noctuidae), *B. fusca* (Noctuidae), *Helicoverpa armigera* (Noctuidae), *Eublemma gayneri* (Noctuidae) en *Nola phaeocraspis* (Nolidae). Die voorkoms van Lepidoptera-skade op blare, stamme en koppe was altyd betekenisvol minder in Bt-mielielande as in lande met vatbare mielies. Hierdie studie verskaf 'n databasis van Lepidoptera wat voed op Bt-mielies in Suid-Afrika. Die nie-teiken

Lepidoptera-spesies wat direk bloedgestel word aan Bt-toksiene, is ook geïdentifiseer. 'n Ekologiese model is gebruik om 'n voorlopige risiko-analise te ontwikkel vir Bt-mielies om prioriteitspesies te identifiseer vir navorsing en monitering, asook spesies waarvan die risiko groot is en wat moontlik weerstand teen Bt kan ontwikkel.

Sleutelwoorde: *Busseola fusca*, *Chilo partellus*, Lepidoptera, MON810, nie-teiken spesies, stamruspers, transgeniese mielies, weerstand.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Entomogenic bacteria, such as *Bacillus thuringiensis* (Bt), have been identified as the most successful group of organisms, from which genes can be used in genetic transformation of crops for pest control. Since the first genetically modified tobacco plants expressing foreign proteins were obtained in 1984, transgenic plants have been produced for more than 100 plant species. The global area under transgenic crops has increased from 1.7 million hectares in 1996 to 44.2 million hectares in 2000. In 1999, 11.7 million hectares of Bt crops were grown by farmers in ten countries (James, 2004).

Since 1999 the use of Bt crops have increased rapidly and in 2004, ten years after the commercialization of genetically modified crops, the global area of Bt crops continued to grow for the ninth consecutive year at a sustained growth rate of 20%. The estimated global area of approved Bt crops for 2004 was 81.0 million hectares, up from 67.7 million hectares in 2003. The increase of 13.3 million hectares in Bt crop area between 2003 and 2004, is the second highest on record. In 2004, there were fourteen biotech mega-countries, growing 50 000 hectares or more. These countries were in order of area, USA, Argentina, Canada, Brazil, China, Paraguay, India, South Africa, Uruguay, Australia, Romania, Mexico, Spain and the Philippines (James, 2004). In South Africa 155 000 hectares of Bt white maize was planted in 2004, a 25 fold increase from when it was first introduced in 2001 (James, 2004).

In South Africa a 25% increase was reported in the combined area of GM maize, soybean and cotton to 0.5 million hectares in 2004. Growth continued in the sales of both Bt white maize used for food, and yellow Bt maize used for feed. Globally, Bt maize is projected to have the highest percentage growth rate for the near term as maize demand increases and as more beneficial traits become available and is approved (James, 2004).

The successes of genetically engineered crops have engendered sharp debate on the future use of these crops. The degree to which this technology will be adopted depends on its relative advantages compared to currently deployed insect control methods. As with any new farming practice, producers need to consider advantages and disadvantages of genetically modified crops (Meeusen & Warren, 1989). Bt maize offers many advantages to producers, but there are some concerns that cannot be ignored, especially from an environmental viewpoint. The adoption of this technology will always be influenced by uncertainties. Maize containing the Bt gene provide a useful test case for consideration of the advantages and disadvantages that Bt crops may hold.

1.2 How does Bt work?

From an environmental viewpoint it is important to know where the Bt gene comes from, which organisms are affected and how this may influence physiology of Lepidoptera larvae. *Bacillus thuringiensis* is a gram-positive bacterium, common in soil, characterized by its ability to produce insecticidal crystal proteins during sporulation (Höfte & Whiteley, 1989; Lambert, Buysse, Decock, Jansens, Piens, Saey, Seurinck, Van Audenhove, Van Rie, Van Vliet & Peferoen, 1996). Since 1901, when Ishiwata (1901) discovered “soto bacillus” as a pathogen of the silkworm *Bombyx mori* Linnaeus (Lepidoptera: Bombycidae), many Lepidoptera-specific *B. thuringiensis* strains have been isolated and characterized (Wasano, Saitoh & Ohba, 1997). Berliner (1915) (cited by Sharma, Sharma, Seetharama & Ortiz, 2000) isolated it from diseased larvae of *Ephestia kuhniella* (Zeller) (Lepidoptera: Pyralidae) and designated it as *B. thuringiensis*. These crystal proteins have a specific toxic activity against certain Lepidoptera, Diptera and Coleoptera larvae and have been used in spray formulations under field conditions for the past 40 years. *Bacillus thuringiensis* is the most important biological insecticide with annual sales of US \$90 million. There are 67 registered Bt products with more than 450 different formulations (Sharma, Sharma, Seetharama & Ortiz, 2000).

Transgenic maize plants produce Bt toxin at a high level throughout the growing season. The Bt gene encodes for a crystalline protein, known as a *Cry* protein. The promoter of the Bt gene inserted into the maize genome turns on the production of an inactive form of the toxin in maize cells (Van Rie, Jansens, Höfte, Degheele & Van Mellaert, 1989). Bt maize hybrids have been genetically engineered to contain the *Cry* genes which subsequently produce *Cry* proteins in the plant's leaves, stem and pollen (Bhatia, Grant & Powell, 1999). Once ingested by a target insect, crystalline inclusions dissolve in the larval midgut releasing *Cry* proteins (Van Rie *et al.*, 1989). *Cry* proteins are protoxins (the inactive toxin) that are proteolytically converted into smaller toxic polypeptides inside the insect midgut (Höfte *et al.*, 1989). The toxin binds to specific receptors on the epithelial cells of the larval gut and ruptures cell walls, leading to subsequent paralysis of the gut and eventual death of the insect (Kumar, Sharma & Malik, 1996). In one to two days, the larvae die from septicemia as spores and gut bacteria proliferate in their hemolymph (Hall, 2004).

1.3 Diversity of the Lepidoptera complex on maize in South Africa

Various Lepidoptera species occur on maize in South Africa. Annecke and Moran (1982) listed ten Lepidoptera species that have pest status on maize (Table 1.2). Although the list does not include all the Lepidoptera species that feed on maize, it provides an overview of the species which are of primary importance. Kroon (1999) reported other Lepidoptera species that feed on maize seedlings and on maize during the pre-flowering and post-flowering stages (Table 1.3).

Agrotis subalba (Walker) (Lepidoptera: Noctuidae), *A. longidentifera* (Hampson) (Lepidoptera: Noctuidae), *A. spinifera* (Hübner) (Lepidoptera: Noctuidae) and *A. segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) are various cutworm species that occur in South Africa. The common cutworm *A. segetum* occurs throughout Southern Africa and is the cutworm with the highest abundance in maize production areas. Environmental conditions influence the hatching time of eggs and duration of subsequent

stages. Larvae moult five times and the last larval instar is followed by a pupal stage from which moths emerge. The life cycle takes approximately 50 days during the summer (Du Plessis, 2000).

The stem borers, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) and *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) are of economical importance in South Africa. Especially *B. fusca* and *C. partellus* cause serious yield losses in maize (Van den Berg, 1997). The maize stem borer, *B. fusca*, is one of the most serious pests of maize and can cause yield losses of 10-60% under favorable conditions (Kfir, 1998). The Chilo borer, *C. partellus*, is better known as a pest of sorghum in South Africa but in recent years has become important in maize production due to an increase in its geographical distribution (Van Rensburg, 2000). The Chilo borer is less injurious than *B. fusca*, but due to differences in larval feeding behavior it is more difficult to control than *B. fusca*, resulting in economically important yield losses (Van Rensburg, 2000).

Busseola fusca and *C. partellus* occur in maize in the Lowveld and Highveld regions and the western maize production areas of South Africa. The pink stem borer, *S. calamistis*, occurs in coastal areas but is becoming increasingly important in the interior of the country. Since 1995 *S. calamistis* has been observed on sweet corn and maize under both center pivot irrigation systems and dryland conditions in the North West and Northern Provinces where it causes stand reductions (Van den Berg & Drinkwater, 2000).

The African bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is regarded as the most important pest of agricultural crops in South Africa because of its wide host range. It is a pest of various grain crops including maize (Du Plessis & Van den Berg, 1999). It attacks maize plants at any growth stage but is only present in large numbers on maize ears during the post-flowering period.

The lorey leafworm (false bollworm), *Acantholeucania (Mythimna) loreyi* (Dup.) (Lepidoptera: Noctuidae) is an insect pest of maize and rice (Hill, 1987). Farmers in South Africa are largely unfamiliar with this pest. In some cases *A. loreyi* damage is

confused with damage caused by the African bollworm and is therefore also known as the False Bollworm.

1.4 Potential advantages of transgenic crops

From an environmental and human-health perspective, the use of genetically modified crops also promises benefits. Many broad-spectrum insecticides reduce the impact of biological control agents that help to control insect and mite pests. Studies have indicated that Bt maize is compatible with biological control and has little effect on natural enemies of pests (Bessin, 2005).

Control of Lepidopteran pests with Bt endotoxins provides four advantages from the grower's perspective. Firstly, control is no longer affected by the weather. The crop is protected even if the field conditions do not allow spray equipment to enter into fields (Meeusen *et al.*, 1989). A second and related advantage is the protection of plant parts that are difficult to reach with insecticide spraying, or the protection of new growth that emerges after spray applications like tillers and ears of maize (Meeusen *et al.*, 1989).

Thirdly, the crop is protected continuously in the field and scouting may no longer be needed. The problem of realizing the presence of lepidopteran pests too late is consequently eliminated (Bessin, 2005). Chemical control of stem borers is complicated because of cryptic feeding deep inside plant whorls where larvae do not easily come into contact with insecticides. A further complication in the chemical control of *C. partellus* is the overlapping of generations owing to staggered pupation and the recurrence of infestation of the same planting at later crop growth stages (Van Rensburg & Van den Berg, 1992). No special application equipment is necessary for insecticide application in the post-flowering period when a Bt crop is planted. Bt crops do not require any specialized equipment and could therefore be effective on farms of all sizes (Bessin, 2005).

Finally, and most likely the most important advantage is the reduction in insecticide applications. A reduction in pesticide application also reduces the potential pesticide drift onto other crops or environmentally sensitive areas (Meeusen *et al.*, 1989). Because the active Bt toxin material is produced directly in the crop tissue, concerns such as spray drift and groundwater contamination are obviated (Meeusen *et al.*, 1989). The use of transgenic crops reduces the use of insecticides and minimizes the impact of these chemicals on non-target organisms and has positive health consequences for farm workers (Barton & Dracup, 2000).

In the Makhathini Flats region of Kwa-Zulu Natal, South Africa, 95% of smallholder (1-3 hectares) cotton producers grew rainfed Bt cotton during the 1999/2000 growing season. The farmers that adopted Bt cotton reported higher yields, reduced insecticide use and a reduction in labor inputs (Ismael, Bennett & Morse, 2001). A typical farmer, often a woman, is now spared 12 days of arduous spraying, saves more than a 1000 liters of water used for spraying, and walks 100 km less per year (Conway, 2004). The University of Pretoria studied the first three seasons of Bt white maize production by 175 small-scale farmers across six sites in South Africa. During the first season yield increases between 21 and 62 percent with an average of 32% was reported with Bt maize above the conventional isoline (Gouse, 2005). Despite a lower than normal rainfall and stem borer pressure in 2002/3, small-scale farmers in KwaZulu Natal enjoyed a statistically significant yield increase of 16% due to better stem borer control with Bt maize. Bt maize adopting-farmers were better off than farmers who planted conventional hybrids, despite the additional technology fee in terms of seed costs (Gouse, 2005).

1.4.1 Environmental and safety benefits

In conclusion the minimizing use of insecticides leads to an increase in biodiversity of non-target insects and, possibly, also birds. The Bt proteins are not toxic to other animals or humans. However, the potential for the development of insect resistance to Bt needs to be carefully monitored (Thomson, 2002).

1.5 Potential disadvantages and uncertainties about transgenic crops

This technology will and have become a major component of insect control strategies. A proper perspective of its potential demands a close look at the limitations and uncertainties, which may reduce its impact on agriculture.

1.5.1 Seed cost and variation in effectiveness

Bt maize seed is more expensive than comparable non-Bt seed. Bt maize is only an advantage when a specific insect pest is present and there is no advantage to plant seed with the Bt gene if the specific pest is not present. Stem borer populations can vary in abundance from year to year and their importance in a given season is not predictable. This is evident from research done by Van Rensburg *et al.* (1985) on *B. fusca*. The seasonal abundance of *B. fusca* moths at five localities in the maize production area of South Africa was monitored by means of Robinson light traps during the 1970's and 1980's. Geographical variation in the flight patterns was shown to exist between localities from east to west. Both the onset and magnitude of the three seasonal moth flights seem to be governed by climatic factors (Van Rensburg, Walters & Giliomee, 1985). In some production areas and during certain growing seasons infestation may be severe or very late.

Variation in effectiveness of Bt maize against the target pest *B. fusca* was observed during the 1998/99 season in South Africa. Considerable stem damage was caused by *B. fusca* in commercial plantings of Bt maize, without leaf feeding damage being visible during the vegetative stages of plant development. This indicated that larvae may survive on some less toxic plant part subsequent to the vegetative stages (Van Rensburg, 2001). While all Bt hybrids control the first generations of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) extremely well, there is a large range of effectiveness against the second generation (Bessin, 2005). Thus Bt maize cannot be effectively utilized if the history of a certain region and pest that occur there is not known.

1.5.2 Cross-pollination of Bt maize

Maize is wind pollinated and can be cross-pollinated with maize pollen from fields within several hundred meters. This may present a problem for producers of food grade maize or non-Bt maize when it is important to keep the grain Bt free. Care should be taken to reduce Bt contamination through cross pollination (Bessin, 2005). Luna *et al.* (2001) conducted experiments to investigate the duration of pollen viability and the effectiveness of isolation distance for controlling gene flow. In this experiment the theoretical, maximum distance that viable pollen could move was 32 km if pollen was transported linearly at the maximum average afternoon wind speeds for the specific location, viability was maintained for 2 h, and pollen settling rate was ignored. Cross pollinations that occurred at a maximum distance of 200 m from the source planting was observed (Luna, Figueroa, Baltazar, Gomez, Townsend & Schoper, 2001).

In Central America two principal concerns regarding the introgression of transgenes into wild maize relatives and land races have been raised. These are possible genetic erosion and increased weediness of maize (Garcia, Figueroa, Gomez, Townsend & Schoper, 1998). The wild relatives of maize, collectively referred to as teosinte are found within the tropical and subtropical areas of Mexico. Maize and teosinte have homologous (functionally identical) chromosomes and hybridize readily. Modern maize is widely considered to be descended from annual teosinte (Garcia *et al.*, 1998). Furthermore, wild plants could become more difficult to control because of their inherent resistance to some of the environmental or cultural challenges they would have faced without the transgene.

1.5.3 Effect of Bt on non-target species

It is difficult to make generalizations about the non-target effects of the *B. thuringiensis* bacteria because of the number of strains and toxins that have been isolated for insect control (Hellmich, Prasifka & Anderson, 2004). Crops that produce these toxins to control some key pests are planted on millions of hectares. The toxins are produced in Bt plants throughout the entire growing season. Thus, target and non-target arthropods have the opportunity to encounter Bt toxins on a continuous basis. This has raised the issue of

whether widespread adoption of Bt crops reduces arthropod abundance and diversity (Sisterson *et al.*, 2004).

Many studies of the impact of Bt crops on non-target organisms have examined the interaction of one or few species under laboratory conditions. Translating laboratory results to the field may however be problematic because toxin doses used in the laboratory may be higher than the doses that the arthropods encounter in the field. The species interactions examined may also not be common in the field, and highly mobile species may spend only a fraction of their lifetime in Bt fields. Despite these limitations, laboratory studies can provide valuable insights into potential effects on non-target organisms (Sisterson *et al.*, 2004).

One of the most important examples of the adverse effects that Bt could have on a non-target organism is that of the Monarch butterfly, *Danaus plexippus* Linnaeus (Lepidoptera: Danaidae) and its host plant, tropical milkweed, *Asclepias curassavica* (Asclepiadaceae). Losey *et al.* (1999) exposed larvae of the monarch butterfly, *D. plexippus*, to leaves of tropical milkweed dusted with pollen from Bt maize. When compared to larvae that fed on leaves with no pollen or leaves with pollen from non-Bt maize, larvae consuming leaves treated with Bt maize pollen consumed less material, weighed less, and had higher mortality. It was subsequently suggested that maize pollen drifting onto the monarch's primary host plant, could pose a danger to the monarch population in areas of the United States where Bt maize is grown (Losey, Rayer & Carter, 1999).

This above mentioned laboratory study resulted in numerous field studies conducted on *D. plexippus*. These studies showed that *D. plexippus* exposure to pollen under field conditions was very low. Monarch larvae developing within or very close to Bt maize fields during pollen shed may encounter small quantities of Bt pollen on the surface of milkweed leaves. However, field studies demonstrated that the amount of Bt pollen deposited on milkweed leaves within maize fields or a short distance away was below the threshold amount that is eaten and which could harm monarch butterfly larvae.

Researchers have found that small monarch larvae can safely consume milkweed leaves containing up to 1100 Bt pollen grains per square centimeter leaf surface (Anon, 2001). This level of pollen deposition was rarely encountered under field conditions.

Although the *Cry* proteins produced in transgenic maize are considered to be specific, some side effects of these toxins on non-target species have been reported (Bourguet, Chaufaux, Micoud, Delos, Naibo, Bombarde, Marque, Eychenne & Pagliari, 2002). The first instar of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), a non-target lepidopteran pest, was shown to contain only one fourth of the concentration after feeding on Bt maize (Obrist, Dutton, Romeis & Bigler, 2006). Dutton *et al.* (2003) suggested that the negative effects observed on *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) were most likely not caused by susceptibility of the predator to the toxin but were due to toxin effects on the larvae of *S. littoralis*.

This result has important implications for agriculture. In this case only one of the large number of insects existing inside or in the vicinity of cultivated fields was studied. It cannot be excluded that many other moths and butterflies, including endangered ones, may be at risk from Bt maize.

1.5.4 Resistance development of target pests

Transgenic crops are grown on >62 million hectares worldwide (Tabashnik, Carrière, Dennehy, Morin, Sisterson, Roush, Shelton & Zhao, 2003). This huge interest in Bt crops has magnified the risk of target insect pest species rapidly adapting and becoming resistant to this class of toxin (Génissel, Augustin, Courtin, Pilate, Lorme & Bourguet, 2003). A concern already voiced has to do with the rate at which resistance will develop in the target insect population. For crops containing the Bt endotoxin, history provides some comfort that it will not be rapid. Products based on the endotoxin have been in use for over a quarter century without reports of significant resistance development in the field (Meeusen *et al.*, 1989). Bessin (2005) predicted that European corn borer, *O. nubilalis* populations have the potential to develop tolerance or become resistant to Bt

endotoxin as more acreage is planted with Bt hybrids. Producers need to prevent the development of resistance by using specific approved resistance management strategies.

Target pests exposed to Bt crops can potentially develop resistance to the Bt toxin. Resistance development occurs repeatedly with conventional chemical insecticides and is thus possible with Bt crops (Thomson, 2002). Basically, the higher the level of resistance of host plants, the stronger the selection pressure on the pest to develop resistance. Populations of the diamondback moth, *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae) and Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), for example, have been reported to show resistance to spray formulations of Bt (Mellet, Schoeman, Broodryk & Hosf, 2004). Sayyed *et al.* (2003) hypothesized that Bt toxin produced by transgenic crops could have nutritionally favorable effects that increase the fitness of resistant insects eating such crops. This idea was based on increased pupal weight of resistant larvae of *P. xylostella* that were fed leaf discs treated externally with a Bt toxin (Sayyed, Cerda & Wright, 2003). Tabashnik and Carrière (2004) however summarized evidence from diamondback moth and other pests showing that the Bt toxins in transgenic crops do not enhance performance of resistant insects. For instance, tobacco hornworm, *Manduca sexta* Linnaeus (Lepidoptera: Sphingidae) larvae that feed on diets with low nicotine concentration are very susceptible to the Bt bacterium, whereas they are unaffected by the bacterium when feeding on a diet with high nicotine concentration.

A study carried out in China showed that the toxin content in Bt cotton varieties changed significantly over time, depending on the part of the plant, the growth stage and the variety. The researchers pointed out that such variability in toxin expression could accelerate the development of pest resistance to the toxin (Ho, 2005). Researchers in India found that the amount of Cry1Ac protein varied across cotton varieties and between different plant parts and that an increased number of *H. armigera* larvae survived on Bt cotton bolls (Sharma & Pampapathy, 2006). The evidence that pests can overcome Bt toxin in cotton also suggest the possibility that Lepidoptera that feed on Bt maize can develop resistance.

As far as known, no pest population has evolved resistance in the field to a Bt crop (Tabashnik *et al.*, 2003). Tabashnik *et al.* (2003), reported in laboratory and greenhouse tests, that at least seven resistant laboratory strains of three pests, i.e. *P. xylostella*, *Pectinophora gossypiella* (Saunders) and *H. armigera*, have completed development on Bt crops. The success of Bt crops to date exceeds the expectations of many, but does not preclude resistance problems in future. Chaufaux *et al.* (2001) reported that all selected strains of *O. nubilalis* developed significantly increased tolerance after chronic exposure to the Cry1Ab toxin in laboratory studies. These results suggest that low levels of resistance are common among widely distributed *O. nubilalis* populations (Chaufaux, Seguin, Swanson, Bourgeut & Siegfried, 2001).

From the above information it is clear that the production of Bt crops have potential advantages and disadvantages. The greatest advantage is that Bt is an alternative for wide spectrum insecticides applied for stem borer control. Although there are great advantages one cannot overlook the possible disadvantages. If disadvantages are ignored it could result in worse problems and development of new pests.

1.6 Aspects of Bt maize in South Africa

An application to the South African Department of Agriculture during 1989 to perform field trials with genetically modified cotton, initiated the South African biosafety process and the first trials with transgenic crops on the African continent (Gouse, 2005). In 1997 South Africa became the first country in Africa to commercially produce transgenic crops (Gouse, Pray, Kirsten & Schimmelpfennig, 2005). To date the commercial release of insect-resistant (Bt) cotton and maize as well as herbicide-tolerant (RR) soya-beans, cotton and maize have been approved. Farmers started adopting Bt cotton varieties during the 1997/98 season and Bt yellow maize during the 1998/99 season. Bt white maize was introduced during the 2001/2 season and 2002/3 saw the first season of large-scale Bt

white maize production (Gouse, 2005). The areas planted under transgenic crops in South Africa are presented in Table 1.1.

Yellow maize is grown in large quantities and is primarily used as animal feed and as an input in the food industry in South Africa (Gouse *et al*, 2005). The initial spread of Bt yellow maize was quite slow in 2000/1, with farmers planting less than 3% of the total maize area under Bt maize. Possible reasons for this were that the Bt hybrids were not well adapted to the local production conditions and that many farmers did not foresee a significant productivity increase from the use of Bt seed. Many farmers believe that if they manage to plant at the recommended time, in order to escape periods of peak stem borer moth flights, their crop would suffer limited damage whether they plant Bt maize or not. The third reason was the farmers' concern that they might not be able to sell their harvest because of consumer concerns about genetically modified food (Gouse, 2005). Gouse *et al.* (2005) found that commercial maize farmers benefited economically from the use of Bt maize. Despite paying more for seeds, farmers who adopted Bt maize enjoyed increased income from Bt maize compared to conventional maize through savings on pesticides and increased yield due to better pest control. Based on the findings of three years of research it was concluded that small-scale subsistence farmers in South Africa can benefit from the use of genetically modified insect resistant maize (Gouse, 2005). In this research where the focus was on the production side of Bt maize the scenario looks promising. However, the possible environmental impacts of planting Bt should not be ignored.

In a study conducted during the 1998/99 growing season considerable stem damage caused by *B. fusca* was observed in commercial plantings of Bt maize. This indicated that larvae may survive on some less toxic plant parts subsequent to the vegetative growth stages (Van Rensburg, 2001). The Bt protein concentration in silks appears to be low enough to allow survival of some larvae until completion of the first two instars, after which the ear tips and husk leaves serve as important feeding sites. The upper stem appears to be less toxic than the lower stem, providing a site for early penetration of stems by young larvae, culminating in the eventual successful penetration of the lower

stem at later stages of plant and larval development. Increased larval survival resulted in a significant increase in the incidence of ear damage, which appears to be most pronounced within the first 14 days after infestation. Observed damage did not result in significant yield losses, but does not exclude the possibility that currently used Bt hybrids may suffer economically important yield losses at the high levels of natural infestation often experienced with late planting dates (Van Rensburg, 2001).

Transgenic maize (MON 810), expressing *Cry1Ab* is also highly effective in controlling *C. partellus* (Van Rensburg, 2001). Under greenhouse conditions in India Bt maize provided effective protection against *C. partellus*, even under high levels of larval infestation. (Singh, Channappa, Deeba, Nagaraj, Sukavaneaswaran & Manjunath, 2005).

1.7 The high dose/refuge strategy

The main environmental threat of Bt crops is that their widespread cultivation could lead to insect resistance to Bt toxins. The high dose/refuge resistance management plan may make theoretical sense, but the practical situation is still uncertain because there are huge gaps in knowledge about pest genetics and about insect and plant ecology (Renner, 1999).

The high dose/refuge strategy is based on a combination of transgenic plants producing high doses of toxin, with the presence of nearby non-Bt plants or refuges. The purpose of the high dose is to kill off as many pests as possible. The purpose of the refuge is to produce pest individuals that survive on the particular crop. The goal is to make sure that a rare, resistant insect that survives on the Bt crops does not produce completely resistant offspring by mating with another toxin-resistant insect. Instead, susceptible individuals from the refuge are expected to mate with toxin-resistant individuals that survive on the engineered plants. Offspring from these matings are expected to have only a low to moderate level of toxin resistance and should not be able to survive on plants with high Bt toxin levels (Renner, 1999; Gould, 2000).

The basis of integrated resistance management is that growers must plant sufficient acreage of non-Bt crops to serve as “refuges” for pests. This decreases the selection pressure for the development of Bt resistant insects and ensures that Bt susceptible pests will be available as mates for Bt resistant insects, should they develop (Thomson, 2002). In Africa it should be possible to use this system on commercial farms but it remains to be seen how effectively it can be managed among small-scale farmers. The authorities in charge of regulating the use of GM crops in these countries will have to pay particular attention to this (Thomson, 2002).

1.8 Biosafety aspects

There is currently no information on non-target effects of GM maize that could be used in the development of risk assessment methods and biosafety protocols in South Africa. Issues such as ecological risks and management change impacts as well as consequences of extensive GM plantings on biodiversity in South Africa urgently need to be addressed (McGeoch & Pringle, 2005). In their publication on risk assessment regarding GM maize in Africa, Hilbeck & Andow (2004) indicated that assessment of biodiversity and non-target impacts of GM maize, as well as life-table studies on prominent non-target insects are critical steps in environmental risk assessments.

An important step in research on the impact of transgenic maize is to identify target and non-target lepidopterans that occur in maize fields. Such a species checklist will provide important information on Lepidoptera species diversity and would identify species that need further investigation. This study will provide base line data on Lepidoptera in South Africa. Information on which Lepidoptera species are exposed to Bt maize will be provided to identify sensitive species that could be likely candidates for developing resistance to Bt toxin. There is a need for research on environmental issues, including potential impacts on non-target Lepidoptera and beneficial species because information on ecological impacts of these crops on the environment is hard to find.

Changes in the abundance of target pests may shift the pest status of other non-target herbivores that could become secondary pests. This could result in changes in the biodiversity and abundance of natural enemies, and lead to negative consequences on the biological control functions on other non-target herbivores.

Apart from the target pests of Bt maize no information is available on non-target Lepidoptera species, that feed on maize and that could be exposed to the Bt toxin. This is the case with *S. calamistis*, a stem borer of which larval behavior differs from other borer species and which could possibly result in survival on Bt maize.

1.9 Objectives

The objectives of this study were to assess the diversity of both target and non-target Lepidoptera species that feed on maize, and to determine the effect of Bt-maize on the biology of one non-target Lepidoptera species. This information was subsequently used to select non-target Lepidoptera species for ecological risk analysis in South Africa. These objectives are reported on in the following chapters:

- The succession of Lepidoptera species and damage caused in Bt- and non-Bt maize under field conditions in South Africa.
- Diversity and host plant range of Lepidoptera that occur on Bt- and non-Bt maize in South Africa.
- The effect of Bt maize on *Sesamia calamistis* (Lepidoptera: Noctuidae) in South Africa.
- Non-target Lepidoptera species selection for ecological risk assessment of Bt maize in South Africa.

This study provided base line data on biodiversity of Lepidoptera and information on which Lepidoptera species are directly exposed to Bt maize, was obtained. It also provided the first attempt at a risk assessment model for Bt maize in South Africa This

information will in future play a role in the development of resistance management strategies if resistance to Bt maize develops in certain pests. This study will serve as a basis from which to provide early-warning of possible future changes in the abundance of target pests that may shift over time, resulting in changes in pest status of non-target herbivores.

1.10 References

Annecke, D.P. & Moran, V.C. 1982. Insects and mites of cultivated plants in South Africa. The Butterworth Group. Durban/Pretoria.

Anonymous. 2001. Bt corn and the monarch butterfly. Council for Biotechnology Information. Good ideas are growing. www.whybiotech.com accessed 24 January 2006.

Barton, J.E. & Dracup, M. 2000. Genetically modified crops and the environment. *Agronomy Journal* 92: 797 – 803.

Bessin, R. 2005. Bt-corn for corn borer control. University of Kentucky College of Agriculture. <http://www.uky.edu/Agriculture/Entomology/entfacts/fldcrops/ef118.htm>. accessed 13 October 2005.

Bhatia, J., Grant S.E. & Powell, D.A. 1999. Backgrounder: genetically-engineered Bt containing field corn. *Technical Report* 10: 1 – 12.
<http://www.foodsafetynetwork.ca/gmo/updated-bt-backgrounder.htm> accessed 20 October 2005.

Bourguet, D., Chaufaux, J., Micoud, A., Delos, M., Naibo, B., Bombarde, F., Marque, G., Eychenne, N. & Pagliari, C. 2002. *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (*Zea mays*). *Environmental Biosafety Research* 1: 49 – 60.

Chaufaux, J., Seguin, M., Swanson, J.J., Bourguet, D. & Siegfried, B.D. 2001. Chronic exposure of the European corn borer (Lepidoptera: Crambidae) to Cry1Ab *Bacillus thuringiensis* toxin. *Journal of Economical Entomology* 94: 1564 – 1570.

Conway, G. 2004. GM Crops – an international perspective on the economic and environmental benefits. pp. 3 – 24. GM Crops – Ecological Dimensions (Van Emden, H.F. & Gray, A.J.) The Association of Applied Biologists. *Aspects of Applied Biology* 74.

Dutton, A., Romeis, J. & Bigler, F. 2003. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt maize expressing Cry1Ab as a case study. *BioControl* 48: 611 – 636.

Du Plessis, H. 2000. Common cutworm – a pest of grain crops. *Crop Protection Series no. 19*. ARC-Grain Crop Institute. Potchefstroom. South Africa.

Du Plessis, H. & Van den Berg, J. 1999. African bollworm – a pest of grain crops. *Crop Protection Series no. 16*. ARC-Grain Crop Institute. Potchefstroom. South Africa.

Garcia, M. C., Figueroa, J.M., Baltazar, B.M., Gomez, R.L., Townsend, R. & Schoper, J.B. 1998. Seed physiology, production and technology. Pollen control during transgenic hybrid maize development in Mexico. *Crop Science* 38: 1597 – 1602.

Génissel, A., Augustin, S., Courtin, C., Pilate, G., Lorme, P. & Bourguet, D. 2003. Initial frequency of alleles conferring resistance to *Bacillus thuringiensis* poplar in a field population of *Chrysomela tremulae*. *Proceeding of the Royal Society of London* 270: 791 – 797.

Gould, F. 2000. Testing Bt refuge strategies in the field. *Nature Biotechnology* 18: 266 – 267.

Gouse, M. 2005. Aspects of biotechnology and genetically modified crops in South Africa. Science, Technology and Globalization Project. Agricultural Biotechnology for Development – socioeconomic issues and institutional challenges. Belfer Center STPP. Kennedy School of Government.

http://bcsia.ksg.harvard.edu/BCSIA_content/documents/SouthAfricaGouse.pdf accessed 21 May 2006.

Gouse, M., Pray, C.E., Kirsten, J. & Schimmelpfennig, D. 2005. A GM subsistence crop in Africa: the case of Bt white maize in South Africa. *International Journal of Biotechnology* 7: 84 – 94.

Hall, H. 2004. A controversy in our corn: Is Bt corn safe? BioTeach. <http://www.bioteach.ubc.ca/Bioengineering/index.htm> accessed 20 Augustus 2004.

Hellmich, R.L., Prasifka, J.R. & Anderson, P.L. 2004. Effects of Bt plants on non-target herbivores. pp. 75 – 80. In GM Crops – Ecological Dimensions (Edi: Van Emden H.F. & Gray, A.J.) The Association of Applied Biologists. *Aspects of Applied Biology* 74.

Hilbeck, A. & Andow, D.A. 2004. Environmental risk assessment of genetically modified organisms: Volume 1. A case study of Bt maize in Kenya. CAB International, Wallingford, UK.

Hill, D.S. 1987. Agricultural insect pests of the tropics and their control. Second edition. Cambridge University Press. United Kingdom.

Ho, M. 2005. Scientists confirm failures of genetically engineered Bt-crops. *Organic Consumers Association*. www.i-sis.org.uk/SCFOBTC.php accessed 6 October 2005.

Höfte, H. & Whiteley, H.R. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Reviews* 53: 242-255.

Ismael, Y., Bennett, R. & Morse, S. 2001. Biotechnology in Africa: The adoption and economic impact of Bt cotton in the Makhathini Flats, Republic of South Africa. Biotechnology conference for Sub-Saharan Africa. 26th – 27th September, Johannesburg, South Africa.

James, C. 2004. Global status of commercialized biotech/GM crops. *ISAAA Briefs* 32: 1-11.

Kfir, R. 1998. Maize and grain sorghum: Southern Africa. (ed. A. Polaszek). African cereal stem borers. Economic importance, taxonomy, natural enemies and control. CAB International. Pp 29 – 37.

Kroon, D.M. 1999. Lepidoptera of Southern Africa. Host-plants and other associations. A Catalogue. *Lepidopterists' Society of Africa*.

Kumar, P.A., Sharma, R.P. & Malik, V.S. 1996. The insecticidal proteins of *Bacillus thuringiensis*. *Advances in Applied Microbiology* 42: 1 – 43.

Lambert, B., Buysse, L., Decock, C., Jansens, S., Piens, C., Saey, B., Seurinck, J., Van Audenhove, K., Van Rie, J., Van Vliet, A. & Peferoen, M. 1996. A *Bacillus thuringiensis* insecticidal crystal protein with a high activity against members of the family Noctuidae. *Applied and Environmental Microbiology* 62: 80 – 86.

Losey, J.E., Rayor, L.S. & Carter, M.E. 1999. Transgenic pollen harms monarch larvae. *Nature (London)* 399: 214.

Luna, S.V., Figueroa, J.M., Baltazar, B.M., Gomez, R.L., Townsend, R. & Schoper, J.B. 2001. Maize pollen longevity and distance isolation requirements for effective pollen control. *Crop Science* 41: 1551 – 1557.

McGeoch, M.A. & Pringle, K.L. 2005. Science and advocacy: the GM debate in South Africa. *South African Journal of Science* 101: 7 – 9.

Mellet, M.A., Schoeman, A.S., Broodryk, S.W. & Hofs, J.L. 2004. Bollworm occurrences in Bt and non-Bt cotton fields, Marble Hall, Mpumalanga, South Africa. *African Entomology* 12: 107 -115.

Meeusen, R.L. & Warren, G. 1989. Insect control with genetically engineered crops. *Annual Review of Entomology* 34: 373 – 381.

Obrist, L.B., Dutton, A., Romeis, J. & Bigler, F. 2006. Biological activity of Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. *BioControl* 51: 31 – 48.

Renner, R. 1999. Will Bt-based pest resistance management plans work? *Environmental Science and Technology* 33: 410 – 415.

Sayyed, A.H., Hugo, C. & Wright, D.J. 2003. Could Bt transgenic crops have nutritionally favourable effects on resistant insects? Ideas and Perspectives. *Ecology Letters* 6: 167 – 169.

Sharma, H.C. & Pampapathy, G. 2006. Influence of transgenic cotton on the relative abundance and damage by target and non-target insect pests under different protection regimes in India. *Crop Protection* 25: 800 – 813.

Sharma, H.C., Sharma, K.K., Seetharama, N. & Ortiz, R. 2000. Prospects for using transgenic resistance to insects in crop improvement. *Electronic Journal of Biotechnology* 3: 76 – 95.

Singh, R., Channappa, R.K., Deeba, F., Nagaraj, N.J., Sukavaneaswaran, M.K. & Manjunath, T.M. 2005. Tolerance of Bt corn (MON 810) to maize stem borer, *Chilo partellus* (Lepidoptera: Pyralidae). *Plant Cell Reports* 24: 556 – 560.

Sisterson, M.S., Biggs, R.W., Olson, C., Carrière, Y., Dennehy, T.J. & Tabashnik, B.E. 2004. Arthropod abundance and diversity in Bt and non-Bt cotton fields. *Environmental Entomology* 33: 921 – 929.

Tabashnik, B.E. & Carrière, Y. 2004. Bt transgenic crops do not have favorable effects on resistant insects. *Journal of Insect Science* 4:1 -3.

Tabashnik, B.E., Carrière, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M. & Zhao, J. 2003. Insect resistance to transgenic Bt crops: lessons from the laboratory and field. *Journal of Economic Entomology* 96: 1031 – 1038.

Thomson, J.A. 2002. Genes for Africa. Genetically modified crops in the developing world. UCT Press. Cape Town. South Africa.

Van den Berg, J. 1997. Economy of stem borer control in sorghum. *Crop Protection Series no. 2*. ARC-Grain Crops Institute. Potchefstroom. South Africa.

Van den Berg, J. 1997. Stem borers in sorghum. *Crop Protection Series no. 3*. ARC-Grain Crops Institute. Potchefstroom. South Africa.

Van den Berg, J. & Drinkwater, T.W. 2000. Pink stem borer. *Crop Protection Series no. 20*. ARC-Grain Crops Institute. Potchefstroom. South Africa.

Van Rensburg, J.B.J. 2001. Larval mortality and injury patterns of the African stalk borer, *Busseola fusca* on various plant parts of Bt-transgenic maize. *South African Journal of Plant and Soil* 18: 62 – 69.

Van Rensburg, J.B.J. 2000. The Chilo borer in maize. *Crop Protection Series no. 22*. ARC-Grain Crops Institute. Potchefstroom. South Africa.

Van Rensburg, J.B.J. & Van den Berg, J. 1992. Stem borers in grain sorghum: II. Yield loss compensation in relation to borer attack. *South African Journal of Plant and Soil* 9: 81 – 86.

Van Rensburg, J.B.J., Walters, M.C. & Giliomee, J.H. 1985. Geographical variation in the seasonal moth flight activity of the maize stalk borer, *Busseola fusca* (Fuller), in South Africa. *South African Journal of Plant and Soil* 2: 123 -126.

Van Rie, J., Jansens, S., Höfte, H., Degheele, D. & Van Mellaert, H. 1989. Specificity of *Bacillus thuringiensis* δ -endotoxins. Importance of specific receptors on the brush border membrane of the mid-gut of target insects. *European Journal of Biochemistry* 186: 239 - 247.

Wasano, N., Saitoh, H. & Ohba, M. 1997. A high homology exists in N-terminal amino acid sequences of δ -endotoxins between Lepidoptera-specific and Coleoptera-specific *Bacillus thuringiensis* strains. *Letters in Applied Microbiology* 24: 438 – 440.

Table 1.1 Percentage and estimated areas (hectares) planted to Bt transgenic crops in South Africa (Gouse, 2005).

Crop	1999/2000	2000/2001	2001/2002	2002/2003	2003/2004
% Bt cotton	50%	<40%	70%	70%	81%
Bt cotton area	13 200	12 000	25 000	18 000	30 000
% Bt yellow maize	3%	5%	14%	20%	27%
Bt yellow maize area	50 000	75 000	160 000	197 000	250 000
% Bt white maize	0	0	0.4%	2.8%	8%
Bt white maize area	0	0	6 000	55 000	175 000

Table 1.2 Lepidoptera pests of maize in South Africa (Annecke & Moran, 1982).

	Common name	Scientific name	Family
Stem borers	Maize stem borer	<i>Busseola fusca</i> (Fuller)	Noctuidae
	Pink stem borer	<i>Sesamia calamistis</i> (Hampson)	Noctuidae
	Sorghum stem borer	<i>Chilo partellus</i> (Swinhoe)	Pyralidae
Cutworms	Black cutworm	<i>Agrotis ipsilon</i> (Hufnagel)	Noctuidae
	Brown cutworm	<i>Agrotis longidentifera</i> (Hampson)	Noctuidae
	Common cutworm	<i>Agrotis segetum</i> (Denis & Schiffermüller)	Noctuidae
	Grey cutworm	<i>Agrotis subalba</i> (Walker)	Noctuidae
Bollworms	African bollworm	<i>Helicoverpa armigera</i> (Hübner)	Noctuidae
Army worms	Army worm	<i>Spodoptera exempta</i> (Walker)	Noctuidae
	Lesser army worm	<i>Spodoptera exigua</i> (Hübner)	Noctuidae

Table 1.3 Other Lepidoptera species that occur on maize in South Africa (Kroon, 1999).

	Family name	Scientific name
1.	Tortricidae	<i>Crociosema plebejana</i> (Zeller)
2.	Arctiidae	<i>Alpenus investigatorum</i> (Karsch)
3.	Arctiidae	<i>Alpenus maculosus</i> (Stoll)
4.	Noctuidae	<i>Borolia torrentium</i> (Guenée)
5.	Lymantriidae	<i>Bracharoa mixta</i> (Snellen)
6.	Pyralidae	<i>Chilo orichalcociliellus</i> (Strand)
7.	Pyralidae	<i>Marasmia trapezalis</i> (Guenée)
8.	Notodontidae	<i>Phalera lydenburgi</i> (Distant)
9.	Arctiidae	<i>Spilosoma lutescens</i> (Walker)
10.	Noctuidae	<i>Spodoptera littoralis</i> (Boisduval)
11.	Noctuidae	<i>Trichoplusia orichalcea</i> (Fabricius)
12.	Hesperiidae	<i>Zenonia zeno</i> (Trimen)
13.	Hesperiidae	<i>Borbo borbonica</i> (Boisduval)
14.	Hesperiidae	<i>Borbo gemella</i> (Mabille)
15.	Hesperiidae	<i>Pelopidas mathias</i> (Fabricius)

CHAPTER 2: THE SUCCESSION OF LEPIDOPTERA SPECIES AND DAMAGE CAUSED IN BT- AND NON-BT MAIZE UNDER FIELD CONDITIONS IN SOUTH AFRICA

2.1 Abstract

Several stem borer species occur on maize in South Africa with *Busseola fusca* and *Chilo partellus* being the most important. Transgenic maize containing event MON 810 expressing *Cry1Ab* protein is the only Bt maize event registered for use against these stem borers in South Africa. This study was done to assess the possible effects of Bt maize on the incidence of Lepidoptera under field conditions on commercial maize farms. Four field experiments were conducted during the 2004/5 and 2005/6 cropping seasons in which the succession of different Lepidoptera species and incidences of damaged plants were monitored over time. Monitoring was done at different growth stages from the early whorl to soft dough stages of crop development. Three to nine hundred maize plants per field were inspected for Lepidoptera larvae and damage recorded. Infestation levels and incidence of damage were compared between Bt and non-Bt maize fields. The following Lepidoptera species were recorded on maize: *B. fusca*, *C. partellus*, *Sesamia calamistis*, *Helicoverpa armigera* and *Acantholeucania loreyi*. Only *B. fusca*, *H. armigera* and *A. loreyi* were recorded on Bt maize. The incidence of Lepidoptera-infested plants and infestation levels were generally lower in Bt fields than in non-Bt fields, possibly indicating that these species may be affected by Bt maize. Results showed that *B. fusca* larvae were able to survive on Bt maize at low infestation levels.

2.2 Introduction

The most important Lepidopterous pests of maize in South Africa are the stem borers *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). The maize stem borer, *B. fusca* is noted for its injuriousness to maize and in epidemic seasons it may cause severe yield losses despite chemical control (Van Rensburg, 1999). Annual yield losses due to *C. partellus* have never been estimated. This species is less injurious than *B. fusca* but due to pronounced differences in habits it is often more difficult to control chemically, resulting in economically important yield losses (Van Rensburg, 2000). Genetically modified (Bt) maize containing the *Cry1Ab* gene that encodes a protein with insecticidal activity is used for control of these stem borers on approximately 425 000 ha in South Africa (Gouse, 2005).

Bt maize was initially developed to control *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae). *Ostrinia nubilalis* was reported to be one of the most damaging insect pests of maize throughout the USA, and severe yield losses have also been reported in Europe (Castanera & Orteg, 2005). Various events of the Bt genes are available, exhibiting high levels of toxicity to *O. nubilalis* and southwestern corn borer, *Diatraea grandiosella* (Dyer) (Lepidoptera: Pyralidae) in the USA (Sharma, Sharma, Seetharama & Ortiz, 2000). Van Rensburg (1999) concluded after evaluation of Bt maize for resistance to *B. fusca* and *C. partellus* that the various events of the *Cry1Ab* gene were not equally effective against *B. fusca*. MON 810 was found to be superior to the other events and *C. partellus* was more susceptible than *B. fusca* to the same Bt-events (Van Rensburg, 1999).

No research has been done on the effect of Bt maize on other Lepidoptera that feed on maize in South Africa. Several Lepidoptera species that attack maize can attain pest status in South Africa (Table 1.1) (Annecke & Moran, 1982; Hill, 1987). These Lepidoptera species are directly exposed to Bt toxin by feeding on Bt maize and may therefore be affected by the toxin.

The objectives of this study were to determine the incidence of plants damaged by Lepidoptera on Bt and non-Bt maize fields over time, and to assess the possible effects of Bt maize on other Lepidoptera species that feed directly on maize under field conditions.

2.3 Material and methods

Four experiments were conducted in which the succession of Lepidoptera species and incidence of plant damage in maize fields were monitored over time. Three experiments were conducted on commercial farms where the non-Bt maize fields that were monitored were planted as the prescribed refuges for the Bt fields. One experiment was conducted on a research farm. Planting dates of the Bt and non-Bt fields differed at most by three days. The same sampling procedures were followed in non-Bt and adjacent Bt maize fields in order to compare data between fields.

Because damage caused by the different Lepidoptera species cannot easily be distinguished, the data presented on incidence of damaged plants was compiled. However, at one site in this study, data could be separated for *H. armigera* and *A. loreyi* since larvae were present on plants.

2.3.1 Experiment 1: Succession study (2004/2005 season)

During the 2004/5 cropping season an experiment was conducted under dryland conditions on a farm at Wolmaransstad (S 27°00.830; E 025°56.780; 1469m above sea level (a.s.l.)) in the North-West province. In this experiment one 20 hectare Bt field (CRN 78-15B) and an adjacent 50 hectare non-Bt field (iso-hybrid: CRN 3505) was planted.

The incidence of plants damaged by different Lepidoptera species were recorded over time and compared between fields. Plants were inspected for damage at five different plant growth stages, from the seedling to the soft dough stage. On the first three sample dates (early to late whorl stages) three blocks each of Bt and non-Bt maize were marked

inside the larger fields. Each block consisted of 20 rows of 100m in length and an inter-row spacing of 1.5m (0.3 ha). These blocks were inspected for any species of Lepidoptera and damage symptoms were recorded. Larvae detected on damaged plants were reared in petri-dishes on the plant part that larvae were found on until the moth stage. Since it was much more time consuming to inspect large plants the number of plants that was inspected was reduced after the flowering stage. With the last two sampling dates (post-flowering stage) three hundred maize plants per field (three replicates of 100 plants each) were inspected for any species of Lepidoptera and damage symptoms recorded. Stem borer species were determined by dissecting ten randomly selected plants exhibiting stem borer damage. Larvae were collected from infested plants and reared until the moths appeared. Stem borer larvae were reared on Bt- or non-Bt maize stems, depending on which they were found. Moths were pinned and preserved to facilitate identification.

2.3.2 Experiment 2: Succession study (2005/2006 season)

During the 2005/6 season two experiments were conducted, one under center pivot irrigation at Castello farms (S 26°21.416; E 027°06.565; 1482m a.s.l.) near Potchefstroom in the North-West province, and the other under dry land conditions on the farm Hartlam (S 27°47.197; E 028°28.571; 1648m a.s.l.) at Reitz in the Free State province. The cultivars planted at Castello were CRN 78-15B (Bt) and SC 710 (non-Bt). The Bt cultivar, CRN 4760B was planted at Hartlam and Phb 3442 (non-Bt) as control. The incidence of damaged plants could have been influenced by the fact that the non-Bt cultivars used as controls were not the iso-hybrids of the Bt hybrids, but this effect is expected to be minimal. Nine hundred maize plants (three replicates of 300 plants each) were inspected for Lepidoptera larvae and damage recorded at different growth stages from the early whorl stage to post flowering. Ten randomly selected plants exhibiting stem borer damage symptoms were dissected to determine which stem borer species were present.

2.3.3 Experiment 3: Succession study (2005/2006 seasons)

A field experiment was conducted on the Syferkuil research farm (S 23°50.103; E 029°41.558) of the University of Limpopo near Polokwane in the Limpopo Province.

The experiment consisted of a randomized block design with two Bt maize hybrids and their non-Bt iso-hybrids, each replicated three times. The four cultivars used were CRN 78-15 (Bt), its non-Bt iso-hybrid CRN 3505, Pan 6012 (Bt) and its non-Bt iso-hybrid Pan 6804. Each hybrid was planted in a block that consisted of 11 rows of 10m in length with a 1.0m inter-row spacing. The blocks were separated by a 2m inter-block space. Only the nine middle rows were used for data collection while the outer rows served as side rows. Plant stand per row was determined when plants were six weeks old.

Maize plants were inspected for Lepidoptera larvae and the incidence of damage recorded 6, 9, 13, 16 and 20 weeks after plant emergence. Larvae observed on plants were collected and reared to adults. The number of damaged plants was expressed as a percentage of the number of plants in each row, and a mean determined for each replicate.

In order to determine which stem borer species occurred on plants and at which infestation levels, a total of 10 plants that exhibited stem borer damage symptoms were selected from the two side rows of each block at each sampling date and dissected.

2.4 Data analysis

The data were analysed separately for each site to compare infestation levels and incidence of damage between Bt and non-Bt fields. At the Wolmaransstad site, damage symptoms was separated for *H. armigera*, *A. loreyi* and stem borers since larvae of the first two species were present on plants. This was not possible at Polokwane and only damage symptoms could be recorded. Repeated measures analysis of variance (ANOVA) was used to compare the incidence of the Lepidoptera damaged plants in Bt- and non-Bt fields at different growth stages during the cropping season. Statistical analysis was done

with Statistica software (version 7.1). In cases where non-significance were indicated by ANOVAs, the independent t-test and the Mann-Whitney U test were used to compare damage of Bt- and non-Bt maize at a specific growth stage.

2.5 Results

2.5.1 Experiment 1: Succession study (2004/2005 season)

Damage caused by lepidopteran was observed on both Bt and non-Bt hybrids from the late whorl stage to the soft dough stage of crop development.

The incidence of *H. armigera* damage was significantly higher in the non-Bt than Bt maize field ($F_{(4, 16)} = 15.254$, $P = 0.000025$) (Fig. 2.1). The incidence of damaged plants during the flowering stage was 0.7% on Bt and 4.3% on non-Bt plants. The incidence of damaged plants during the milk stage was 8.3% on Bt- and 80% on non-Bt plants. The incidence of *A. loreyi* damaged plants during flowering did not differ significantly and was 0.4% on both Bt and non-Bt plants ($F_{(2,8)} = 4.169$, $P = 0.057478$) (Fig. 2.2). A significant difference however was observed during the milk stage with the incidence of infested plants being 0.9% and 2.7% on the Bt and non-Bt field respectively ($t_{(2,6)} = -5.014$; $P = 0.0209$).

The incidence of stem borer damage increased over time and differed significantly between the various growth stages ($F_{(4,16)} = 88.899$, $P < 0.000001$) (Fig. 2.3). Although stem borer damage was present during the pre-flowering stages it increased rapidly to 2.8% on Bt and 12% on non-Bt plants during the flowering stage. Damage was significantly lower on Bt plants but was observed to be 12.5% infested plants at the soft dough stage (Fig. 2.3). The only stem borer that was recovered from Bt maize was *B. fusca*. It can therefore be assumed that all whorl damage symptoms could be ascribed to *B. fusca*. No *C. partellus* were ever recovered from Bt maize.

2.5.2 Experiment 2: Succession study (2005/2006 season)

At the Castello site, the first stem borer damage symptoms were only observed during the post-flowering stage. *Busseola fusca* was the only stem borer species recovered from damaged Bt plants. The incidence of damage was significantly lower on Bt than non-Bt maize ($F_{(2,8)} = 85.399$, $P = 0.000004$) with 0.77 % on Bt maize during the milk stage compared to the 13.11 % on non-Bt maize (Fig. 2.4). The stem borer infestation level could not be determined on non-Bt maize during the soft dough stage because ears were removed from plants for use as green maize.

At Reitz stem borer damage was observed on both Bt and non-Bt maize plants from the mid-whorl stage to the milk stage (Fig. 2.5). There were no significant differences in the incidence of damaged plants between Bt and non-Bt maize fields ($F_{(2,8)} = 1.121$, $P = 0.3721$). During the mid-whorl stage stem borer infestation was 2.4% on Bt- and 6.0% on the non-Bt field but did not differ significantly ($t_{(3,5)} = -1.735$; $P = 0.13$). The only stem borer that was recovered from Bt maize was *B. fusca*.

2.5.3 Experiment 3: Succession study (2005/2006 seasons)

Stem borer damage was observed on all four the cultivars (Fig. 2.7). The incidence of stem borer damaged plants was significantly lower in Bt plots than in non-Bt plots for both the CRN 78-15B ($F_{(4,16)} = 60.398$, $P < 0.000001$) and Pan 6012B ($F_{(4,16)} = 43.248$, $P < 0.000001$) hybrids. The highest incidence of stem borer damaged plants was observed nine weeks after plant emergence when 32.3% of Bt and more than 70% of non-Bt plants showed symptoms of stem borer feeding. Stem borer damage symptoms on Bt plants were however limited to minute pin-hole type feeding lesions that were difficult to spot. The latter damage symptoms were characteristic of 1st instar feeding. The incidence of damage that was observed 16 weeks after emergence was lower than at the earlier samplings because of the difficulty to observe damage on old plants and because plants were physiologically mature and leaves started drying off. At the hard dough stage (20 weeks after emergence) symptoms of stem borer infestation on ears and stems was 16.4% on Pan 6804 (non-Bt) and 9.1% on CRN 3505 (non-Bt), with few Bt plants exhibiting only superficial damage.

Helicoverpa armigera and *A. loreyi* damage was observed on ears from 13 weeks after plant emergence onwards. The number of damaged plants was not significantly higher in non-Bt than Bt maize plots for Pan 6012B and Pan 6804 ($F_{(2,8)} = 3.463$, $P = 0.082506$). Similar incidences of damaged plants were also observed in plots with CRN 78-15B (Bt) and its non-Bt counterpart CRN 3505 ($F_{(2,8)} = 2.485$, $P = 0.144732$) (Fig. 2.6). However, significant differences were observed on Pan 6012B and Pan 6804, sixteen ($t_{(2.2)} = -3.90$; $P = 0.0495$) and twenty ($t_{(4)} = -3.41$; $P = 0.0495$) weeks after emergence. The incidence of damaged plants differed significantly between CRN 78-15 (Bt) and CRN 3505 (non-Bt) 13 ($t_{(2)} = -2.13$; $P = 0.0369$) and 16 ($t_{(2)} = -1.71$; $P = 0.0495$) weeks after emergence.

Three stem borer species were recovered from dissected non-Bt maize plants. At 6, 9 and 13 weeks after emergence 100% of the recovered larvae were *C. partellus*. Sixteen weeks after emergence 97.4% was *C. partellus* and 2.6% *B. fusca*. At the last sampling date 60% of the larval population consisted of *C. partellus*, 20% of *B. fusca* and 20% of *S. calamistis*.

2.6 Discussion

Damage was observed on Bt and non-Bt plants throughout plant development of plants. The general tendency was a sharp increase in the number of damaged plants from the flowering period onwards and in some instances the presence of larvae on plants.

Except for the high incidence of stem borer damage observed in research Bt plots at Polokwane (34.3 %) stem borer damage on farmers' fields ranged between 3 and 12.5 % on Bt fields and from 3.4 to 19.7 % on non-Bt fields. Although the incidence of damage on Bt plants at Polokwane was high, the damage lesions were minute (pinholes < 1mm in diameter) and could only be recognized after intensive inspection. Survival of stem borers at Polokwane was only observed on non-Bt maize. This result showed that the Bt event used against *C. partellus* in South Africa is highly effective under field conditions in spite of severe infestation pressure. The presence of *B. fusca* on Bt maize during both

seasons in the North West - and the Free State provinces confirms observations by Van Rensburg (2001) that larvae may survive on less toxic plant parts during the reproductive stages. The increased incidence of stem borer damage symptoms after tasseling of maize have been ascribed to increased larval migration from the whorl and flag-leaf furl at the onset of tasseling (Van Rensburg, Walters & Giliomee, 1988). The Bt-protein concentration in silks was reported to be low enough to allow survival of some larvae until completion of the first two instars, after which the ear tips and husk leaves serve as important feeding sites (Van Rensburg, 2001). The fact that only superficial damage to Bt maize plants was observed in the Limpopo province can be ascribed to the dominance of *C. partellus* and low incidence of *B. fusca* during the early part of the season in this region (Rebe *et al.*, 2004). Because *C. partellus* is highly susceptible to Bt maize with event MON 810 (Singh, Channappa, Deebe, Nagaraj, Sukavaneaswaran & Manjunath, 2005; Van Rensburg, 1999) no significant stem borer damage was observed on Bt plants.

Sesamia calamistis was only recorded on non-Bt maize during very late plant growth stages at the Polokwane site in the Limpopo Province. Although this species has been indicated to be increasing in importance in South Africa, its infestation levels are low and pest outbreaks confined to isolated incidences. *Sesamia calamistis* was reported to be highly susceptible to Bt maize with event MON 810 (Van den Berg & Van Wyk, in press).

Damage caused by *H. armigera*, was lower on Bt than non-Bt maize with the general pattern of increase in larval numbers and damage after flowering. *Helicoverpa armigera* and *A. loreyi* associated damage as well as larval numbers at one site increased during the post-flowering period. Non-target effects of feeding on Bt maize have been reported for several Noctuidae species. The interaction of *H. armigera* with Bt maize may also alter the seasonal dynamics of *H. armigera* and its pest status (Fitt *et al.*, 2004). *Helicoverpa armigera* is considered to be the main non-target pest of concern for resistance risk in Kenya where it is consistently associated with maize (Andow & Hillbeck, 2004). The same could apply to this species in South Africa because of its wide occurrence on maize. Tabashnik *et al.* (2003) reported that resistant strains of *H. armigera* on Bt cotton in

Australia completed larval development in the field when the *Cry1Ac* concentration declined at the end of the season in Australia (Tabashnik *et al.*, 2003; Akhurst *et al.*, 2003). Fitt *et al.* (2004) reported that there is an absence of information on the level of expression and efficacy of *Cry* proteins in reproductive structures of the Bt maize events evaluated to date. It is therefore not possible to assess the possible impact of Bt maize on *H. armigera* larvae and the associated resistance risk. Because survival of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) on commercial Bt maize does occur in the USA, there will likely be some pertinent resistance risk associated with *H. armigera* on Bt maize in South Africa.

The polyphagous nature of *H. armigera* and exposure to Bt toxin in maize could accelerate resistance development in this pest in areas where Bt maize is planted in close proximity to or in rotation systems with Bt cotton. *Helicoverpa armigera* is exposed to Bt toxin when feeding on ears of maize. Exposure to *Cry1Ab* during feeding would add to selection pressure in areas where maize and Bt cotton expressing *Cry1Ac* is planted. The *Cry1Ab* and *Cry1Ac* toxins are similar in their mode of action and are widely reported to confer cross-resistance (Fitt *et al.*, 2004). Resistance risks posed by *H. armigera* are difficult to assess. As a highly mobile and polyphagous species, it would be necessary to assess the level of geographic separation of maize, cotton and vegetable production systems. Polyphagy and mobility will operate to reduce selection pressure of Bt maize on *H. armigera*, although localized intense selection may occur.

Eizaguirre *et al.* (2005) also reported a delay in developmental times of *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae) larvae at reduced concentrations of Bt. They also reported that this observation is consistent with earlier reports on other Lepidoptera such as *Spodoptera exigua* (Hübner) (Stapel *et al.*, 1998), *Plodia interpunctella* (Hübner) (Giles *et al.*, 2000), *Pseudoplusia includens* (Walker) (Achfaq *et al.*, 2001), *H. armigera*, *Spodoptera littoralis* (Obrist *et al.*, 2006), and *Spodoptera frugiperda* (Smith) (Adamczyk *et al.*, 2001). In most of these cases, developmental delay was attributed to a decrease in food intake, a cause that could partially explain the development delay in larvae fed with sublethal Bt concentrations (Eizaguirre, Tort, López

& Albajes, 2005). This delay may affect the rate of Bt resistance development. Gene flow studies on *Ostrinia nubilalis* (Hübner) done by Bourguet *et al.* (2000) pointed out that resistant individuals that feed on high-doses Bt in maize may develop more slowly than susceptible moths. This temporal delay in development may lead susceptible *O. nubilalis* to complete mating before resistant moths emerged (Bourguet, Bethenod, Pasteur & Viard, 2000). The effect of Bt maize on diapause induction and development of borers also may delay the onset of flights of adults emerging from Bt fields. This delay could decrease the efficacy of non-Bt refuges as adults from Bt fields would have less probability of mating with adults from non-Bt fields than with adults from the same field (Eizaguirre *et al.*, 2005).

The damage symptoms caused by *A. loreyi* to maize ears and leaves are similar to that of *H. armigera* which made it difficult to distinguish between these two species when larvae were not present. Leaves of the lower section of Bt maize plants during the post-flowering stage were sometimes skeletonized by larvae. Hill (1987) described *A. loreyi* to be a main pest on maize and rice. The older larvae become gregarious and feed voraciously, eating entire leaves and sometimes the whole plant. In this study results showed that *A. loreyi* numbers and incidence of damage was to some extent affected by Bt toxin and that it has the ability to survive on Bt maize. This could possibly be because Bt maize toxin decreases with time which makes it possible for larvae to feed on leaves after flowering. No *A. loreyi* larvae were observed on maize seedlings and the effect that leaf feeding on young plants could have on larvae is unknown.

Because of their omnipresence and abundance, *H. armigera* and *A. loreyi* can be considered to be important non-target lepidopteran herbivores on maize in South Africa. Any non-target species feeding on the transgenic plant or parts of the plant may come into contact with the transgene and its product. Some non-target herbivores are potential secondary pests of the crop. While on the one hand, Bt maize may reduce target pest species to insignificant levels, it may have another effect on non-target pest species. For example, other pests may increase (secondary pests), decrease or their status be unchanged.

2.7 Conclusions

This study showed that *H. armigera* and *A. loreyi* occur in significant numbers on Bt maize in South Africa and that they should be considered as sensitive species because of their exposure to Bt maize. The lower incidences of Lepidoptera damaged plants observed on Bt fields indicated that Bt toxin ingested through direct feeding most likely had a negative effect on survival of *H. armigera* and *A. loreyi*. Results showed that *B. fusca* was present at low levels in Bt maize. No survival of *C. partellus* was observed on Bt maize, indicating the extreme susceptibility of this pest to MON 810. This high susceptibility of *C. partellus* may lead to local extinction in regions where wild hosts of this pest does not occur. It may also have a subsequent negative effect on populations of natural enemies of this pest. More information is required about *H. armigera* ecology in the region.

2.8 References

Adamczyk, J.J., Hardee, D.D., Adams, L.C. & Sumerford, D.V. 2001. Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry 1A(d) δ -endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *Journal of Economic Entomology* 94: 284 – 290.

Akhurst, R.J., James, W., Bird, L.J. & Beard, C. 2003. Resistance to the Cry1Ac δ -endotoxin of *Bacillus thuringiensis* in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 96: 1290 – 1299.

Andow, D.A. & Hillbeck, A. 2004. Bt maize, risk assessment and the Kenya case study. (eds.: Hillbeck & Andow). Environmental risk assessment of genetically modified organisms: Vol. 1. A case study of Bt maize in Kenya. CAB International. Wallingford. UK.

Anneck, D.P. & Moran, V.C. 1982. Insects and mites of cultivated plants in South Africa. The Butterworths Group. Durban/Pretoria.

Ashfaq, M., Young, S.Y. & McNew, R.W. 2001. Larval mortality and development of *Pseudoplusia includens* (Lepidoptera: Noctuidae) reared on a transgene *Bacillus thuringiensis*-cotton cultivar expressing Cry 1Ac insecticidal protein. *Journal of Economical Entomology* 94: 1053 – 1058.

Bourguet, D., Bethenod, M.T., Pasteur, N. & Viard, F. 2000. Gene flow in the European corn borer *Ostrinia nubilalis*: implications for the sustainability of transgenic insecticidal maize. *Proceedings of the Royal Society of London* 267: 117 – 122.

Castanera, P. & Orteg, F. 2005. Environmental implications of Bt maize in Spain: monitoring corn borer resistance and non-target impacts. Internet Forum. Monitoring of

Genetically Modified Plants. Session 3.

www.gmsciencedebate.org.uk/topics/forum/pdf/0090b.pdf accessed 15 February 2006.

Eizaguirre, M., Tort, S., López, C. & Albajes, R. 2005. Effects of sublethal concentrations of *Bacillus thuringiensis* on larval development of *Sesamia nonagrioides*. *Journal of Economical Entomology* 98: 464 – 470.

Fitt, G.P., Andow, D.A., Carrière, Y., Moar, W.J., Schuler, T.H., Omoto, C., Kanya, J., Okech, M.A., Arma, P. & Maniania, N.K. 2004. Resistance risks and management associated with Bt maize in Kenya. (eds.: Hillbeck & Andow). Environmental risk assessment of genetically modified organisms: Vol. 1. A case study of Bt maize in Kenya. CAB International. Wallingford. UK.

Giles, K.L., Hellmich, R.L., Inverson, C.T. & Lewis, L.L. 2000. Effects of transgenic *Bacillus thuringiensis* maize grain on *B. thuringiensis*-susceptible *Plodia interpunctella* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 93: 1011 – 1016.

Gouse, M. 2005. Aspects of biotechnology and genetically modified crops in South Africa. Science, Technology and Globalization Project. Agricultural Biotechnology for Development – socioeconomic issues and institutional challenges. Belfer Center STPP. Kennedy School of Government.

http://bcsia.ksg.harvard.edu/BCSIA_content/documents/SouthAfricaGouse.pdf 21 May 2006.

Hill, D.S. 1987. Agricultural insect pests of the tropics and their control. Second edition. Cambridge University Press. United Kingdom.

Obrist, L.B., Dutton, A., Romeis, J. & Bigler, F. 2006. Biological activity of Cry 1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure to the predator *Chrysoperla carnea*. *BioControl* 51: 31 – 48.

Rebe, M., Van Den Berg, J. & McGeogh, M.A. 2004. Colonization of cultivated and wild graminaceous host plants by *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) under field conditions. *African Entomology* 12: 187 – 199.

Sharma, H.C., Sharma, K.K., Seetharama, N. & Ortiz, R. 2000. Prospects for using transgenic resistance to insects in crop improvement. *Electronic Journal of Biotechnology* 3: 76 – 95.

Singh, R., Channappa, R.K., Deeba, F., Nagaraj, N.J., Sukavaneaswaran, M.K. & Manjunath, T.M. 2005. Tolerance of Bt corn (MON 810) to maize stem borer, *Chilo partellus* (Lepidoptera: Pyralidae). *Plant Cell Reports* 24: 556 – 560.

Stapel, J.O., Waters, D.J., Ruberson, J.R. & Lewis, W.J. 1998. Development and behavior of *Spodoptera exigua* (Lepidoptera: Noctuidae) in choice test with food substrates containing toxins of *Bacillus thuringiensis*. *Biological Control* 11: 29 – 37.

Statistica 7.1. 2005. StatSoft, Inc. Tulsa. USA.

Tabashnik, B.E., Carrière, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M. & Zhao, J. 2003. Insect resistance to transgenic Bt crops: Lessons from the laboratory and field. *Journal of Economic Entomology* 96: 1031 – 1038.

Van den Berg, J. & Van Wyk, A. In press. The effect of Bt maize on *Sesamia calamistis* (Lepidoptera: Noctuidae) in South Africa. *Entomologia Experimentalis et Applicata*.

Van Rensburg, J.B.J. 1999. Evaluation of Bt-Transgenic maize for resistance to the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) in South Africa. *South African Journal of Plant and Soil* 16: 38 – 43.

Van Rensburg, J.B.J. 2000. The Chilo borer in maize. *Crop Protection Series no 22*. ARC-Grain Crops Institute. Potchefstroom, South Africa.

Van Rensburg, J.B.J. 2001. Larval mortality and injury patterns of the African Stalk borer, *Busseola fusca* on various plant parts of Bt-transgenic maize. *South African Journal of Plant and Soil* 18: 62 – 69.

Van Rensburg, J.B.J., Walters, M.C. & Giliomee, J.H. 1988. Ecology of the maize stalk borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 77: 255-269.

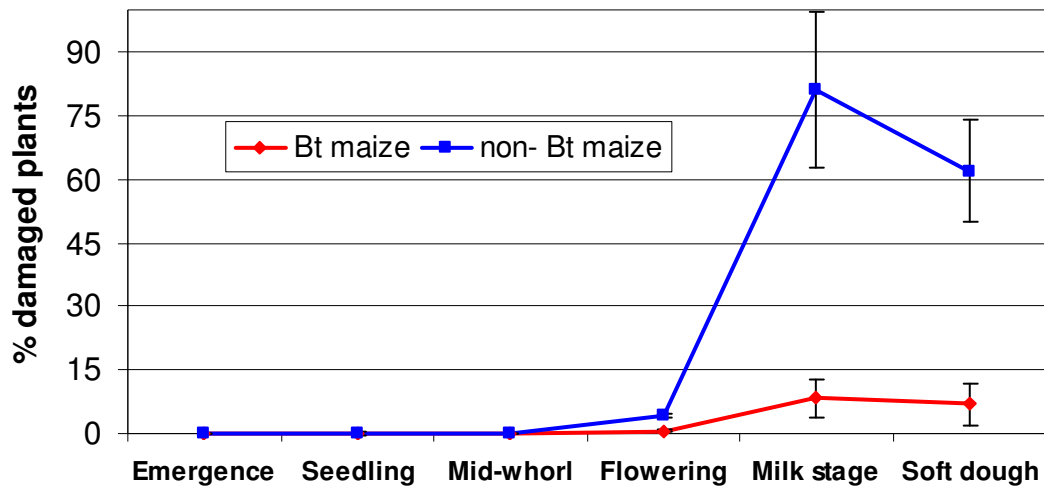


Figure 2.1 The incidence of *Helicoverpa armigera* damage at different growth stages of Bt and non-Bt maize plants at Wolmaransstad (Bars = standard error).

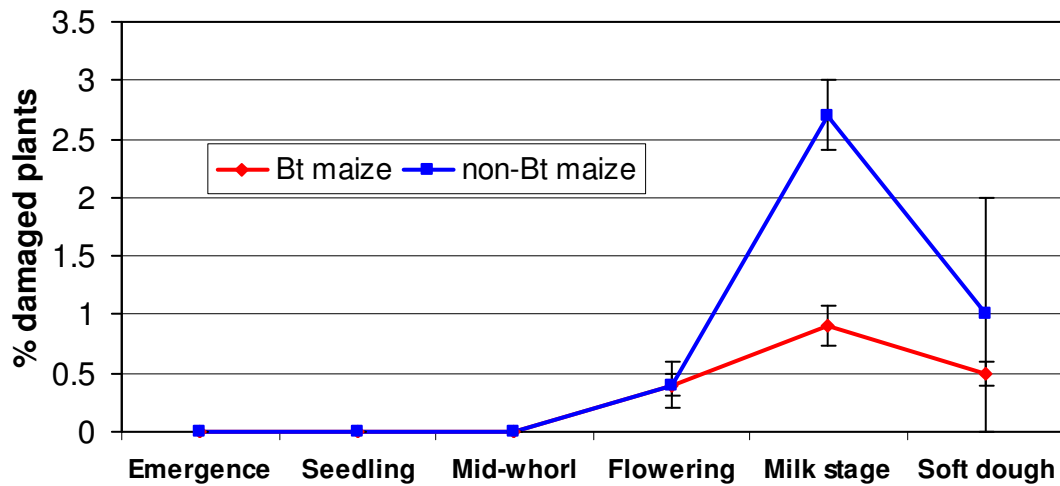


Figure 2.2 The incidence of *Acantholeucania loreyi* damage at different growth stages of Bt and non-Bt maize plants at Wolmaransstad (Bars = standard error).

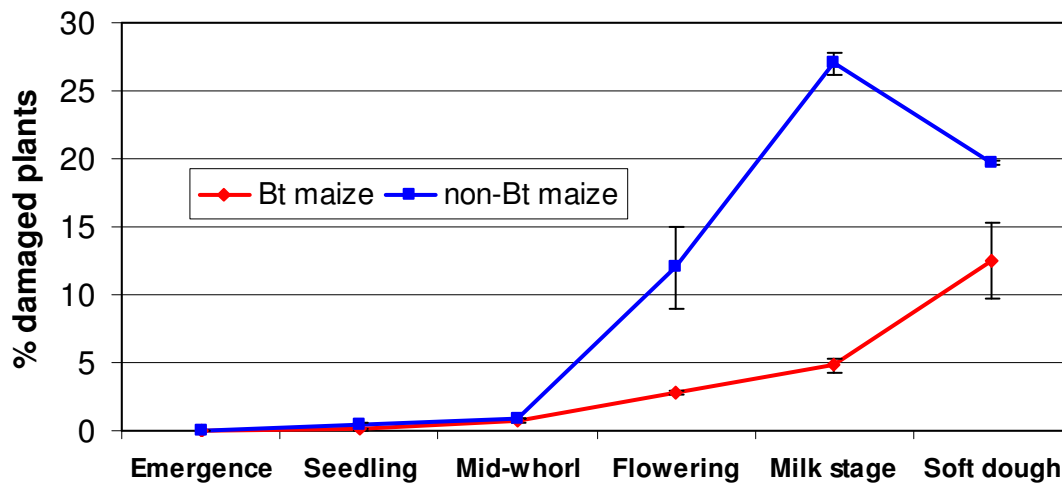


Figure 2.3 The incidence of *B. fusca* damage at different growth stages of Bt and non-Bt maize plants at Wolmaransstad (Bars = standard error).

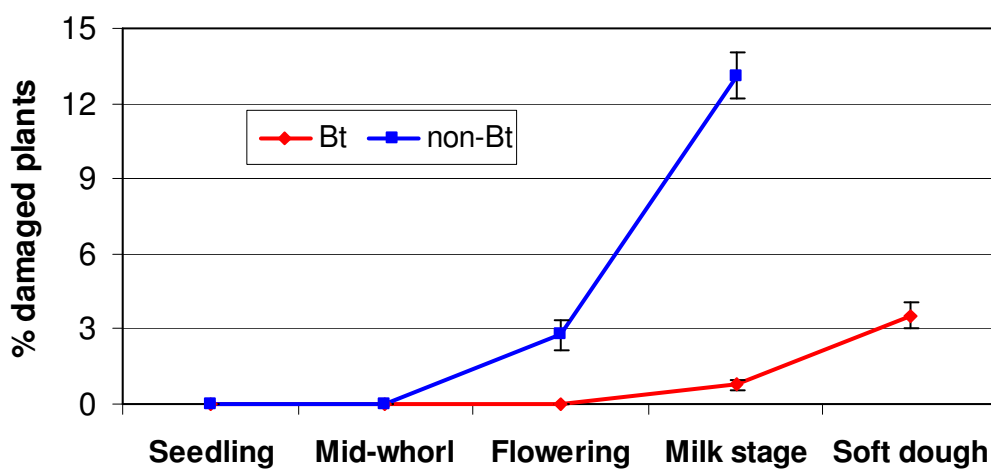


Figure 2.4 The incidence of *B. fusca* damage at different growth stages of Bt and non-Bt maize plants at Potchefstroom (Castello farmers) (Bars = standard error).

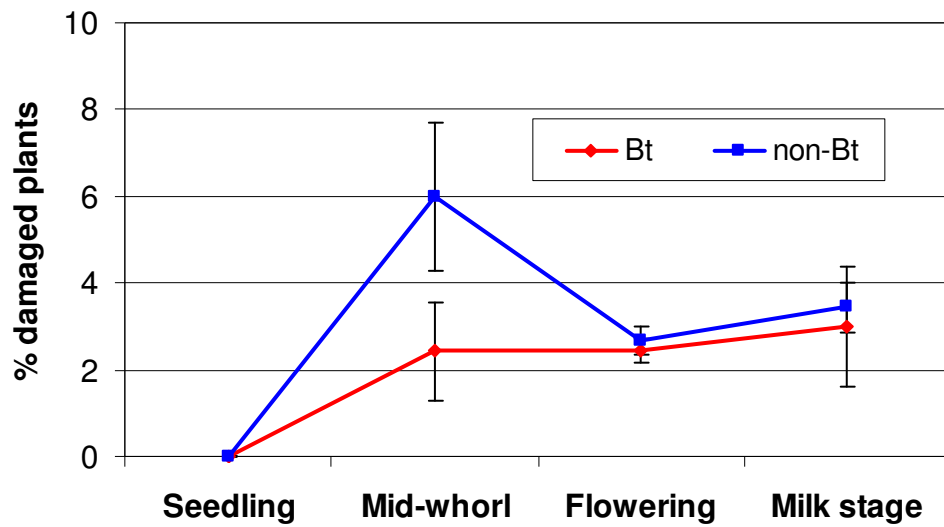


Figure 2.5 The incidence of *B. fusca* damage at different growth stages of maize plants in a Bt maize field and non-Bt maize refuge at Reitz (Hartlam) (Bars = standard error).

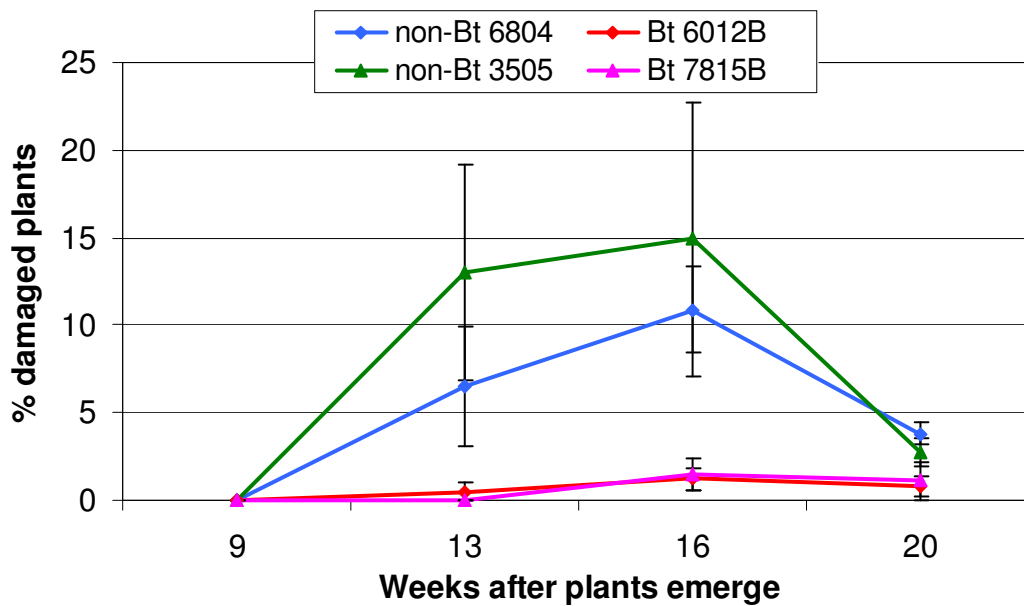


Figure 2.6 The incidence of *H. armigera* and *A. loreyi* damage over time at Polokwane during the 2005/6 season (Bars = standard error).

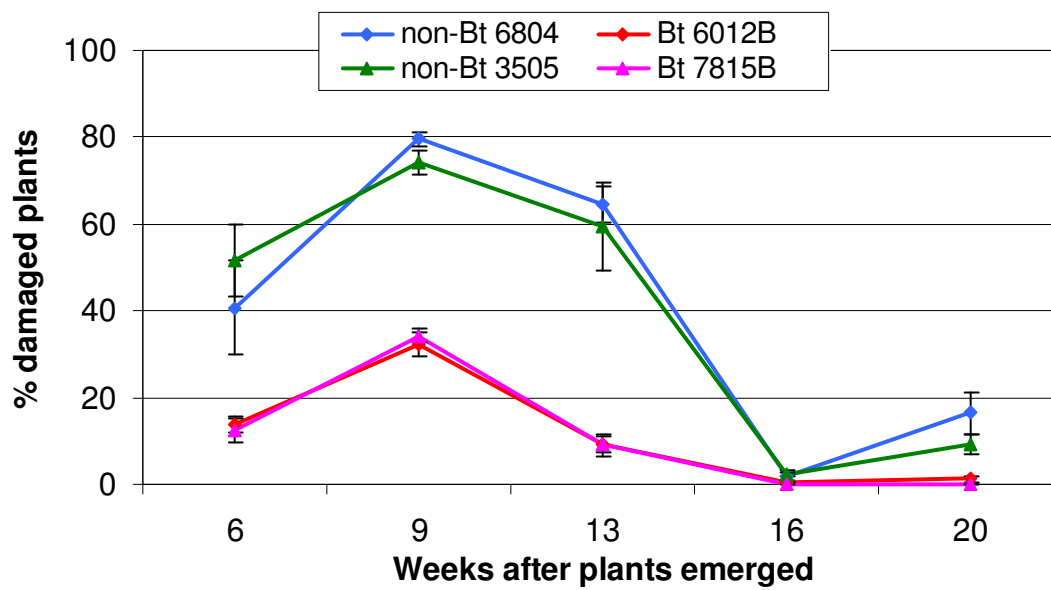


Figure 2.7 The incidence of stem borer damage in two Bt hybrids and iso-hybrids at Polokwane during the 2005/6 season (Bars = standard error).



Figure 2.8. Maize stem borer (*Busseola fusca*) moth.



Figure 2.9. *Busseola fusca* larvae and damage to maize ear.



Figure 2.10. *Chilo partellus* moth.



Figure 2.11. *Chilo partellus* larvae and damage to maize stem.



Figure 2.12. *Helicoverpa armigera* moth.



Figure 2.13. *Helicoverpa armigera* larva on a maize ear.



Figure 2.14. *Acantholeucania loreyi* moth.



Figure 2.15. *Acantholeucania loreyi* larva damaging silks of a maize ear.

CHAPTER 3: DIVERSITY AND HOST PLANT RANGE OF LEPIDOPTERA THAT OCCUR ON BT- AND NON-BT MAIZE IN SOUTH AFRICA

3.1 Abstract

The use of Bt maize could directly or indirectly affect non-target organisms. Assessment of the impacts of Bt crops is hampered by the lack of even the most basic checklist of the species present in most systems. The maize agro-ecosystem is of particular interest because of the large area planted with Bt maize used for control of *Busseola fusca* and *Chilo partellus* in South Africa. No information is available on which non-target Lepidoptera feed on maize and that could possibly be exposed to Bt toxin. The aim of this study was to provide baseline data on the diversity of Lepidoptera species that feed on or are closely associated with maize. Surveys were conducted on maize fields at 24 localities in four provinces during the 2004/5 and 2005/6 cropping seasons. Maize plants were inspected for Lepidoptera larvae that were feeding on it. Between 300 and 900 maize plants per field were inspected. Larvae were collected and reared to the moth stage. Fifteen species of Lepidoptera were recorded on maize. Eleven species were recorded to feed on non-Bt maize while pupae of four other species were also found on maize. Six of these species were also recorded to feed on Bt maize. Species that were recorded on Bt maize and reared to the adult stage on Bt maize tissue all belonged to the Noctuidae, except for one Nolidae species. These species were *Acantholeucania loreyi*, *Agrotis segetum*, *B. fusca*, *Helicoverpa armigera*, *Eublemma gayneri* and *Nola phaeocraspis*. Limited information is available on host plants of some of these species and one species, *Eublemma gayneri*, has previously been reported to have only two host plants, *Acacia karroo* (Fabaceae), a tree species and *Sorghum* sp. (Poaceae).

3.2 Introduction

Agro-ecosystems in which transgenic crops such as Bt maize is grown, consists largely of monocultures interspersed by many unwanted monocotyledonous and dicotyledonous plants that vary in their spatial and temporal distribution inside crop fields. These plants play an important role in ecology of herbivorous arthropods inside crop fields (Norris & Kogan, 2000) and may in many cases be the perfect hosts of arthropods. However, removal of host plants of arthropods through cultivation practices often results in their feeding on crops due to lack of availability of other food sources. More than 70 families of arthropods that are potential crop pests can primarily be associated with weeds (Norris & Kogan, 2000). Absence of these host plants may force many Lepidoptera species to move from their preferred host plant to the crop that is growing on the field.

Most pest management technologies have effects on non-target arthropod species both within and outside the units that are being managed. The goal of insect pest management is to maintain economically important insect pest species below threshold densities. These management tactics interfere with the ability of insects to survive, reproduce or exploit resources, and the impacts of these tactics are very rarely confined to the target species only (Losey, Hufbauer & Hartzler, 2003).

An example of a non-target effect that Bt maize could have is that of the monarch butterfly (*Danaus plexippus*), and its host plant, common milkweed (*Asclepias curassavica*). Losey *et al.* (1999) found that larval survival of *D. plexippus* feeding on leaves dusted with pollen from Bt maize was significantly lower than survival on leaves dusted with pollen of untransformed maize or on leaves with no pollen (Losey, Rayor, Maureen & Carter, 1999).

Laboratory assessments of the effects of Bt maize on native Lepidoptera done by Peacock *et al.* (1998) in the USA showed significant mortality for 27 of the 42 species evaluated. Significant reductions in numbers of larvae of non-target lepidopterans following the use of *B. thuringiensis* can indirectly affect other animals that rely on lepidopteran larvae as a

primary source of food (Peacock, Schweitzer, Carter & Dubois, 1998). Mortality rates of 100 % in the target species may result in localized host depletion for parasitoids specific to the target pest, and possibly also in a reduction in prey for generalist natural enemies, when compared to populations in unsprayed non-GM crops (Schuler, 2004). Pons *et al.* (2005) on the other hand indicated *Cry1Ab* toxin to be very selective in its action and indicated that the impact of Bt maize on non-target arthropods is expected to be minimal. In the latter study Bt maize did not affect the incidence of cutworms (*Agrotis segetum*) or wireworms (*Agriotes lineatus*) (Pons *et al.*, 2005). Although the *Cry* proteins produced in transgenic maize are considered to be specific, some side effects of these toxins on non-target species have been reported (Bourguet *et al.*, 2002).

The first step towards a comprehensive insect management program that would provide adequate pest suppression, maintenance of ecological services, and minimal impact on rare species is a detailed assessment of which insect species are likely to exist in the managed system (Losey *et al.*, 2003). Assessment of the impacts of management strategies is hampered by the lack of even the most basic checklist of the species present in most systems (Losey *et al.*, 2003). The maize agro-ecosystem is of particular interest because of the large area planted with maize and the use of transgenic maize expressing the *Cry1Ab* gene that encodes a *B. thuringiensis* endotoxin. Genetically modified maize containing *Cry1Ab* is used for control of two Lepidoptera species in South Africa, i.e. *B. fusca* (Fuller) and *C. partellus* (Swinhoe) (Van Rensburg, 1999). Several other Lepidoptera also feed on maize and could potentially be affected by Bt maize. No information is available on non-target Lepidoptera that feed on maize and that could possibly be exposed to Bt toxin in South Africa.

The aim of this study was to provide baseline data on lepidopteran species that feed on maize and that are likely to be exposed to Bt toxin.

3.3 Material and methods

Two types of field surveys were conducted. First, collection surveys were done during which Bt- and non-Bt maize fields were visited. During these surveys larvae were collected to compile a checklist of species likely to be found on maize. Field surveys were also conducted during which damage symptoms were quantified. At some sites no damage or larvae of any species were observed on Bt maize.

3.3.1 Collection surveys

A survey was conducted during which Bt- and non-Bt maize fields at 24 localities in four provinces were sampled during the 2004/5 and 2005/6 cropping seasons. Sampling was done in the in North-West Province (Wolmaransstad, Makwassie and Potchefstroom), the Free State Province (Reitz and Oranjeville), Gauteng Province (Carltonville and Bronkhorstspuit) and the Limpopo Province (Warmbad, Naboomspruit, Crecy, Polokwane and Thohoyandou).

Sampling was done once during the milk - soft dough stages of development on each field since field observations (Chapter 2) showed that Lepidoptera infestation on maize increased during the post-flowering stages of plant growth. Maize plants were inspected for Lepidoptera larvae that were feeding on it. In the first season 300 maize plants (three replicates of 100 plants each) and in the second season 900 maize plants (three replicates of 300 plants) were inspected in each maize field. Each replicate consisted of 100 maize plants within a row that were randomly selected 20 to 50 meters inside the field.

Lepidoptera larvae that were found on maize were reared on the specific part of the maize plant that it was found on. This was done in the laboratory at 20 to 25 °C. Food was changed when necessary. Larvae were reared until moths emerged. Moths were preserved for identification purposes.

Lepidoptera species that occurred on maize were grouped into different guilds based on the different plant tissues they were feeding on. The following guilds were recognized:

seedling and leaf feeders; stem borers; two post-flowering guild species that feed on maize tassels and ears (including silks) respectively; and a visitors guild. Lists were compiled of the lepidopteran species that were found on maize, the different guilds to which each species belong to and a host plant range list of lepidopteran species recorded in this survey that was not previously recorded on maize.

At Polokwane and Venda, the percentage non-Bt maize plants infested by *Eublemma gayneri* and *Nola phaeocraspis* were determined, as well as on Bt plants at Potchefstroom and Warmbad. Data on *E. gayneri* and *N. phaeocraspis* infestation at these sites were compiled in tables because the incidence of damage was determined on Bt or non-Bt fields only and could not be compared to other fields.

Surveys conducted at Warmbad (S 25°14.686, E 028°17.979) in the Limpopo province during the 2004/5 and 2005/6 seasons were done on maize planted under center pivot irrigation systems. Three Bt maize fields were inspected. The same fields were inspected the second season but were planted with different Bt hybrids. In the 1st season all three the fields were planted with the same Bt hybrid, Pioneer 32A05B. The 2nd season CRN 78-15B was planted on one field and the other two fields with PAN 6013B. The number of damaged ears were determined and expressed as a percentage of the total number of ears produced.

3.3.2 Field surveys – comparison of infestation levels between Bt and non-Bt fields

Sampling was done once during the milk - soft dough stages of development on each field. Sampling was conducted and damage was recorded at Wolmaransstad, Potchefstroom, Reitz, Oranjeville and Carletonville. Sampling during the 2004/5 season was done by inspecting 300 maize plants (three replicates of 100 maize plants) and during the 2005/6 season with 900 maize plants (three replicates of 300 maize plants) for lepidopteran damage in Bt- and adjacent non-Bt maize fields. Each replicate were randomly selected in the maize field 20 – 50 m inside the field. This procedure was used

for all the sites except for at Lukaskraal and Castello where seedlings were inspected for damage caused by *Agrotis segetum*, *Spodoptera exigua* and *Helicoverpa armigera*.

During the 2004/5 cropping season an experiment was conducted under dryland conditions on a farm at Lukaskraal (S 27°00.830, E 025°56.780) in the North-West province. In this experiment one 20 hectare Bt field and an adjacent 50 hectare non-Bt field was planted. The Bt hybrid was CRN 7815 (Bt) and its non-Bt iso-hybrid CRN 3505. The incidence of plants damaged by *A. segetum* were recorded and compared between fields. Plants were inspected for damage during the seedling stage. Three blocks each of Bt and non-Bt maize were marked inside the larger maize fields. Each block consisted of 20 rows of 100m in length and an inter-row spacing of 1.5m (0.3 ha). Larvae were collected from infested plants and reared until the moths appeared. Moths were pinned and preserved to facilitate identification.

A survey was also conducted at Castello farms (S 26°21.416; E 027°06.565) during the 2005/6 season. Maize seedlings were inspected for larvae in a Bt (CRN 78-15B) and non-Bt (SC 710) field in two adjacent centre-pivot irrigation systems. Three rows of 100m length were inspected at three localities inside each field.

Sampling was conducted once during the post-flowering stage on farms at Ntsu (S 26°52.749, E 027°00.880) and Rooibult (S 27°00.830, E 025°56.780) during the 2005/6 season in centre-pivot irrigation fields. The Bt cultivar (CRN 8012B) with its iso-hybrid (CRN 8010) was planted at both sites. Stem borer damage was determined at both sites. *Helicoverpa armigera* damage was determined by sampling once during the milk stage on the farm Hartlam (S 27°47.197; E 028°28.571) under dryland conditions (Bt cultivar CRN 4760B and non-Bt cultivar Phb 3442).

During the 2004/5 season sampling was conducted once during post-flowering at the following localities: Vaalbank, Hartlam, Nog-'n-Stuk, Potchefstroom (drip irrigation), Noric and Wildfontein. Cultivars planted at Vaalbank were Phb 33A13 (non-Bt) and Phb 33A14B (Bt) under dryland conditions. The first site at Reitz, Hartlam were planted with

CRN 4760B (Bt) and Phb 3442 (non-Bt) as control, and the second site, Nog-‘n-Stuk with Phb 33A14B (Bt) and Phb 32D99 (non-Bt) under dryland conditions. The cultivars planted at Potchefstroom under drip irrigation in strip plots were Phb 33A14B with its iso-hybrid 33A14, Phb 33P67B with iso-hybrid 33P66 and 32D96B with iso-hybrid 32D99. Cultivars used at Noric were CRN 78-15B and iso-hybrid CRN 3530 under centre-pivot irrigation. At Wildfontein Pan 6013B (Bt) and Pan 6335 (non-Bt) were planted under dryland conditions.

3.4 Data analysis

Only means were calculated for sites where no comparisons could be made between Bt and non-Bt fields. Other data were analysed separately for each site comparing infestation levels and incidence of damage between Bt and non-Bt maize fields. The two-by-two table was used to determine significance by using the Chi-square analysis. Statistical analysis was done with Statisica software (version 7.1). The 95% confidence interval, of the mean percentage infestation on individual fields, was determined by using the odds-ratio.

3.5 Results

3.5.1 Collection surveys

Fifteen species of Lepidoptera were recorded on maize plants. Eleven species were observed to feed on non-Bt maize while pupae of four species were also found on maize plants (Table 3.1). Six of these species were recorded to feed on Bt maize and were reared on Bt maize until the adult stage.

These 15 species could be divided into four guilds: seedling and leaf feeders, stem borers, a post-flowering guild that feeds on maize tassels, ears and silk, and a visitors guild (Table 3.2). Seven of the species recorded during this study are known pests of maize

(Annecke & Moran, 1982). Three of these were stem borers (*B. fusca*, *C. partellus* and *S. calamistis*) while two can be regarded as leaf feeders (*A. loreyi* and *Spodoptera exigua*). *Agrotis segetum* is a pest of maize seedlings while *H. armigera* usually attacks plants during the reproductive stages when it feeds on maize ears and silks. The stem borers can however also belong to both the leaf feeding- and post-flowering guilds since they feed on maize plants from the early whorl stage onwards and also damage the stem and ears during the post-flowering stages. The other four species that were recorded were assigned to the ear and silk guild. These species were *Eublemma gayneri* (Lepidoptera: Noctuidae), *Nola phaeocraspis* (Lepidoptera: Nolidae), *Chlorissa stibolepidae* (Lepidoptera: Geometridae) and *Chrysodeixis acuta* (Lepidoptera: Noctuidae) (Table 3.2).

Bt maize feeding species that belonged to the seedling and leaf-feeding guilds were *A. segetum*, *H. armigera* and *A. loreyi*. The stem boring-guild was represented by *B. fusca* while the post flowering-guild was represented by *A. loreyi*, *H. armigera*, *E. gayneri* and *N. phaeocraspis* (Table 3.2). The latter two species are both 1st reports on maize and were successfully reared on Bt maize up to the moth stage. First instars of *C. partellus* and *S. calamistis* were recorded on Bt maize but were highly susceptible to Bt plants and could not be reared further than the 2nd instar. *Sesamia calamistis* (Van den Berg & Van Wyk, In press) and *C. partellus* (Van Rensburg, 1999; Singh *et al.*, 2005) has been reported to be highly susceptible to Bt maize.

Species that were recorded at extremely low numbers or as pupae were assigned to the visitors guild, even though larvae of two species were reared successfully on maize. These species were *Chlorissa stibolepidae* (one individual) and *Chrysodeixis acuta* (two individuals at two localities). One pupa each of *Athetis pigra*, *Caradrina atriluna*, *Simplicia extinctalis* and *Cornutiplusia circumflexa* were found on maize but it cannot be assumed that these species feed on maize during the larval stages.

Of the six species that were newly recorded on maize only two, i.e. *E. gayneri* and *N. phaeocraspis*, were recorded in high numbers and at several localities. The percentage

plants infested by *E. gayneri* and *N. phaeocraspis* at different localities are provided in Table 3.4. Larvae of the latter two species never occurred at infestation levels higher than one larva per ear. The percentage plants infested with *E. gayneri* larvae ranged between 0.11 - 3.67 %. The highest infestation level of *N. phaeocraspis* was recorded in Venda with an incidence of 2.0 % infested plants on non-Bt maize. Both these species were recorded on maize ears and silks. It seemed if larvae were feeding on the silks after pollination when silks became brown. After feeding on silks larvae moved to the husk leaves below the dry silks where they use the silks to construct a cocoon for pupation.

The incidence of Bt plants infested with *H. armigera*, *A. loreyi*, *E. gayneri* and the mean percentage damage are provided in Table 3.5. The incidence of *H. armigera* infestation was low with only 1.67 % during the 2004/5 cropping season. That of *A. loreyi* ranged between 0 and 5.7 %. The incidence of *E. gayneri* infested plants ranged between 0 and 6.3 % over the two seasons. The mean percentage ears damaged by the complex of post-flowering Lepidoptera ranged between 0 and 17.4 % over the two seasons. It was not possible to separate damage symptoms caused by the different species.

3.5.2 Field surveys – comparison between Bt- and non-Bt fields

Infestation levels of economically important species are provided elsewhere (Chapter 2). The incidence of seedling damage caused by *A. segetum* at Lukaskraal, and *S. exigua* and *H. armigera* seedling damage at Castello were significantly higher on non-Bt than Bt fields (Table 3.6). A significant difference between the incidence of damaged plants on Bt and non-Bt fields was observed at all the sites except for stem borers and *A. loreyi* at Nog-‘n-Stuk, and *H. armigera*, stem borers and *A. loreyi* at Potchefstroom Site 3. The incidence of plants damaged by *H. armigera* for both seasons ranged between 0 and 9 % on Bt- and 0.3 to 52 % on non-Bt maize. The incidence of stem borer damage was higher on non-Bt than Bt fields at all the sites except for Rooibult where the incidence of stem borer damaged plants during post-flowering was significantly higher on Bt fields. The incidence of stem borer damaged plants ranged between 0 and 7.67 % on Bt- and 1 to 16

% on non-Bt maize. The mean total percentage of Lepidoptera damaged plants recorded during the two-year survey on Bt maize was 2.6% and 10% on non-Bt maize (Table 3.6).

3.6 Discussion

Kroon (1999) noted that finding larvae on a particular plant does not imply that it was its naturally selected food source. Larvae could just be wandering over a particular plant at a given time as part of normal migration behavior. The observation of pupae attached to maize plants indicates that there could be some kind of association with the crop, even if this is only an indirect association through another host plant that occurs in the maize ecosystem. The larvae could have fed on a weedy host plant inside the maize field after which it migrated to maize to find a suitable pupation site. Further research is necessary to determine if the Lepidoptera species of which pupae were found on maize plants could also feed and survive on maize.

No host plant records for *Athetis pigra*, *Caradrina atriluna*, *Chlorissa stibolepidae* and *Nola phaeocraspis* could be found in the extensive host plant list published by Kroon (1999) (Table 3.3) or elsewhere in the literature. These species have not been described in the literature to feed on maize and this report on their association with maize is therefore new. Extensive host plant ranges have been reported for *B. fusca* (Fitt *et al.*, 2004), *C. partellus* (Fitt *et al.*, 2004; Seshu Reddy, 1983), *S. calamistis* (Fitt *et al.*, 2004; Gounou & Schulthess, 2004; Seshu Reddy, 1983), *H. armigera* (Liu *et al.*, 2004), *A. segetum* (Kroon, 1999) and *S. exigua* (Kroon, 1999) and it can be said that these species are all polyphagous. *Acantholeucania loreyi* is the only lepidopteran pest of maize with a narrow host range, reported only on *Eleusine* sp., *Setaria* sp. and *Zea mays* (Kroon, 1999). Ajayi *et al.*, (2001) reported *Mythimna* (*Acantholeucania*) sp. on sorghum but did not provide a species name.

Only the stem borers *B. fusca* and *C. partellus* are target species of Bt maize in South Africa. The other species listed in Table 3.1 can be regarded as non-target Lepidoptera

that are, through feeding, directly exposed to Bt toxin and of which susceptibility to Bt maize have not yet been evaluated. The two stem borers, *B. fusca* and *C. partellus* are the dominant pest species of maize in South Africa and in Kenya where the introduction of Bt maize is in an advanced stage. According to Fitt *et al.* (2004) these are high risk species for resistance development in Kenya. This can also be the case for *B. fusca* in South Africa but because of its high susceptibility to Bt maize. *Chilo partellus* cannot be considered to be a high risk species in South Africa where event MON810 is used in all Bt maize hybrids because no survival of this species has been observed. Even in cases where Bt events are less effective against *B. fusca* the same events are highly effective against *C. partellus* (Van Rensburg, 1999).

In this study the incidence of *A. segetum* damaged plants in Bt and non-Bt maize fields were very low during the 2004/5 season. Evidence from this study suggest that, *A. segetum* is susceptible to Bt maize (MON 810) expressing *Cry1Ab*. This cutworm species is not a target pest of Bt maize in South Africa. Pons *et al.* (2005) in Spain, report that Bt maize did not affect the incidence of *A. segetum* because the *Cry1Ab* toxin is considered to be very selective in its action and the impact of Bt maize on non-target arthropods is therefore expected to be minimal. *Agrotis segetum* lives in the soil and eats at the base of stems of maize seedlings, which is formed by leaf sheaths and tightly rolled leaves inside the sheaths. Consequently, it may ingest Bt-toxin. However, Pons *et al.* (2005) did not find a significant difference in the incidence of *A. segetum* larvae in Bt and non-Bt plots. One reason for this could be the low level of Bt toxin in the stem of Event Syngenta 176, which he used in his study. Other authors have noted that Bt maize does not provide good control of some Noctuidae species including *A. ipsilon* (Pons, Lumbierres, Lopez & Albajes, 2005). According to De Maagd *et al.* (2003) so far there are no reports of any *Cry* protein having significant activity against *A. ipsilon*. One recently published study reported some effect on survival and larval growth when *Cry1Ac* and *Cry1J* was administered to neonate *A. ipsilon* larvae, but only at very high doses. *Cry9Ca* was reported to be active against *A. segetum* (De Maagd, Weemen-Hendriks, Molthoff & Niamov, 2003). However, some species of the family Noctuidae such as *Spodoptera frugiperda* and *Agrotis* spp. were reported to be insensitive to insecticidal crystal proteins

(Lambert, Buysse, Decock, Jansens, Piens, Saey, Seurinck, Van Audenhove, Van Rie, Van Vliet & Peferoen, 1996).

Helicoverpa armigera is a highly polyphagous insect with 172 host plant species from 40 families that have been recorded in Australia and approximately 200 species from 30 families in China (Liu, Li, Gong & Wu, 2004). The reproductive parts of cotton, maize and legumes are highly suitable foods for *H. armigera* (Liu, Li, Gong & Wu, 2004). *Helicoverpa armigera* is sensitive to a range of Bt toxins, with Cry1Ac being the most effective (Romeis, Sharma, Sharma, Das & Sarmah, 2004). So far, the only Bt transgenic crop that has been commercialized for the control of *H. armigera* is cotton. Several other crops have successfully been transformed to express different *Cry* genes for protection against this pest namely tobacco, brinjal, potato and tomato (Romeis *et al.*, 2004). In South Africa, cotton competes with maize for area under irrigation. In the 1999/2000 season 13 200 hectares of cotton were planted which increased to 30 000 hectares in the 2003/2004 season (Gouse, 2005). If farmers change to a different crop like Bt maize or use it in crop rotation systems with Bt cotton it could lead to increased exposure of *H. armigera* to Bt toxin with a consequent resistance development.

Spodoptera littoralis (Lepidoptera: Noctuidae) is partly affected by Cry1Ab (Dutton, Klein, Romeis & Bigler, 2002) and recent studies have shown that transgenic crops can have negative effects on non-target herbivores, with potential implications for higher trophic levels. Vojtech *et al.* (2005) have shown that *S. littoralis* larvae were negatively affected by Bt maize (MON 810) in terms of developmental time and survival. Studies on the parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) showed that survival, development times and cocoon weights were significantly negatively affected if *S. littoralis* host larvae had been fed Bt maize (Vojtech, Meissle & Poppy, 2005). Studies on the predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) provide the most probable explanation that *C. carnea* is affected by feeding on a food source of reduced quality rather than by the toxin itself (Obrist, Dutton, Romeis & Bigler, 2006). Obrist *et al.* (2006) concluded that the negative impacts of *C. carnea* following

consumption of *S. littoralis* that fed on Bt maize resulted from a reduction in the prey quality, because the lepidopteran larvae themselves were affected by the Bt toxin.

In an assessment of biodiversity and non-target impacts in Kenya, Birch *et al.* (2004) identified *H. armigera* and *Spodoptera* spp. as being the most important non-target herbivores in maize and recommended that *Spodoptera* spp. be studied as a key non-target herbivore. *Spodoptera exigua* was recorded during this survey but only on non-Bt maize. Indications are therefore, that its populations are also negatively affected by Bt maize and that it could be listed as a key non-target herbivore in South Africa.

The association of *E. gayneri* with maize is very interesting and evidence suggests that it probably has a narrow host range. If this species is negatively affected by Bt maize, it could have a negative impact on its ecology. According to Kroon (1999) the only recorded host plant of this species was *Acacia karoo* (Fabaceae), a tree species that commonly grows around maize fields in South Africa (Kroon, 1999). Ajayi *et al.*, (2001) reported *E. gayneri* on sorghum as an insect pest damaging sorghum panicles in West and central Africa. One to nine head caterpillars that include *H. armigera*, *Mythimna* (*Acantholeucania*) sp., *Pyroderces* sp. and *E. gayneri* were reported per sorghum panicle in west Africa (Ajayi, Sharma, Tabo, Ratnadass & Doumbia, 2001). *E. gayneri* is also reported as an insect pest of sorghum and pearl millet panicles in Africa (Harris, 1995). While it could previously be said that *E. gayneri* was oligophagous, it could now be said to be polyphagous since it was recorded on several other host plants in a different plant family (Poaceae). As many as 99 species in 19 families of Lepidoptera are associated with *Acacia karoo* (Kroon, 1999). The geometrid moth, *Chlorissa stibolepidae* which was found in this study on maize in the sub-tropical areas of Venda is also reported to use *Acacia karoo* as host plant (Kroon, 1999).

The Lepidoptera host plant complex inside maize fields is very complex. Weed-associated Lepidoptera species inside maize fields are exposed to the pollen of Bt maize and in this way could be exposed to the toxin. However, not all Bt maize hybrids express

the Bt toxin in its pollen (Dutton *et al.*, 2003). Bt toxin is expressed in pollen of hybrids with event MON810.

Athetis pigra, *Caradrina atriluna*, *Simplicia extingalis* and *Cornutiplusia circumflexa* were grouped into the visitors guild since only their pupae were found on maize plants. These pupae most probably originated from larvae that were feeding on weeds inside maize fields. The importance of the possible indirect effect of Bt maize on weed-associated Lepidoptera should not be underestimated. Losey, Rayor and Carter (1999) found that larval survival of the monarch butterfly, *D. plexippus* was reduced after feeding on leaves of milk weed dusted with Bt pollen.

The common non-target species found on maize during this study can be included in a post-release non-target effects testing programme, as suggested by Birch *et al.* (2004) in Kenya. These species are could possibly be exposed to Bt toxin, and can be used to guide the design of the exposure system in test protocols as suggested by Birch *et al.* (2004). This information provides knowledge on which species is likely to be directly exposed to the plant-expressed Bt toxin. *Helicoverpa armigera* is closely associated with maize when cotton is not readily available. *Spodoptera* spp. are more polyphagous and can survive on a broader range of host plants. For sub-lethally affected lepidopteran herbivore species, it is important to determine whether or not their pest status will change on Bt maize compared to non-transgenic maize varieties.

In contrast to insecticides, physiological susceptibility to the Bt toxin varies widely within lepidopteran families and even within genera. This extreme variability essentially makes it impossible to establish a phylogenetic pattern that could guide the risk assessment process (Losey *et al.*, 2003). Thus, a full assessment of any lepidopteran species for which no data on susceptibility to Bt exists may require standardized toxicity testing. Once a group of species is selected, risk assessment could proceed by gathering data on: (1) the relative importance of the maize ecosystem as a habitat for the host plant; (2) the relative importance of that plant for the Lepidoptera species and (3) the susceptibility of the species to impact on the management tactic being assessed (Losey *et*

al., 2003). The vast majority of lepidopteran species found in maize fields are “neutral” or “value unknown” species (Andow & Hillbeck, 2004). The remaining challenging questions are: what is the role of this other “neutral” or “value unknown” non-target lepidopterans, and how will the agroecosystem function without these species if they are eliminated by the Bt toxin?

3.7 Conclusions

Lepidopteran species can be recognized as a functional group consisting of target and non-target primary consumers of maize plants. Once a functional group is established non-target primary consumers species must be identified. These non-target primary consumers are plant-consuming species that are not the target of the transgene, e.g. all the species mentioned above, except for *B. fusca* and *C. partellus*. Target primary consumers like *B. fusca* and *C. partellus* cannot be regarded as unimportant because another functional group, the natural enemies, can be indirectly affected if their hosts (stem borers) are affected. In order to provide a rational approach to support the selection of species for studies of non-target effects, a series of selection matrices should be developed (Fitt *et al.*, 2004). Through knowledge of occurrence, presence, abundance and linkage each species can then be ranked for its maximum potential exposure to Bt toxin (Birch *et al.*, 2004). The question remains if a target or non-target lepidopteran that is not affected will pass the Bt toxin on to higher trophic levels, possibly affecting any of its natural enemies. Will a natural enemy species providing an important ecological service elsewhere, decline in numbers because a main non-target herbivore prey source is adversely affected? (Birch *et al.*, 2004).

This study provided base line information on Lepidoptera species that occur on maize in South Africa and that are exposed to Bt maize. Future surveys should be conducted to include all maize production areas in South Africa and to determine the effect of Bt maize on the non-target Lepidoptera identified during this study.

3.8 References

Ajayi, O., Sharma, H.C., Tabo, R., Ratnadass A. & Doumbia, Y.O. 2001. Incidence and distribution of the sorghum head bug, *Eurystylus oldi* Poppius (Heteroptera: Miridae) and other panicle pests of sorghum in West and Central Africa. *Insect Science and its Application* 21: 103 – 111.

Andow, D.A. & Hillbeck, A. 2004. Bt maize, risk assessment and the Kenya case study. (eds.: Hillbeck & Andow). Environmental risk assessment of genetically modified organisms: Vol. 1. A case study of Bt maize in Kenya. CAB International. Wallingford. UK.

Anneck, D.P. & Moran, V.C. 1982. Insects and mites of cultivated plants in South Africa. Butterworths, Durban.

Birch, A.N.E., Wheatley, R., Anyango, B., Arpaia, S., Capalbo, D., Getu Degaga, E., Fontes, E., Kalama, P., Lelmen, E., Lovei, G., Melo, I.S., Muyekho, F., Ngi-Song, A., Ochieno, D., Ogwang, J., Pitelli, R., Schuler, T., Sétamou, M., Sithanantham, S., Smith, J., Van Son, N., Songa, J., Sujii, E., Tan, T.Q., Wan, F.H. & Hillbeck, A. 2004. Biodiversity on non-target impacts: a case study of Bt maize in Kenya. (eds.: Hillbeck & Andow). Environmental risk assessment of genetically modified organisms: Vol. 1. A case study of Bt maize in Kenya. CAB International. Wallingford. UK.

Bourgeat, D., Chaufaux, J., Micoud, A., Delos, M., Naibo, B., Bombarde, F., Marque, G., Eychenne, N. & Pagliari, C. 2002. *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (*Zea mays*). *Environmental Biosafety Research* 1: 49 – 60.

De Maagd, R. A., Weemen-Hendriks, M., Molthoff, J.W. & Naimov, S. 2003. Activity of wild-type and hybrid δ -endotoxins against *Agrotis ipsilon*. *Archives of Microbiology* 179: 363 – 367.

Dutton, A., Romeis, J. & Bigler, F. 2003. 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., 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.

Fitt, G.P., Andow, D.A., Carrière, Y., Moar, W.J., Schuler, T.H., Omoto, C., Kanya, J., Okech, M.A., Arma, P. & Maniania, N.K. 2004. Resistance risks and management associated with Bt maize in Kenya. (eds.: Hillbeck & Andow). Environmental risk assessment of genetically modified organisms: Vol. 1. A case study of Bt maize in Kenya. CAB International. Wallingford. UK.

Gounou, S. & Schulthess, F. 2004. Spatial distribution of lepidopterous stem borers on indigenous host plants in West Africa and its implications for sampling schemes. *African Entomology* 12: 171 – 178.

Gouse, M. 2005. Aspects of biotechnology and genetically modified crops in South Africa. Science, Technology and Globalization Project. Agricultural Biotechnology for Development – socioeconomic issues and institutional challenges. Belfer Center STPP. Kennedy School of Government.

http://bcsia.ksg.harvard.edu/BCSIA_content/documents/SouthAfricaGouse.pdf 21 May 2006.

Harris, K.M. 1995. World review of recent research on panicle insect pests of sorghum and pearl millet. (eds.: Nwanze & Youm). Panicle insects of sorghum and pearl millet: Proceedings of an International Consultative Workshop, 4 – 7 October 1993, ICRISAT Sahelian Center, Niamey, Niger. Patancheru 502 324, Andhra Pradesh, India: International Crop Research Institute for the Semi-Arid Tropics.

Kroon, D.M. 1999. Lepidoptera of Southern Africa. Host-plants and other associations. A Catalogue. Lepidopterists' Society of Africa. The Honorary Secretary. Jukskei Park. South Africa.

Lambert, B., Buysse, L., Decock, C., Jansens, S., Piens, C., Saey, B., Seurinck, J., Van Audenhove, K., Van Rie, J., Van Vliet, A. & Peferoen, M. 1996. A *Bacillus thuringiensis* insecticidal crystal protein with a high activity against members of the family Noctuidae. *Applied and Environmental Microbiology* 62: 80 – 86.

Liu, Z., Li, D., Gong, P. & Wu, K. 2004. Life table studies of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), on different host plants. *Environmental Entomology* 33: 1570 – 1576.

Losey, J.E., Hufbauer, R.A. & Hartzler, R.G. 2003. Enumerating lepidopteran species associated with maize as a first step in risk assessment in the USA. *Environmental Biosafety Research* 2: 247-261.

Losey, J.E., Rayor, L.S. & Carter, M.E. 1999. Transgenic pollen harms monarch larvae. Artificial Organism. Macmillan. *Nature* 399, 214.

Norris, R.F. & Kogan, M. 2000. Interaction between weeds, arthropod pests, and their natural enemies in managed ecosystems. *Weed Science* 48: 94-158.

Obrist, L.B., Dutton, A., Romeis, J. & Bigler, F. 2006. Biological activity of Cry 1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. *BioControl* 51: 31 – 48.

Peacock, J.W., Schweitzer, D.F., Carter, J.L. & Dubois, N.R. 1998. Laboratory assessment of the effects of *Bacillus thuringiensis* on native Lepidoptera. *Environmental Entomology* 27: 450 – 457.

Pons, X., Lumbierres, B., Lopez, C. & Albajes, R. 2005. Abundance of non-target pest in transgenic Bt maize: A farm scale study. *European Journal of Entomology* 102: 73 – 79.

Romeis, J., Sharma, H.C., Sharma, K.K., Das, S. & Sarmah B.K. 2004. The potential of transgenic chickpeas for pest control and possible effects on non-target arthropods. *Crop Protection* 23: 923 – 938.

Schuler, T.H. 2004. GM crops: good or bad for natural enemies? Aspects of Applied Biology 74, GM crops – Ecological dimensions, pp. 81 – 90. Association of Applied Biologists, Wellesbourne, UK.

Seshu Reddy, K.V. 1983. Studies on the stem borer complex of sorghum in Kenya. *Insect Science and its Application* 4: 3 – 10.

Singh, R., Channappa, R.K., Deebe, F., Nagaraj, N.J., Sukaveneswaran, M.K. & Manjunath, T.M. 2005. Tolerance of Bt corn (MON 810) to maize stemborer, *Chilo partellus* (Lepidoptera: Pyralidae). *Plant Cell Reports* 24: 556 – 560.

Statistica 7.1. 2005. StatSoft, Inc. Tulsa. USA.

Van den Berg, J. & Van Wyk, A. In press. The effect of Bt maize on *Sesamia calamistis* (Lepidoptera: Noctuidae) in South Africa. *Entomologia Experimentalis et Applicata*.

Vojtech, E., Meissle, M. & Poppy, G.M. 2005. Effects of Bt maize on the herbivore *Spodoptera littoralis* (Lepidoptera: Noctuidae) and the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae). *Transgenic Research* 14: 133 – 144.

Van Rensburg, J.B.J. 1999. Evaluation of Bt-transgenic maize for resistance to the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) in South Africa. *South African Journal of Plant Soil* 16: 38 – 43.

Table 3.1 Lepidopteran species observed to feed on maize during two growing seasons.

Scientific name	Family	Feeding on Bt maize with no survival	Feeding on Bt maize with survival	Feeding on non-Bt maize
Recognized Lepidopteran pests of maize in South Africa				
<i>Acantholeucania loreyi</i> (Dup.)	Noctuidae	✗	✓	✓
<i>Agrotis segetum</i> (Denis & Schiff)	Noctuidae	✗	✓	✓
<i>Busseola fusca</i> (Fuller)	Noctuidae	✗	✓	✓
<i>Chilo partellus</i> (Swinhoe)	Pyralidae	✓	✗	✓
<i>Helicoverpa armigera</i> (Hübner)	Noctuidae	✗	✓	✓
<i>Sesamia calamistis</i> (Hampson)	Noctuidae	✓	✗	✓
<i>Spodoptera exigua</i> (Hübner)	Noctuidae	✗	✗	✓
Newly recorded Lepidoptera species on maize				
<i>Athetis pigra</i> (Guenée)	Noctuidae	✗	✗	**
<i>Caradrina atriluna</i> (Guenée)	Noctuidae	✗	✗	**
<i>Chlorissa stibolepidae</i> (Butler)	Geometridae	✗	✗	✓
<i>Chrysodeixis acuta</i> (Walker)	Noctuidae	✗	✗	✓
<i>Eublemma gayneri</i> (Roths.)	Noctuidae	✗	✓	✓
<i>Nola phaeocraspis</i> (Hmps)	Nolidae	✗	✓	✓
<i>Simplicia extinctalis</i> (Zeller)	Noctuidae	✗	✗	**
<i>Cornutiplusia circumflexa</i> (Linnaeus)	Noctuidae	✗	✗	**

** no feeding observed, pupa found on maize and moths emerged.

Table 3.2 Different guilds of Lepidoptera species that occurred on maize.

	Seedling/leaf feeders	Stem borers	Tassel feeders	Ear and silk feeders	Visitors
<i>Acantholeucania loreyi</i>	✓			✓	
<i>Agrotis segetum</i>	✓				
<i>Busseola fusca</i>	✓	✓	✓	✓	
<i>Chilo partellus</i>	✓	✓	✓	✓	
<i>Helicoverpa armigera</i>	✓			✓	
<i>Sesamia calamistis</i>	✓	✓		✓	
<i>Spodoptera exigua</i>	✓				
<i>Athetis pigra</i>					✓
<i>Caradrina atriluna</i>					✓
<i>Chlorissa stibolepidae</i>				✓	
<i>Chrysodeixis acuta</i>				✓	
<i>Eublemma gayneri</i>				✓	
<i>Nola phaeocraspis</i>				✓	
<i>Simplicia extintalis</i>					✓
<i>Cornutiplusia circumflexa</i>					✓

Table 3.3 Host plant range of Lepidoptera species that were recorded on maize. *

Lepidoptera species	Host plant scientific name	Host plant family	Host plant common name
<i>Athetis pigra</i>	-	-	-
<i>Caradrina atriluna</i>	-	-	-
<i>Chlorissa stibolepidae</i>	-	-	-
<i>Chrysodeixis acuta</i>	<i>Canna</i> sp.	Cannaceae	Lilies
	<i>Cestrum auranticum</i>	Solanaceae	Orange cestrum
	<i>Lonicera</i> sp.	Caprifoliaceae	“Kanfer”
	<i>Lycopersicon</i> sp.	Solanaceae	Tomato
	<i>Musa</i> sp.	Musaceae	Banana
	<i>Nicotiana</i> sp.	Solanaceae	Tobacco
	<i>Phaseolus vulgaris</i>	Fabaceae	French bean
	<i>Plectranthus</i> sp.	Lamiaceae	“Vliëebos”
	<i>Solanum</i> sp.	Solanaceae	Potato
	<i>Solanum tuberosum</i>	Solanaceae	Potato
	<i>Syncolostemon densiflorus</i>	Lamiaceae	-
	<i>Trema</i> sp.	Ulmaceae	Pigeon wood
<i>Eublemma gayneri</i>	<i>Acacia karoo</i>	Fabaceae	Sweet thorn
<i>Nola phaeocraspis</i>	-	-	-
<i>Simplicia extinctalis</i>	<i>Acacia mearnsii</i>	Fabaceae	Black wattle
	<i>Medicago sativa</i>	Fabaceae	Lucerne
	<i>Olea</i> sp.	Oleaceae	Olive
<i>Cornutiplusia circumflexa</i>	<i>Eschscholzia</i> sp.	Papaveraceae	-
	<i>Heliotropium</i> sp.	Boraginaceae	“Brakganna”
	<i>Pittosporum viridiflorum</i>	Pittosporaceae	-
	<i>Solanum tuberosum</i>	Solanaceae	Potato

* Host range compiled by Kroon (1999).

Table 3.4 Percentage maize plants infested with *Eublemma gayneri* and *Nola phaeocraspis* larvae at different localities (values in brackets are actual numbers).

	Season	Polokwane (non-Bt)	Venda (non-Bt)	Potchef- stroom (Bt)	Warmbad (Bt)
<i>Eublemma gayneri</i>	2004/5	-	-	-	3.67 (33)
	2005/6	3.61 (19)	2.90 (29)	0.11 (1)	0.15 (3)
<i>Nola phaeocraspis</i>	2005/6	1.52 (8)	2.00 (20)	-	0.15 (3)

Table 3.5 Mean percentage Bt plants of which ears were infested by larvae of *Helicoverpa armigera*, *Acantholeucania loreyi*, *Eublemma gayneri* and the incidence of plants exhibiting ear damage in three center pivot irrigation systems at Warmbad (an average of two ears per maize plant were recorded) (values in brackets are standard errors).

	% infestation by <i>Helicoverpa</i> <i>armigera</i>	% infestation by <i>Acantholeucania</i> <i>loreyi</i>	% infestation by <i>Eublemma</i> <i>gayneri</i>	% damaged ears
Season 2004/2005				
Field 1	1.67 (± 0.88)	0	4.33 (± 0.67)	7.00 (± 1.15)
Field 2	1.67 (± 0.67)	0	6.33 (± 0.88)	1.67 (± 0.88)
Field 3	0.67 (± 0.67)	0.33 (± 0.33)	0.33 (± 0.33)	0.67 (± 0.67)
Season 2005/2006				
Field 1	0.78 (± 0.32)	5.67 (± 1.19)	0.11 (± 0.11)	17.44 (± 2.81)
Field 2	0	3.78 (± 0.72)	0.22 (± 0.15)	10.11 (± 0.92)
Field 3	0	0	0	0

Table 3.6 The incidence of maize plants damaged by stem borers, *Agrotis segetum*, *Helicoverpa armigera*, *Spodoptera exigua* and *Acantholeucania loreyi* at different localities in the maize production area of South Africa.

Field	Species	% damage (Bt)	% damage (non-Bt)	Chi-square	P-value	95% confidence interval
2004/5 Cropping season						
Lukaskraal	<i>A. segetum</i>	0.22	0.75	17.65	0.0001	(1.90; 6.26)
Vaalbank	<i>H. armigera</i>	9	16	7.38	0.0066	(1.24; 3.41)
	Stem borers	0.3	3	5.93	0.0149	(1.33; 82.19)
Hartlam	<i>H. armigera</i>	3	10	13.27	0.0030	(1.89; 9.31)
	Stem borers	0	2	4.21	0.0402	-
Nog-'n -Stuk	<i>H. armigera</i>	6.6	52	148.22	0.0001	(9.26; 25.53)
	Stem borers	0.3	1	0.81	0.3691	(0.49; 32.94)
	<i>A. loreyi</i>	1	0.3	0.81	0.3691	(0.49; 32.94)
Potchefstroom Site 1. 33A13; 33A14B	<i>H. armigera</i>	3	18	35.33	0.0001	(3.65; 16.79)
	Stem borers	1	5	6.93	0.0085	(1.49; 18.19)
Potchefstroom Site 2. 33P66; 33P67B	<i>H. armigera</i>	5	7	1.45	0.2289	(0.81; 3.22)
	Stem borers	4	16	23.38	0.0001	(2.48; 9.60)
Potchefstroom Site 3. 32D99; 32D96B	<i>H. armigera</i>	3	15	23.14	0.0001	(2.53; 10.36)
	Stem borers	1	2	0.45	0.5018	(0.50; 8.15)
	<i>A. loreyi</i>	0.3	0	0	1	-
Noric	<i>H. armigera</i>	3	19	34.47	0.0001	(3.32; 13.32)
	Stem borers	1	5	6.05	0.0139	(1.38; 17.04)
Wildfontein	<i>H. armigera</i>	4	34	84.43	0.0001	(6.51; 22.74)
	Stem borers	5	14	11.93	0.0006	(1.59; 5.27)
	<i>A. loreyi</i>	0	6	15.5	0.0001	-
2005/6 Cropping season						
Castello	<i>H. armigera</i>	0	0.3	7.13	0.0076	-
	<i>S. exigua</i>	0	0.3	5.15	0.0232	-
Rooibult	Stem borers	7.67	5	4.95	0.026	(1.06; 2.31)
Ntsu	Stem borers	3.78	13.11	49.5	0.00001	(2.59; 5.69)
Hartlam	<i>H. armigera</i>	2.7	7	17.44	0.00001	(1.70; 4.44)
Mean total percentage		2.59	10.07			



Figure 3.1. *Eublemma gayneri* moth.



Figure 3.2. *Eublemma gayneri* larva feeding on maize ear.



Figure 3.3. *Nola phaeocraspis* moth.



Figure 3.4. *Nola phaeocraspis* larva feeding on maize silks.



Figure 3.5. *Chrysodeixis acuta* larva feeding on maize husk leaves.

CHAPTER 4: THE EFFECT OF BT MAIZE ON *SESAMIA CALAMISTIS* (LEPIDOPTERA: NOCTUIDAE) IN SOUTH AFRICA

4.1 Abstract

Bt maize (event MON810) expressing *Cry1Ab* insecticidal proteins was introduced for control of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), and *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in South Africa after its development for control of stem borers in North America. In the light of the reportedly lower toxicity of Bt maize to certain Noctuidae borers, the effect of Bt maize was evaluated on *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae). The characteristic larval behavior of *S. calamistis* may result in reduced exposure to Bt toxin and subsequent high levels of survival. Larvae do not feed in plant whorls like other borer species but penetrate stems directly from behind leaf sheaths where eggs are laid. Greenhouse and laboratory bioassays were done with three Bt maize hybrids and their iso-hybrids. “Whole plant” methods were used and potted plants artificially infested with eggs or larvae and survival recorded over time. Larval survival was also determined on different plant parts (whorls, stems, tillers and ears) over time. Bt maize was shown to be highly toxic to *S. calamistis*. No larvae survived longer than 12 to 18 days on Bt maize plants in any of the experiments. Adults did not differentiate between Bt and non-Bt plants in oviposition choice experiments. *Sesamia calamistis* is polyphagous and occurs in mixed populations with other borer species with which it shares many parasitoid species in Africa. The ecological impact of local extinction of *S. calamistis* caused by this highly effective transgenic event is therefore not expected to be great.

4.2 Introduction

Bt maize was initially developed for the control of two noctuid stem borers in North America i.e., *Diatraea grandiosella* (Dyar) (Lepidoptera: Crambidae) (Archer, Patrick, Schuster, Cronholm, Bynum & Morrison, 2001) and *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) (Ostlie, Hutchison & Hellmich, 1997) before it was introduced for control of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in South Africa. Although the Bt maize (event MON810) that is used in South Africa effectively controls *B. fusca*, survival of this species on certain plant parts has been reported (Van Rensburg, 1998; Van Rensburg, 2001). This event is, however, reported to cause 100% mortality of *C. partellus* (Van Rensburg, 1998) (Singh, Channappa, Deeba, Nagaraj, Sukaveneswaran & Manjunath, 2005). The stem borer, *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae), which occurs widely throughout sub-Saharan Africa, was not intended as a target organism for Bt maize in South Africa and the toxicity of Bt maize to this species has not yet been determined.

The African stem borer, *B. fusca* is an indigenous species that occurs throughout sub-Saharan Africa while *C. partellus* is exotic and occurs widely through eastern and southern Africa (Overholt & Maes, 2000). *Sesamia calamistis* on the other hand is only economically important in western Africa (Ajala, Kling, Schulthess, Cardwell & Odiyi, 2001). It does not often attain economically important status in eastern and southern Africa in spite of its wide occurrence on several crops (Harris, 1962) (Overholt & Maes, 2000). *Sesamia calamistis* can be regarded as one of the stem borer species with the widest distribution in Africa although it occurs at very low infestation levels and forms a small proportion of the total stem borer population outside of western Africa. The contribution of *S. calamistis* to the total stem borer population varies over time and between regions but has been reported to be small. In South Africa *S. calamistis* was initially only recorded as a pest of maize in the coastal belt of the Western Cape region but its importance has since the 1990s been reported to be on the increase in maize on the

Highveld regions (< 1300 m above sea level), especially in irrigated maize (Van den Berg & Drinkwater, 2000).

Although the general biology of *S. calamistis* is similar to that of other stem borers there is one major difference in larval behaviour. An outstanding characteristic of its larval behaviour is that neonate larvae do not migrate to plant whorls after egg hatch. Eggs are laid between leaf sheaths and the stem (like *B. fusca*) but newly hatched larvae feed on the leaf sheath for a short time before penetrating the stem directly (Shanower, Schulthess & Bosque-Pérez, 1993) (Ajala *et al.*, 2001). Larvae therefore seldom leave the protection of the stem except when they migrate to other plants (Harris, 1962). This aspect of its biology may affect the effectiveness of Bt against *S. calamistis* since larvae have minimal feeding on whorl and leaf tissue. Oviposition at late plant growth stages results in larval infestation of maize ears. These larvae feed on husk leaves and then penetrate the ears (Annecke & Moran, 1982) (Kalule, Ogenga-Latigo & Okoth, 1997) (Van den Berg *et al.*, 2001). Considering the feeding habits of stem borers and the expression of the Bt-toxin in maize it can be assumed that all major plant-chewing herbivores ingest the toxin upon feeding on Bt maize, given that all plant parts fed upon contain the toxin (Dutton, Romeis & Bigler, 2003). Bt-toxin of event MON810 is expressed in the leaf and pollen of maize but not in stems (Dutton *et al.*, 2003). Increased survival of *B. fusca* when feeding on stem tissue, silk and ears of Bt maize has been ascribed to the absence or reduced expression of the toxin in certain plant parts (Van Rensburg, 2001).

The aim of this study was to determine the effect of Bt maize on *S. calamistis*. This was done by studying moth oviposition choice between Bt and non-Bt maize plants and larval survival on Bt maize plants and on different plant parts.

4.3 Material and methods

4.3.1 Oviposition experiments

A two-choice experiment was conducted to determine the oviposition selection of moths when presented with a choice between Bt and non-Bt maize plants.

The experiment was conducted in muslin cloth cages measuring 0.80m × 0.70m × 1.2m. Six cages (replicates) were placed in a room with natural daylight and in which the temperature varied between 18 – 26 °C. Plants were grown in a greenhouse before they were used in the experiments. Two pots were placed inside each cage. One pot contained a Bt maize plant (Pan 6012B) while the other contained the non-Bt iso-hybrid (Pan 6804). Each two-choice test was replicated six times. Pairs of male and female moths were put separately in 300 ml plastic containers to mate overnight before two females were put into each cage. Moths were allowed to oviposit for two nights before they were removed. Kaufmann (1983) and Usua (1970) reported that *S. calamistis* moths mate on the night of eclosion and lay the majority of their eggs during the subsequent two nights. Egg batches were removed from plants and the numbers of egg batches and eggs per batch recorded for each plant.

4.3.2 Larval survival studies

Eight studies were done to determine larval survival on Bt- and non-Bt maize plants. These studies involved laboratory bioassays where plant material was fed to larvae, followed by greenhouse studies using potted plants. The “whole plant method” suggested by Birch *et al.* (2004) was used to evaluate the effect of the transgene plant and not only the transgene product. The event MON810 is the only registered Bt maize event in South Africa and is present in all the transgenic varieties used in this study. Larvae and moths used in this study were reared on susceptible maize for one or two generations after collecting larvae from maize fields.

Experiment 1: Infestation of potted plants with eggs

Two experiments were conducted using 100 potted plants for each hybrid. In each experiment one Bt maize variety was used together with its non-Bt maize iso-hybrid as control treatment. One experiment was conducted in the open under a hail net using non-Bt (Pan 6804) and Bt (Pan 6012B) maize hybrids. The other experiment was done in a green house with a non-Bt (Phb 3223) and Bt (Phb 31B13) hybrid. In each experiment the non-Bt and Bt plants were kept in two separate blocks to prevent possible migration of larvae between blocks.

Plants were inoculated with *S. calamistis* eggs that were in the black-head stage and ready to hatch within one day. Maize plants were four weeks old at the time of inoculation. Eggs were obtained from a mass reared colony where moths were allowed to oviposit on non-Bt maize plants. Egg batches were removed from plants and divided into smaller groups to facilitate infestation of plants with 10 eggs each. A camel-hair brush was used to put eggs onto plants (10 larvae per plant) between the stem and the last unfolded leaf sheath of the plant. The infestation level of 10 eggs per plant was based on results of Nwosu (1990) who recommend the use of 10-15 larvae per plant when screening maize for resistance to *S. calamistis*.

The number of live larvae per plant was determined over a six-week period when larvae were expected to reach the pupae stage. Nine randomly selected plants of each maize hybrid were dissected at regular intervals after inoculation. In the one experiment plants were dissected at 3, 6, 9, 12, 15, 21, 27, 33 and 44 days after inoculation. In the other experiment plants were dissected at 3, 6, 9, 12, 17, 23, 29 and 40 days after inoculation.

Experiment 2: Infestation of plants with neonate larvae behind leaf sheaths

The experiment was done in a green house using 100 potted plants each of the non-Bt (Phb 3223) and Bt (Phb 31B13) hybrids. The experimental design was a completely randomized block.

Four-week old plants were inoculated with *S. calamistis* neonate larvae. A camel-hair brush was used to put larvae onto plants between the stem and the last unfolded leaf sheath of the plant. An infestation level of 10 larvae per plant was used.

The number of live larvae per plant was determined over a three-week period until no survival of larvae on Bt maize was recorded. Nine randomly selected plants of each hybrid were dissected three, six and twelve days after inoculation.

Experiment 3: Whorl infestation of potted plants with neonate larvae

Since feeding of *S. calamistis* in plant whorls have been reported and infestation of young plants does result in whorl feeding, an experiment was conducted to determine its effect on larval survival. The maize hybrids, Pan 6012B (Bt), and iso-hybrid, Pan 6804 (non-Bt) as well as Phb 3223 (non-Bt) and Phb 31B13 (Bt) were used in this experiment. There were 20 potted plants of each hybrid and plants were four weeks old at the time of infestation. The experiment was done in the open and potted plants were kept under hail nets.

Plants were infested by placing 15 neonate larvae into the whorl of each plant by means of a camel-hair brush. Five randomly selected plants of each hybrid were dissected 4, 8, 11 and 18 days after inoculation. The data collected were similar to that in the above-mentioned study.

4.3.3. Laboratory experiments

Larval survival on different plant parts was evaluated in the laboratory. Test tubes or plastic containers were held under natural day/night conditions in a laboratory where temperatures fluctuated between 20 and 25 °C. The experimental designs were completely randomized.

Experiment 1: Larval survival on stems

Bt-hybrids Pan 6012B and Phb 31B13, and their non-Bt iso-hybrids, Pan 6804 and Phb 3223 were used. Pieces of stem (± 15 cm) of 5-week old plants of which leaf sheaths were removed were placed in glass test tubes (3 x 25 cm). Ten 1st instars were put into each test tube and the tube plugged with cotton wool. Each hybrid was replicated 10 times. Stems were left undisturbed for 19 days when the number of live larvae was recorded.

Another experiment was conducted using the same varieties to determine the survival of larger larvae when feeding on Bt stems. To obtain larger larvae of uniform age, 1st instar larvae were put on susceptible maize until they reached the 4th instar before they were used in the experiment. A longitudinally cut piece of stem (± 15 cm) from which the leaf sheaths were removed was placed in a glass test tube (3 x 25 cm). Each hybrid was replicated 20 times. One larva was put into each test tube and survival and larval mass recorded 1, 3 and 6 days after the start of the experiment.

Experiment 2: Survival on tillers

Tillers in the whorl stage of development of Bt (CRN 78-15B) and non-Bt (SC701) maize plants in the ear stage of development were collected from commercial maize fields. Stems were cut into 15 cm pieces and pieces of which leaf sheaths were not removed placed in test tubes similar to the methods described above and infested with ten 1st instar larvae. Each treatment was replicated 30 times and the number of surviving larvae recorded after 20 days. Since a piece of stem was big enough to sustain larval development for this period, food was not changed and larvae were left undisturbed for the whole period.

Experiment 3: Survival on ears

One non-Bt hybrid (SC701) and two Bt-hybrids (CRN 78-15B and Pan 6013B) were used in this study. Ears were in the soft dough stage and were cut from plants growing in irrigated maize fields. Twenty maize ears of each hybrid were placed singly in plastic containers (15 X 25 cm) lined with absorbent tissue paper at the bottom. The outer two

husk leaves of each ear were removed before putting ears into containers. This was done to remove dirt, aphids and fungal infections that could occur on ears. Each ear was infested by placing 15 neonate larvae below the outer of the remaining husk leaves by means of a camel-hair brush. All ears were left undisturbed in the plastic containers for a period of 24 days after which they were dissected to recover live larvae. The experimental design was completely randomised.

4.4 Data analysis

Repeated measures ANOVA were used to analyse larval survival over time while t-tests (STATISTICA 7.0) were used to analyse laboratory results.

4.5 Results

4.5.1 Oviposition study

No significant differences were observed between the numbers of egg batches, egg batch size and total numbers of eggs laid on the Bt and non-Bt maize plants (Table 4.1.).

4.5.2 Larval survival studies

Experiment 1: Infestation of plants with eggs behind leaf sheaths

In both experiments larval survival decreased rapidly over the first nine days after inoculation (Figures 4.1 & 4.2). Larval survival differed significantly over time between the Bt (Pan 6012B) and non-Bt hybrid (Pan 6804) (F-value $(7, 12) = 37.83$; $P < 0.001$) in the experiment conducted under hail nets. The level of survival also differed significantly in the greenhouse experiment (F-value $(7, 112) = 29.53$; $P < 0.001$). No surviving larvae were recorded on Bt maize plants from 12 days after inoculation onwards in any of the two experiments. The percentage surviving larvae on the non-Bt plants was 25% on Pan 6804 and 22% on Phb 3223 44 days after infestation. Larvae that were recovered from Bt

plants were never larger than 7mm indicating that they did not develop further than the 3rd instar.

Experiment 2: Infestation of plants with neonate larvae behind leaf sheaths

There was a rapid decline in larval numbers on Bt plants over the first six days after infestation (Figure 4.3). No surviving larvae were observed on any of the Bt-plants 12 days after infestation and survival differed significantly between hybrids from day three onwards (F-value $(2, 36) = 68.19$; $P < 0.001$). The percentage surviving larvae on the non-Bt plants was 30% on day 12.

Experiment 3: Infestation of potted plants with neonate larvae in whorls

Larval survival decreased rapidly over the first eight days after inoculation (Figure 4.4). No surviving larvae were recorded on Bt maize plants 18 days after inoculation when the experiment was terminated. Larval survival differed significantly over time between the Bt (Pan 6012B) and non-Bt hybrid (Pan6804) (F-value $(3, 24) = 14.39$; $P < 0.000014$) as well as between the Bt (Phb31B13) and the non-Bt hybrid (Phb3223) (F-value $(3, 24) = 23.45$; $P < 0.001$). The percentage surviving larvae on non-Bt plants in the two experiments was 22 and 36 % respectively on day 18.

4.5.3 Laboratory experiments

Experiment 1: Stem artificial infestation

Survival of neonate larvae differed significantly between Bt and non-Bt hybrids. No survival was observed on Bt maize stems in the glass test tubes 19 days after infestation. Survival on non-Bt stems was 13 % on Pan 6804 (t-value = 3.55; $P < 0.0023$) and 24% on Phb 3223 (t-value = 2.79; $P < 0.012$).

Survival of 4th instars on non-Bt maize stems was 70 % after 10 days while only 5 % (one larvae) survived on Bt maize. Larval mass of 4th instars on Bt plants remained constant between three and six days after commencement of the experiment but increased on non-Bt plants. A slight decrease in mass was observed between 6 and 11 days when larvae started changing into pre-pupae on non-Bt stems (Figure 4.5.). A large difference in

larval mass between Bt and non-Bt feeding larvae was already observed after three days of feeding.

Experiment 2: Tiller artificial infestation

No larvae survived on pieces of tillers of Bt-plants while the percentage survival was 60% on non-Bt stems after 20 days and the difference between levels of survival was highly significant (t-value = 8.63; $P < 0.0001$).

Experiment 3: Ear artificial infestation

Significant differences (t-value = 15.57; $P < 0.0001$) were observed in survival on the Bt and non-Bt plants with no surviving larvae recorded on any of the ears of Bt maize plants. Sixty six percent of larvae survived on non-Bt maize ears after 24 days.

4.6 Damage symptoms of *S. calamistis* larval feeding on susceptible maize

Sesamia calamistis larval feeding damage to maize has been poorly described in the literature. Even though larvae do not migrate to plant whorls as other stem borers do, reference is often made to whorl damage or leaf damage (Nwosu, 1990). Stem damage and stem breakage also occur but have never been put into context. While damage symptoms caused by other stem borer species is first noticed when feeding lesions appear on the furl leaves deep inside the whorl, this is not the case with *S. calamistis* damage. Whether or not whorl damage symptoms appear depends on plant age at time of infestation and egg hatch.

During the first week after egg hatching, larvae spend their time behind leaf sheaths or between the leaf sheath and the stem. During this time they feed mostly on the outer leaf sheath. Towards the end of this period larvae start to make small holes in the inner sheaths or stem. Second instars do sometimes also show boring (leaf mining) behavior inside the outer leaf sheaths and may tunnel in the sheath for up to 10 mm.

If plants are very young (3- 4 weeks) and stems are thin when larval feeding behind leaf sheaths start, larvae may also start feeding on some inner sheaths and the “stem” causing symptoms to grow out and appear like whorl damage symptoms while larvae are actually feeding inside the very young stem. These symptoms appear on whorl leaves as small symmetrical lesions, often in rows of repeating identically-shaped damage lesions in the emerging leaves. These leaf damage symptoms differ from that made by other borers in that there are holes in leaves and not “windows” such as the damage caused by other borer larvae that feed in whorls.

When larger plants are infested (four to six weeks old) whorl damage symptoms appear much later and look less severe. Approximately 10 to 14 days after egg hatch plants exhibit whorl damage caused by larval feeding on the young leaves deep in plant whorls where larvae that bored into stems were feeding. Characteristic girdling of stems by larvae can also be observed at this stage. Visible whorl damage symptoms can be described as pinholes to elongated rounded lesions (2mm x 8mm). Stems may be tunneled into (± 1 cm long). Plants exhibit damage to outside leaf sheaths with scarification and feeding lesions of ± 2 cm in length on the insides of sheaths. Frass build-up caused by feeding of late 2nd to 3rd instars can be observed behind leaf sheaths. Approximately 16 days after infestation long elongated damaged symptoms start to appear at the base of older furl leaves and by this time most larvae occur inside stems with tunnels of 3 to 4 cm at several places in the stems. Three weeks after egg hatching, long elongated damaged symptoms became visible at the base of older furl leaves and most larvae occur inside stems with some tunneling ($\pm 3 - 4$ cm). Larvae that do occur in whorls are usually deep down and at the “base” of the whorl. Stem breakage start to occur three to four weeks after infestation when they break at positions where girdling was severe. In this study, *S. calamistis* larvae took between 40 and 44 days to develop into pupae.

Visible damage on Bt maize appeared only after 12 days. This damage occurred on some of the whorl leaves of plants and was limited to a few extremely small pin holes (<1mm in diameter). No typical whorl damage symptoms were visible. Visible lesions on leaf

sheaths of Bt plants showed 2 to 3 sheaths bored with small pin-hole type lesions approximately 1mm in diameter. No tunneling was observed inside leaf sheaths and only superficial damage was present. Many dead 1st instars were observed behind leaf sheaths. From day 16 onwards Bt-plants grew out of damage symptoms and new leaves appearing in whorls showed no damage. This indicated that no more larvae were feeding on plants and that larval feeding had ceased a week or more previously.

4.7 Discussion

This study showed that there was no difference in oviposition choice of moths between Bt and non-Bt plants and that certain cues used by moths for host recognition were not affected in spite of Bt maize plants not being suitable for offspring survival. Similar results were reported by Pilcher and Rice (2001) who observed that *O. nubilalis* females did not show any oviposition preference towards non-Bt or Bt maize (event 176 and BT11). The importance of determining the success rate of host finding as one of the aspects that needs to be included in the assessment of possible changes in transgenic plants was pointed out by Turlings *et al.* (2005). The latter authors observed that the ratios of caterpillar-induced odor emissions of Bt maize plants were identical to that of non-Bt plants. The total amount of volatiles released by Bt plants was however significantly less than that released by non-Bt plants. Braconidae parasitoids which are attracted to plants emitting these compounds, did not distinguish among odours of the different plants (Turlings, Jeanbourquin, Held & Degen, 2005).

Sesamia calamistis was highly susceptible to Bt maize plants containing event MON 810. The behavioural characteristic of larvae to feed behind leaf sheaths and to enter stems directly did not result in escape of exposure to the toxin. Larval feeding on leaf sheaths therefore resulted in the ingestion of enough toxin to kill larvae before they entered maize stems where Bt-toxin of event MON 810 is reported not to be expressed (Dutton *et al.*, 2003). Larval feeding on different plant parts such as the whorls, ears, stems of post-flowering plants and stems of tillers, which are typically attacked, also caused 100 %

mortality. The superficial feeding on husk leaves covering ears resulted in the death of 1st instars within days. Cadavers of many 1st instars were visible underneath the husk leaves of Bt maize ears where they were put when the experiment was initiated. Minute feeding lesions could be observed on the husk leaves. Feeding of larger larvae (4th instars) on stem tissue of young plants and tillers also resulted in 100 % mortality. The drastic reduction in larval mass of 4th instars feeding on Bt stems indicate that the toxic effect of Bt was immediate. This result is significant since it showed that larger migrating larvae could also be killed by Bt plants. Kaufmann (1983) reported that there is minimum migration of larvae at infestation levels of less than 40 eggs per plant. Ingram (1958) reported that larvae migrate when they reach the 3rd and 4th instars. Even if larvae could somehow escape from susceptible plants or wild host plants that may occur in close proximity to a Bt maize plant, larvae will die once they start feeding on Bt plants.

The high mortality level observed in this study was not expected since it was reported that Bt maize does not effectively control other Noctuidae species such as corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in the United States (Archer *et al.*, 2001; Hilbeck & Andow, 2004). Van Rensburg (2001) also observed survival of *B. fusca* on Bt maize when feeding on stem tissue and silks of maize ears.

It seems that the mortality level that could be expected depends on the toxicity of Bt maize varieties. In a review on risks and management of Bt maize in Kenya, Fitt *et al.* (2004) indicated that the toxicity of currently-available Bt maize varieties in that country was considered to be low and that toxicity depended on the event used. Mugo *et al.* (2005), in leaf disk bioassays observed that not all events were equally efficient against different stem borers and that especially *B. fusca* was difficult to control. This aspect will need careful consideration in risk assessments and decisions on deployment of Bt maize varieties.

4.8 Ecological impacts

The use of transgenic insecticidal Bt crops could directly or indirectly affect non-target organisms that occur in agro-ecosystems and provide significant services to ecosystem functioning. *Sesamia calamistis* is a common stem borer species in both maize and wild host ecosystems and may have an important role in the general ecology of parasitoids of not only itself, but also other stem borers such as *B. fusca*, *C. partellus* and *Eldana saccharina* Walker (Lepidoptera: Pyralidae). It is therefore important to understand the population dynamics of stem borers in cultivated crops, and to have knowledge on the ecological relationships between stem borers, parasitoids and native grasses.

Sesamia calamistis is probably the most widely distributed stem borer species in Africa. It occurs throughout sub-Saharan Africa from the coast up to 2400 m above sea level (Polaszek & Khan, 1998) (Muhammad & Underwood, 2004). It often occurs in mixed populations with other stem borers and together with *B. fusca* and *E. saccharina* present the three indigenous most economically-important borer species on maize. Together with *C. partellus* these species form the complex of stem borers that is targeted by Bt maize. Although *S. calamistis* is not considered a target organism of Bt maize in South Africa, it was listed as a target organism in Kenya (Hilbeck & Andow, 2004). The target pests of Bt maize in Kenya include the mentioned four species and *Chilo orichalcociliellus* (Strand) (Muhammad & Underwood, 2004).

Mortality rates of 100 % can result in localized host depletion for parasitoids specific to the target pest, and possibly also in a reduction in prey for generalist natural enemies, when compared to populations in unsprayed non-GM crops (Schuler, 2004). In the case of *S. calamistis* the impact on parasitoid numbers would most likely not be great since several other stem borer species that also occur in wild host plants also host the same parasitoid species.

Of the approximately 104 parasitoid species reported on stem borers in Africa (Bonhoff, Overholt, Van Huis & Polaszek, 1997), 16 species were reported to attack *S. calamistis*

and another 16 species to attack *Sesamia* sp. In a review on parasitoids of stem borers in Africa, Bonhoff *et al.* (1997) cited only two species as specialist parasitoids on *S. calamistis*, i.e., *Cotesia ruficrus* (Haliday) (Hymenoptera: Braconidae) and *Metagonistylum* sp. (Diptera: tachinidae). Nearly all the parasitoids of *S. calamistis* also attack other borers such as *B. fusca*, *C. partellus*, *C. orichalcociliellus* and *E. saccharina*. General parasitoids that occur on *S. calamistis* and other stem borer species include several Braconid species: *Chelonus curvimaculatus* Cameron, *Co. sesamiae* Cameron, *Dolichogenidea polaszeki* Walker and *Stenobracon rufus* Szepligeti. The Ichneumonidae that occur on several stem borers species are *Pediobius furvus* (Gahan) (Eulopidae), *Procerochasmias nigromaculatus* Cameron and *Xanthopimpla* spp. (Bonhoff *et al.*, 1997). *Sesamia calamistis* plays an important role in the ecology of these stem borer parasitoids. Together with *Co. flavipes* Cameron these parasitoid species account for 83% of parasitism of stem borers in southern coastal Kenya (Zhou, Baumgartner & Overholt, 2001). *Cotesia sesamiae* is also the most abundant parasitoid stem borer in South Africa and is reared from 90 % of parasitized larvae of *B. fusca* (Kfir, 1995) and 75 % of *C. partellus* (Kfir, 1990). Other parasitoids used in a classical biological control approach for *B. fusca* and *C. partellus* and of which the parasitoids also use *S. calamistis* as host, are the larval parasitoid *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) and egg parasitoid *Telenomus isis* Polaszek (Hymenoptera: Scelionidae) (Muhammad & Underwood, 2004).

Resistant plants, whether produced through conventional breeding or biotechnology, can potentially affect natural enemies. Individuals can be affected directly if they are susceptible to the transgene product, or indirectly *via* changes in prey quality, prey behaviour or plant quality (Schuler, 2004). A positive effect of sub-lethal concentrations of Bt toxin on parasitoid biology was observed by Tounou *et al.* (2005). The paralytic effect that Bt toxin has on *S. calamistis* larvae could make it easier for *Co. sesamiae* to successfully attack and oviposit in the host (Tounou Gounou, Borgemeister, Goumedzoe & Schulthess, 2005). *Sesamia calamistis* larval feeding on diets containing sub-lethal concentrations of strains of Bt may actually lead to more larvae being parasitized and to increased parasitoid numbers emerging from parasitized larvae (Tounou *et al.*, 2005).

These observations were made in laboratory studies where larvae were fed artificial diets containing Bt-toxin.

A decrease in numbers of *S. calamistis* in Bt maize fields can be expected to have an indirect effect on parasitoid numbers through reduced pest numbers. Such observations were made by Bourguet *et al.* (2002) on parasitoids of the European corn borer, *O. nubilalis*. In a review of the effect of transgenic crops on biological control agents Romeis *et al.* (2006) concluded that there was no indication of direct effects of Bt plants on natural enemies and that in cases where adverse effects were observed they were due to reduced host quality since the herbivores were susceptible to Bt. Groot and Dicke (2002) also concluded that the effect of Bt-crops on parasitoids and predators was not direct but indirectly *via* sub-optimal food or host death. In this study where feeding on whole-plants resulted in 100 % mortality of *S. calamistis* the effect of Bt maize on natural enemies under field conditions would be indirect since a drastic reduction in larval numbers in maize fields will occur. Larvae that survived on Bt plants for 10 – 18 days were smaller than their counterparts on susceptible maize. These larvae were never larger than the 3rd instars (approximately 10 mm) and were therefore still too small to be parasitized by larval parasitoids such as *Co. sesamiae*.

Cropping systems may also affect the relative importance of *S. calamistis* in different ecological areas. Setamou and Schulthess (1995) showed that there are important relationships between wild hosts, stem borers and their parasitoids. *Sesamia calamistis* can be regarded to be polyphagous and has been recorded on more than 24 host plant species in three plant families (Typhaceae, Cyperaceae and Graminae) (Polaszek & Khan, 1998). Setamou and Schulthess (1995) reported that *S. calamistis* tend not to lay eggs and cannot survive long periods in dry maize stalks and that grasses are in many areas the only suitable hosts during periods between planting seasons and the dry season. If only a single maize crop is planted, for example like in rain-fed maize production systems in southern Africa, moths will emerge from maize and lay eggs on suitable wild host plants when the crop is no longer suitable. *Sesamia calamistis* does not go into diapause like *B. fusca* and moths are active throughout the year. Even under severe dry

season conditions no rest-stage was reported in the life cycle of *S. calamistis* (Harris, 1962). In South Africa whereas *B. fusca* and *C. partellus* go into a facultative diapause and rest stages during the off-season, *S. calamistis* continues its activity. *Sesamia calamistis* could therefore be important in the ecology of natural enemies when other stem borer species are in a rest phase. It is not known if there is large-scale movement of *S. calamistis* between wild and crop habitats. Chabi-Olaye *et al.* (2006) did, however, observe that the comparative numbers of *S. calamistis* in inland valleys of Cameroon increased during the dry season and between maize cropping seasons. *Sesamia* spp. also serve as alternate hosts of egg parasitoids of *B. fusca* during the dry season in inland valleys of Cameroon. Songa *et al.* (2002) recorded higher numbers of stem borers in grasses during non-cropping periods in semi-arid eastern Kenya.

The polyphagous nature of *S. calamistis* does not necessarily protect it against local depletion of a population in a particular area. Local depletion will depend on the presence of wild hosts in the area and gene flow between the maize and wild ecosystem. If *S. calamistis* populations in the wild constitute separate sub-populations that do not move to maize, the wild system cannot be viewed as a refuge that will sustain the maize attacking population. Bourget *et al.* (2000) observed that *O. nubilalis* populations on non-maize plants might constitute separate sub-populations.

4.9 Conclusions

This study, in which plant material and the “whole-plant” method was used to evaluate the effect of the transgene plant on *S. calamistis*, showed that Bt maize (event MON 810) was highly effective for control of this pest. This paper only touched on the potential effect that a highly effective Bt maize variety could have on the natural enemy complex of *S. calamistis*. However, evidence suggests that all Noctuid and Pyralid stem borers are highly susceptible to Bt maize. The impact of localized depletion of *S. calamistis* on parasitoid numbers would most likely not be great since several other stem borer species that also occur in wild host plants also host the same parasitoid species.

4.10 References

Ajala, S.O., Kling, J.G., Schulthess, F., Cardwell, K. & Odiyi, A. 2001. Progress in breeding for resistance to maize stem borers *Sesamia calamistis* and *Eldana saccharina* in west and central Africa. Seventh Eastern and Southern Africa Regional Maize Conference. Integrated Approaches to Higher Maize Productivity in the New Millennium. (ed. by DK Friesen & AFE Palmer) 11-15 February, 2001, pp. 49-54. CIMMYT, KARI, Nairobi, Kenya.

Anneck, D.P. & Moran, V.C. 1982. Insects and mites of cultivated plants in South Africa. Butterworths, Durban.

Archer, T.L., Patrick, C., Schuster, G., Cronholm, G., Bynum, E.D. & Morrison, W.P. 2001. Ear and shank damage by corn borers and corn earworms to four events of *Bacillus thuringiensis* transgenic maize. *Crop Protection* 20: 139 – 144.

Birch, A.N.E., Wheatley, R., Anyango, B., Apraia, S., Capalbo, D., Getu Degaga, E., Fontes, E., Kalama, P., Lelmen, E., Lovei, G., Melo, I.S., Muyekho, F., Ngi-song, A., Ochieno, D., Ogwang, J., Pitelli, R., Schuler, T., Setamou, M., Sithanantham, S., Smith, J., Van Son, N., Songa, J., Sujii, E., Tan, T.Q., Wan, F.H. & Hilbeck, A. 2004. Biodiversity and non-target impacts: a case study of Bt maize in Kenya. Environmental Risk Assessment of Genetically Modified Organisms, Vol. 1: A Case Study of Bt Maize in Kenya (ed. By A Hillbeck & DA Andow) CAB International, Wallingford, UK, pp. 116 – 185.

Bonato, O. & Schulthess, F. 1998. Selecting a character for identifying larval instars of the Lepidopteran stemborers *Sesamia calamistis* Hampson (Noctuidae) and *Eldana saccharina* Walker (Pyralidae) in maize. *Insect Science and its Application* 18: 101 – 103.

Bonhoff, M.J., Overholt, W.A., Van Huis, A. & Polaszek, A. 1997. Natural enemies of cereal stem borers in east Africa: a review. *Insect Science and its Application* 17: 19 – 35.

Bourguet, D., Chaufaux, J., Micoud, A., Delos, M., Naibo, B., Bombarde, F., Marque, G., Eychenne, N. & Pagliari, C. 2002. *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (*Zea mays*). *Environmental Biosafety Research* 1: 49 – 60.

Bourguet, D., Bethenod, M.T., Trouve, C. & Viard, F. 2000. Host-plant diversity of the European corn borer *Ostrinia nubilalis*: what value for sustainable transgenic insecticidal Bt maize? *Proceedings of the Royal Society of London* 267: 1177 – 1184.

Chabi-Olaye, A., Nolte, C., Schulthess, F. & Borgemeister, C. 2006. Role of inland valleys in the management of stem borers and their natural enemies in upland maize fields in the humid forest zone of Cameroon. *Environmental Entomology* 35: 282 – 292.

Dutton, A., Romeis, J. & Bigler, F. 2003. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. *BioControl* 48: 611 – 636.

Fitt, G.P., Andow, D.A., Garrière, Y., Moar, W.J., Schuler, T.H., Omoto, C., Kanya, J., Okeck, M.A., Arama, P. & Maniania, N.K. 2004. Resistance risks and management associated with Bt maize in Kenya. *Environmental Risk Assessment of Genetically Modified Organisms, Vol. 1: A Case Study of Bt Maize in Kenya* (ed. By A Hillbeck & DA Andow) CAB International, Wallingford, UK, pp 209 – 250.

Groot, A.T. & Dicke, M. 2002. Insect-resistant transgenic plants in a multi-trophic context. *The Plant Journal* 31: 387 – 406.

Harris, K.M. 1962. Lepidopterous stem borers of cereals in Nigeria. *Bulletin of Entomological Research* 53: 139 – 171.

Hilbeck, A. & Andow, D.A. 2004. Environmental risk assessment of genetically modified organisms. Vol. 1. A case study of Bt maize in Kenya. CABI, Wallingford, UK.

Ingram, W.R. 1958. The lepidopterous stalk borers associated with gramineae in Uganda. *Bulletin of Entomological Research* 49: 367 – 383.

Kaufmann, T. 1983. Behavioural biology, feeding habits, and ecology of three species of maize stem-borers: *Eldana saccharina* (Lepidoptera: Pyralidae), *Sesamia calamistis* and *Busseola fusca* (Lepidoptera: Noctuidae) in Ibadan, West Africa. *Journal of the Georgia Entomological Society* 18: 259 – 272.

Kalule, T., Ogenga-Latigo, M.W. & Okoth, V.A.O. 1997. Seasonal fluctuations and damage of Lepidopteran stem borers of maize in a major agroecozone of Uganda. *African Crops Science Journal* 5: 385 – 393.

Kfir, R. 1990. Parasites of the spotted stalk borer, *Chilo partellus* (Lepidoptera: Pyralidae) in South Africa. *Entomophaga* 35: 403 – 410.

Kfir, R. 1995. Parasitoids of the African stem borer, *Busseola fusca* (Lepidoptera: Noctuidae), in South Africa. *Bulletin of Entomological Research* 85: 369 – 377.

Mugo, S., De Groote, H., Bergvinson, D., Mulaa, M., Songa, J. & Gichuki, S. 2005. Review: Developing Bt maize for resource-poor farmers – recent advances in the IRMA project. *African Journal of Biotechnology* 4: 1490 – 1504.

Muhammad, L. & Underwood, E. 2004. The maize agricultural context in Kenya. In: Hilbeck, A. & Andow, D.A. (Eds.). Environmental risk assessment of genetically modified organisms: vol.1. A case study of t maize in Kenya. CAB International, Wallingford, UK.

Nwosu, K.I. 1990. Optimum larval population of *Sesamia calamistis* Hmps (Lepidoptera: Noctuidae) for artificial infestation of maize plants. *Insect Science Application* 13: 369-371.

Ostlie, K.R., Hutchison, W.D. & Hellmich, R.L. 1997. (Eds.) Bt corn and European corn borer: Long-term success through resistance management. NCR Publication No. 602, University of Minnesota, St Paul, Minnesota.

Overholt, W.A. & Maes, K.V.N. 2000. Field guide to the maize and sorghum stemborer larvae of eastern and southern Africa. ICIPE Science Press, Nairobi, Kenya.

Pilcher, C.D. & Rice, M.E. 2001. Effect of planting date and *Bacillus thuringiensis* corn on the population dynamics of European corn borer (Lepidoptera: Crambidae). *Journal of Economic Entomology* 94: 730-742.

Polaszek, A. & Khan, Z.R. 1998. Host plants. In: Polaszek, A. Ed. African cereal stem borers: economic importance, taxonomy, natural enemies and control. P. 4 – 10. CAB International, Wallingford, UK.

Romeis, J., Missle, M. & Bigler, F. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxin and biological control. *Nature Biotechnology* 24: 63 – 71.

Schuler, T.H. 2004. GM crops: good or bad for natural enemies? Aspects of Applied Biology 74, GM crops – Ecological dimensions, pp. 81 – 90.

Setamou, M. & Schulthess, F. 1995. The influence of egg parasitoids belonging to the *Telonomus busseolae* (Hymenoptera: Scelionidae) species complex on *Sesamia calamistis* (Lepidoptera: Noctuidae) populations in maize fields in southern Benin. *Biocontrol Science and Technology* 5: 69 – 81.

Shanower, T.G., Schulthess, F. & Bosque-Pérez, N. 1993. Development and fecundity of *Sesamia calamistis* (Lepidoptera: Noctuidae) and *Eldana saccharina* (Lepidoptera: Pyralidae). *Bulletin of Entomological Research* 83: 237-243.

Singh, R., Channappa, R.K., Deebe, F., Nagaraj, N.J., Sukaveneswaran, M.K. & Manjunath, T.M. 2005. Tolerance of Bt corn (MON 810) to maize stemborer, *Chilo partellus* (Lepidoptera: Pyralidae). *Plant Cell Reports* 24: 556 – 560.

Songa, J.M., Overholt, W.A., Mueke, J.M. & Okello, R.O. 2002. Regional distribution of lepidopteran stemborers and their parasitoids among wild grasses in the semi-arid eastern Kenya. *African Crop Science Journal* 10: 183 – 194.

Statistica 7.1. 2005. StatSoft, Inc. Tulsa. USA.

Tounou, A.K., Gounou, S., Borgemeister, C., Goumedzoe, Y.M.D. & Schulthess, F. 2005. Susceptibility of *Eldana saccharina* (Lepidoptera: Pyralidae), *Busseola fusca* and *Sesamia calamistis* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* Cry toxins and potential side effects on the larval parasitoid *Cotesia sesamiae* (Hymenoptera: Braconidae). *Biocontrol Science and Technology* 15: 127 – 137.

Turlings, T.C.J., Jeanbourquin, P.M., Held, M. & Degen, T. 2005. Evaluating the induce-odour emission of a Bt maize and its attractiveness to parasitic wasps. *Transgenic Research* 14: 807 – 816.

Usua, E.J. 1970. Some notes on maize stem borers in Nigeria. *Journal of Economic Entomology* 63: 776 – 778.

Van den Berg, J. & Drinkwater, T.W. 2000. Pink Stem Borer. ARC-Grain Crops Institute. Potchefstroom. *Crop Protection Series no 20*.

Van den Berg, J., Rebe, M., De Bruyn, J. & Van Hamburg, H. 2001. Developing habitat management systems for gramineous stem borers in South Africa. *Insect Science Application* 21: 381-388.

Van Rensburg, J.B.J. 1998. Evaluation of Bt-Transgenic maize for resistance to the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) in South Africa. *South African Journal Plant Soil* 16: 38 – 43.

Van Rensburg, J.B.J. 2001. Larval mortality and injury patterns of the African stalk borer, *Busseola fusca* (Fuller) on various plant parts of Bt-transgenic maize. *South African Journal Plant Soil* 16: 38 – 43.

Zhou, G., Baumgartner, J. & Overholt, W.A. 2001. Impact assessment of exotic parasitoids on stemborer (Lepidoptera) population dynamics in Kenya. *Ecological Applications* 11: 1554 – 1562.

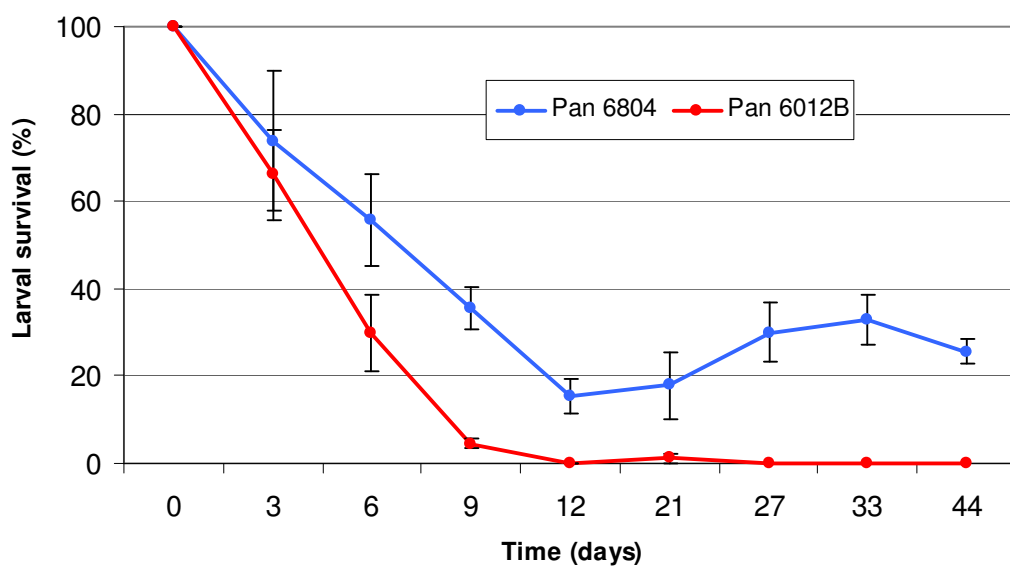


Figure 4.1 Percentage *Sesamia calamistis* larval survival on Bt maize (Pan 6012B) and non-Bt maize (Pan 6804) inoculated with egg batches.

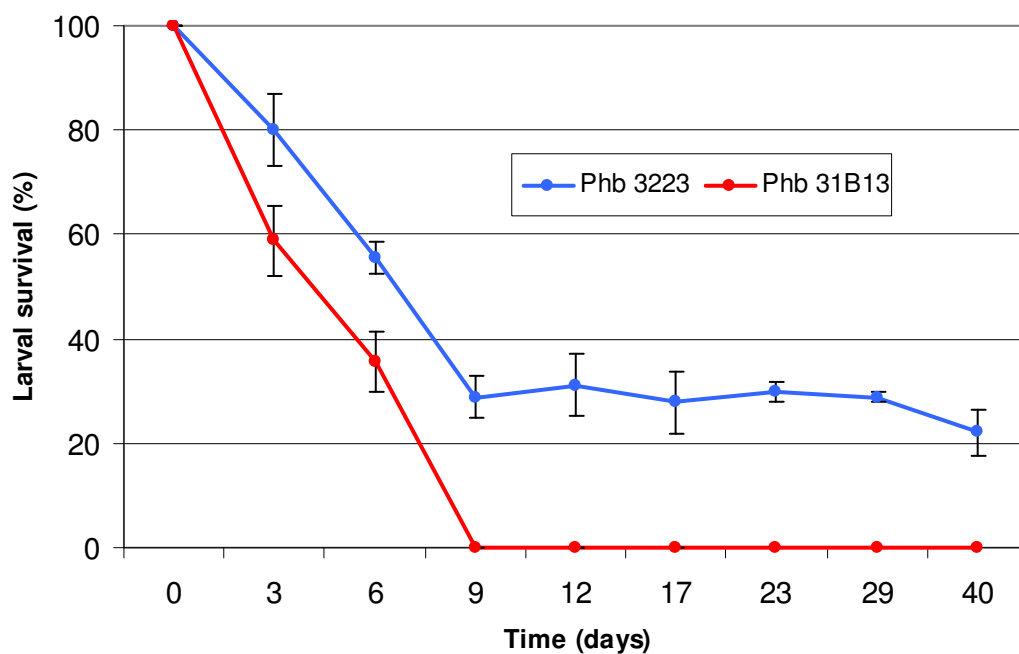


Figure 4.2 Percentage *Sesamia calamistis* larval survival on Bt maize (Phb 31B13) and non-Bt maize (Phb 3223) inoculated with egg batches.

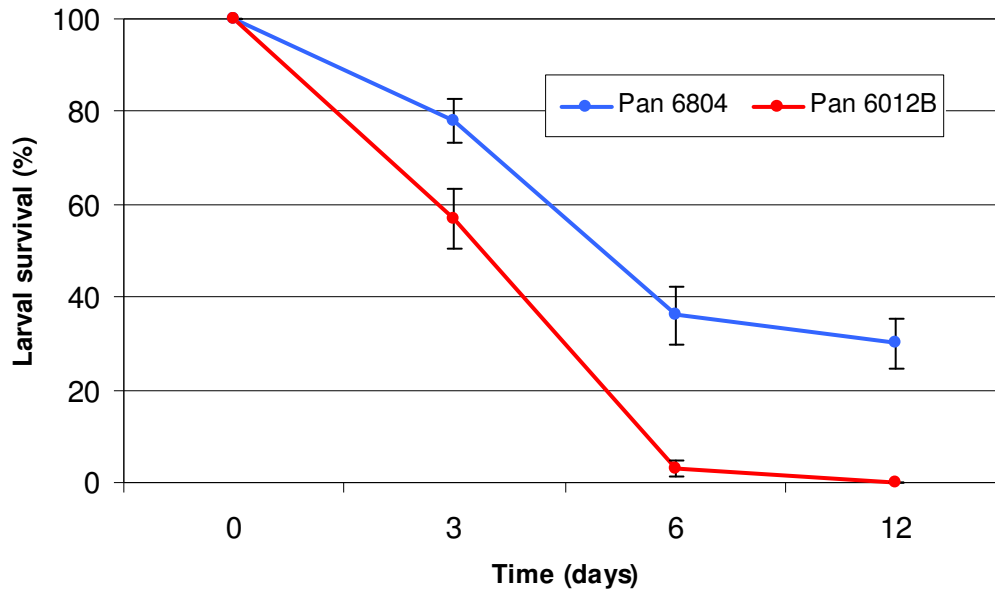


Figure 4.3 Percentage *Sesamia calamistis* larval survival on Bt maize (Pan 6012B) and non-Bt maize (Pan 6804) inoculated with neonate larvae.

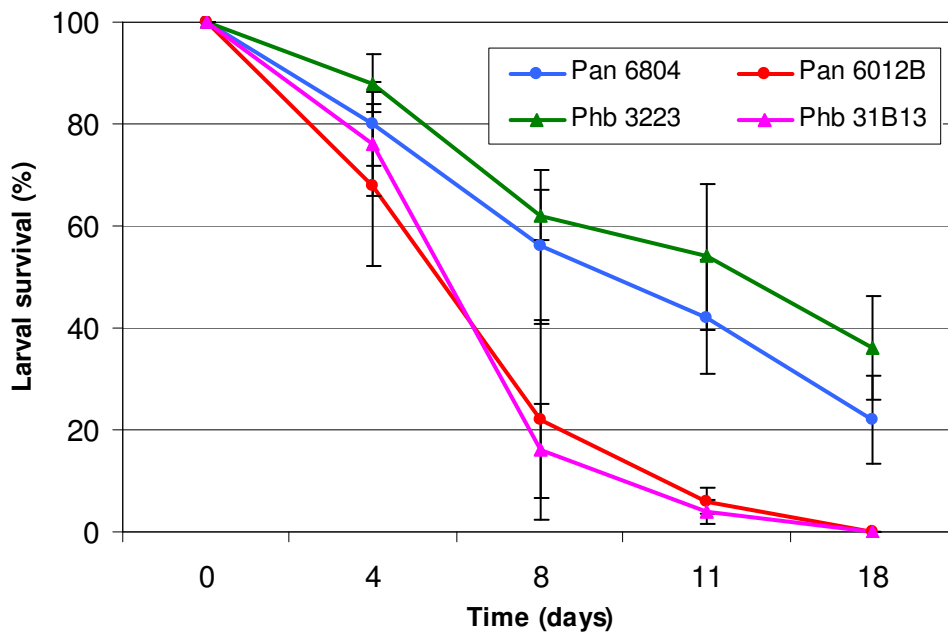


Figure 4.4 Percentage *Sesamia calamistis* larval survival on Bt maize (Pan 6012B and Phb 31B13) and non-Bt maize (Pan 6804 and Phb 3223) inoculated with neonate larvae in the whorl.

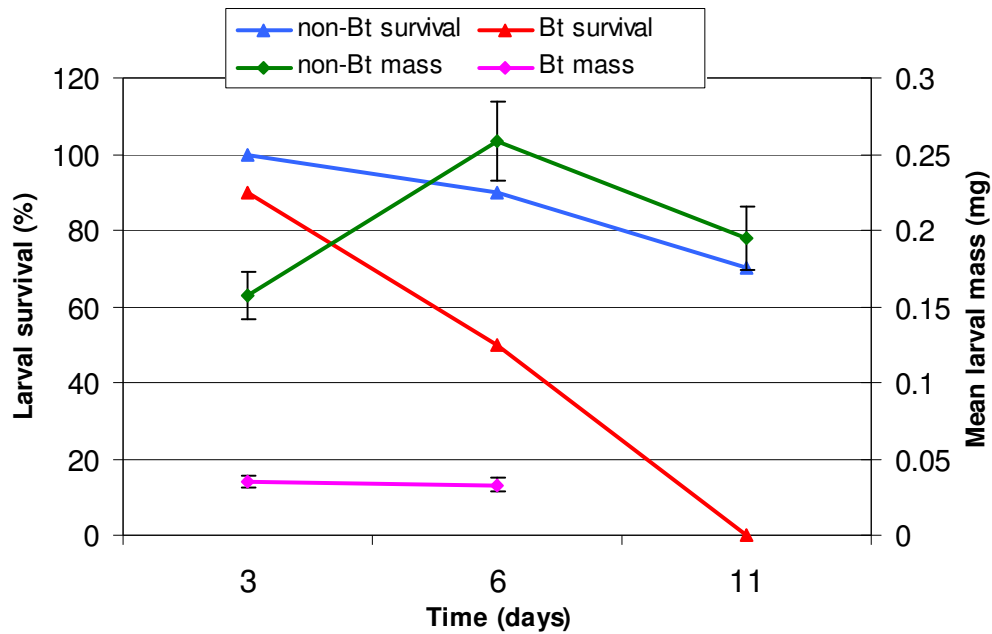


Figure 4.5 Percentage larval survival and mean larval mass of *Sesamia calamistis* on Bt- and non-Bt maize stems inoculated with 4th instars.

Table 4.1 Mean numbers of eggs and egg batches laid on Bt and non-Bt maize plants by *Sesamia calamistis*.

	Pan6804	Pan6012B	t-value	P-value
Number of egg batches	2.5	2.3	0.307	0.765
Number of eggs/batch	138.3	127.3	0.236	0.818
Total number of eggs/plant	304.2	311.7	-0.066	0.949



Figure 4.6. *Sesamia calamistis* first-instar damage to a four-week old Bt maize plant.



Figure 4.7. *Sesamia calamistis* first-instar damage to a four-week old non-Bt maize plant.



Figure 4.8. *Sesamia calamistis* second-instar damage symptoms on the inside surface of a leaf sheath.

CHAPTER 5: SELECTION OF NON-TARGET LEPIDOPTERA SPECIES FOR ECOLOGICAL RISK ASSESSMENT OF BT MAIZE IN SOUTH AFRICA

5.1 Abstract

It is essential to assess the environmental risk that Bt maize may hold and to study its effect on species assemblages that fulfil a variety of ecosystem functions. Ecological theory can be used to improve environmental risk assessment and, by applying it to specific environments, local species can be classified functionally and prioritized to identify potential test species, assessments and end points. Although the stem borers *Busseola fusca* and *Chilo partellus* are the target species of Bt maize in South Africa various other Lepidoptera species could also be directly exposed to Bt toxin. An ecological approach was followed in which the potential effects of direct exposure of Lepidoptera to Bt toxin in maize was investigated. Non-target Lepidoptera that are primary consumers of maize and could therefore be directly exposed to Bt-toxin were identified, and the possible effects that Bt maize could have on these species assessed. A selection matrix was developed in which each species was ranked for its maximum potential exposure to Bt toxin by assessing its occurrence, abundance, presence and linkage in the maize ecosystem. Through use of the selection matrix knowledge gaps were identified for future research and guidance of the design of ecologically realistic experiments. The non-target species with the highest maximum potential exposure to Bt toxin were *Sesamia calamistis*, *Helicoverpa armigera* and *Acantholeucania loreyi*. *Eublemma gayneri* can be considered to be a “value unknown” species in the wild. *Agrotis segetum* and *Spodoptera exigua* were considered to be of lesser importance but should also be considered in pre- and post-release monitoring. A list was compiled of target and non-target Lepidoptera species associated with maize and that could be at risk to develop resistance.

5.2 Introduction

The biodiversity of an agroecosystem is not only important for its intrinsic value, but also because it influences ecological functions that are vital for crop production in sustainable agricultural systems, and for wildlife and the environment surrounding the agroecosystem (Hilbeck *et al.*, 2006). Species assemblages in an agroecosystem fulfil a variety of ecosystem functions that may be harmed if changes occur in these assemblages (Dutton *et al.*, 2003; Birch *et al.*, 2004). For this reason it is essential to assess the environmental risk that the release of a genetically modified (GM) organism may hold and to study its effect on species assemblages. Risk assessment is a process by which risks are identified and the seriousness of the risks is characterized so that decisions can be made on whether or how to proceed with the technology (Andow & Hilbeck, 2004a).

Ecological theory can be used to improve environmental risk assessment and to tailor it to specific environments. In an ecological model for non-target risk assessment, local species can be classified functionally and prioritized using risk based ecological criteria to identify potential test species, assessments and end points (Andow & Hilbeck, 2004b). The environmental risk assessment process described for Bt maize by Birch *et al.* (2004) assessed the possible risks of transgenic crops on biodiversity. In that model it is recommended that species be selected from assemblages, that the potential for risk be identified and that research protocols be developed to assess these risks.

In this study the ecological model was used to select species for future research and to evaluate species for their susceptibility to Bt maize. A case-specific approach suggested by Birch *et al.* (2004) and Andow & Hilbeck (2004a) was followed during which selection of non-target species and the potential effects of direct exposure of Lepidoptera to Bt maize was investigated. No risk assessment for Bt maize was done in South Africa before its release in 1998. As pointed out by McGeoch & Rhodes (2006), the protocols and guide lines for risk assessment of GM crops in South Africa has yet to be developed. In this chapter we relied strongly on methods and examples used by Andow & Hilbeck

(2004a, b) and Hilbeck, Andow & Fontes (2006) in their case studies on Bt maize in Kenya and Bt cotton in Brazil respectively.

5.3 An ecological model for non-target risk assessment

Species selection in the ecological model is case specific, depending on the transgenic crop and its cropping context, and prioritizes species that could be adversely affected by the transgenic crop (Andow & Hilbeck, 2004a, b). Species selection follows four steps: (1) establishing the functional groups according to their ecological role or function in the ecosystem, (2) classifying the non-target species found in the relevant environment into these functional groups, (3) prioritizing these species on the basis of ecological principles, and (4) selecting a number of high-priority species to test (Andow & Hilbeck, 2004b).

5.3.1 Establishing functional groups

Using ecological function allows focus on ecological processes and limit the number of species and functions tested (Birch *et al.*, 2004). Based on ecological function Birch *et al.* (2004) identified primary consumers, secondary consumers, pollinators, decomposers, nutrient recyclers, seed dispersers and species of unknown function as functional groups that needs to be considered in pre-release testing of GM plants. Hilbeck *et al.* (2006) identified the following functional groups for use in Bt cotton risk assessment in Brazil: non-target pest herbivores, pollinators and species of conservation concern, predators, parasitoids, weeds and soil ecosystem functions. These functional groups are not mutually exclusive. For example many species are both secondary pests and non-target primary consumers (Andow & Hilbeck, 2004b).

In this study on maize in South Africa only the functional group of lepidopterous primary consumers of maize was used. Using the guild concept, this functional group was further sub-divided. The Lepidoptera was selected as a group because of the target species,

reported specificity of the *Cry1Ab* protein to Lepidoptera and the taxonomic expertise available to identify Lepidoptera species.

5.3.2 Classifying non-target species

The second step in species selection is classification of the non-target species that occur in association with the crop in the region where the transgenic crop is intended to be released, followed by classification into functional groups, using available information and expertise. Inclusion of species that actually occur in the region generates a case-specific set of potential non-target species (Andow & Hilbeck, 2004b).

Surveys were conducted between January 2004 and May 2006, to assess the diversity of Lepidoptera and to identify non-target primary consumers of maize (Chapter 3). This constituted a functional group of species that is not targeted by the transgene. This list of non-target Lepidoptera is specific to maize and its cropping context in the agro-ecosystem. A list was compiled of Lepidoptera species associated with maize and that have the potential to be affected by Bt maize (Table 3.1).

Fifteen Lepidoptera species were recorded during the two-cropping season survey on maize. Eleven species were recorded to feed on non-Bt maize while pupae of four other species were also found on maize. Six of these species were also recorded to feed on Bt maize. Species were divided into different guilds based on the different plant tissues they were feeding on. The following guilds were recognized: seedling and leaf feeders; stem borers; a post-flowering guild that feed on maize tassels and ears (including silks) respectively; and a visitors guild. The stem borer guild consisted of three species, i.e. *Busseola fusca* (Lepidoptera: Noctuidae), *Chilo partellus* (Lepidoptera: Pyralidae) and *Sesamia calamistis* (Lepidoptera: Noctuidae), while two species can be regarded as leaf feeders (*Acantholeucania loreyi* (Lepidoptera: Noctuidae) and *Spodoptera exigua* (Lepidoptera: Noctuidae)). *Agrotis segetum* (Lepidoptera: Noctuidae) is a pest of maize seedlings while *Helicoverpa armigera* (Lepidoptera: Noctuidae) usually attacks plants during the reproductive stages when it feeds on maize ears and silks. The stem borers can however also belong to both the leaf feeding- and post-flowering guilds since they feed

on maize plants from the early whorl stage onwards and also damage the stem and ears during the post-flowering stages. The other four species that were recorded were assigned to the post-flowering guild. These species were *Eublemma gayneri* (Lepidoptera: Noctuidae), *Nola phaeocraspis* (Lepidoptera: Nolidae), *Chlorissa stibolepidae* (Lepidoptera: Geometridae) and *Chrysodeixis acuta* (Lepidoptera: Noctuidae). Only *B. fusca*, *H. armigera*, *A. loreyi*, *E. gayneri* and *A. segetum* were recorded on Bt maize.

5.3.3 Prioritizing species using ecological principles

Several criteria can be used to prioritize non-target species, including maximum possible exposure, potential adverse effects, and potential exposure. Any non-target organism that feeds on a transgenic plant or part of the plant would come in contact with the transgene and its product (Andow & Hilbeck, 2004b).

The maximum potential exposure of a non-target species to a transgenic crop is based on geographic range, habitat specificity, local abundance, prevalence and temporal association with the crop (Andow & Hilbeck, 2004b). Defining potential exposure of insects to selection by Bt toxin is an important aspect of resistance risk assessment (Fitt *et al.*, 2004). In order to provide a rational and transparent approach to support the selection of species for use in risk assessment analyses, Birch *et al.* (2004) developed a series of selection matrices. In this system each species is ranked for its maximum potential exposure to Bt-toxin by assessing its occurrence, abundance, presence and linkage in the maize ecosystem as well as for potential adverse effects that exposure may have on the non-target species (Birch *et al.*, 2004) (Table 5.1). In this context “occurrence” refers to the presence of a non-target species in the agroecosystem, its geographic range and prevalence. “Abundance” refers to local abundance and prevalence while “presence” involves temporal association with the crop. “Linkage” refers to habitat specificity and association of the non-target species with maize.

Although many species have an unknown ecological function, this does not imply that their ecological function is insignificant. Of the species with unknown ecological function, Andow & Hilbeck (2004a) suggested that those with a high standing biomass or

those that are found in frequent association with the transgenic crop habitat should also be selected for testing. By explicitly considering such species for initial non-target testing, a scientifically justified precautionary approach is introduced into risk assessment.

Data collected during surveys and expert opinion were used to develop selection matrixes in which species were selected on the bases of ecological principles (Table 5.1). The aims with development of these matrixes were to establish options and identify knowledge gaps for future research and possible post-release impact monitoring studies. The non-target Lepidoptera recorded during this study was *A. loreyi*, *A. segetum*, *C. stibolepidae*, *C. acuta*, *E. gayneri*, *H. armigera*, *S. calamistis*, *S. exigua* and *N. phaeocraspis* (Chapter 3). The most common species were *S. calamistis*, *H. armigera*, *A. loreyi* and *E. gayneri*. These species are directly exposed to Bt maize. The most important non-target species according to Table 5.1 was *S. calamistis*, *H. armigera* and *A. loreyi*.

5.3.4 Selecting high-priority species to test

Those species that are given the highest priority should be the candidates for testing. This final selection process is not a purely scientific one, but it should be transparent (Andow & Hilbeck, 2004b).

Knowledge on diversity, survival and infestation levels of Lepidoptera on maize can be used to guide the hazard identification process and development of hypotheses relevant for risk assessment (Birch *et al.*, 2004). From this study five non-target Lepidoptera species (*S. calamistis*, *H. armigera*, *A. loreyi*, *A. segetum*, *S. exigua*) can be recommended for inclusion in post-release monitoring on Bt maize in South Africa. Based on their distribution and the fact that some of these species are well studied, several of these species could also be recommended for inclusion in pre-release testing (Barton & Dracup, 2000) (Birch *et al.*, 2004) and impact assessments. The major hazards associated with these species are that they might become significant secondary pests and may develop resistance because of their continual exposure to Bt toxin.

In this study *S. calamistis* was the only species evaluated in life-table studies, using only maize varieties expressing Cry1Ab protein (event MON810) (Chapter 4). *Sesamia calamistis* was studied because it is a stem borer species that is closely associated with maize and wild host plants that occur in and around the maize cropping system. Although *S. calamistis* occurs at low infestation levels it is ranked as a high priority species (Table 5.1) based on several aspects regarding its biology and ecology. *Sesamia calamistis* can occur during the whole cropping season and causes damage to the leaves, stems and ears of plants. The geographical distribution and prevalence of this species also changed during the pest decade. This species used to be considered a pest in warmer coastal areas but has since 1995 been observed on sweet corn on the Highveld region as well as center pivot irrigation systems in the North West and Northern Provinces where it causes serious stand reductions (Van den Berg & Drinkwater, 2000). The fact that *S. calamistis* is noticed largely on irrigated maize adds to its linkage with Bt maize since this is the preferred crop under center pivot irrigation. The linkage of *S. calamistis* to maize may be weak in the more sub-tropical low-altitude areas where it has several wild host plants and also attacks other crops (Van den Berg *et al.*, 2001). However, its linkage with maize in the Highveld region of South Africa and especially in semi-arid areas where maize is planted under irrigation may be strong since few or no wild hosts are present. This study showed however that *S. calamistis* is highly susceptible to Bt maize (event MON810) and that no survival occurred in laboratory and greenhouse experiments.

The ear-feeding Lepidoptera, i.e. *H. armigera* and *A. loreyi* were also regarded as priority species and received high rankings (Table 5.1). The effects of Bt maize on these non-target species were not studied in controlled experiments and conclusions were based on field observations. These two species were abundant in many Bt and non-Bt maize fields, during the post-flowering stage (Chapter 2). The presence and occurrence of *A. loreyi* on maize was high since it attacked the crop from the seedling to the post flowering stages and was recovered in the majority of fields during surveys. Feeding damage on maize ears are the same as that of *H. armigera* but this species is sometimes also a voracious feeder on maize leaves. The many uncertainties regarding the biology, distribution and host plant range of *A. loreyi* together with its capability to survive on Bt maize contributes

to the importance of these non-target species. The linkage of *A. loreyi* with the maize ecosystem is uncertain and only a few wild host plants of this species are known.

Helicoverpa armigera has the potential to be exposed to Bt-toxin for prolonged periods since its presence inside the maize crop can be from the seedling to soft dough stage. With its high abundance and common occurrence (Table 5.1) this species could become a significant secondary pest. A strong linkage between maize and *H. armigera* is evident because of this species' wide host range and its common association with maize. The information matrix provided in table 5.1, indicates that this species has the highest maximum potential exposure to Bt toxin.

Eublemma gayneri can be considered to be a “value unknown” species in the wild and it is not possible to speculate on the effects that may result should Bt maize have negative or positive effects on their numbers in the wild. It is ranked lower in importance than other species since little is known about it and its linkage with maize is probably not strong. The presence of this species on maize is uncertain and it has only been recovered from silks of plants during the post-flowering stage. Only three host plant species have been reported for this insect (Chapter 3). Using the criteria of Andow and Hilbeck (2004b) *E. gayneri* is a good example of a value-unknown species that could be considered for further testing.

Agrotis segetum and *S. exigua* were considered of lesser importance than the above mentioned non-target species (Table 5.1). These species could however, also be considered in future pre-release impact assessments in countries where they occur and post-release monitoring of pest status and resistance development in South Africa. *Agrotis segetum* is an important maize pest but was found in low abundance and only on maize seedlings. Its presence in the maize system is short and its linkage weak since it is only directly exposed to maize during the seedling stages and because larvae prefer to feed on weeds rather than maize (Van Rensburg, 1994; Drinkwater & Van Rensburg, 1992). *Spodoptera exigua* is considered a species of lesser importance because of its occasional occurrence and low abundance in maize in South Africa. Although the

abundance of *S. exigua* is low in the majority of seasons, solitary larvae often feed on maize seedlings and cause damage resembling that of *C. partellus* (Van Rensburg, 1999b). Outbreaks do occur from time to time, from there the common name, lesser army worm (Van Rensburg, 1999b).

This study (Chapter 4) and others (Van Rensburg 1999a, 2001) showed that Bt maize is highly effective against the target stem borer species, *B. fusca* and *C. partellus* under laboratory and field conditions and that their numbers on plants are reduced to insignificant levels. It is important to keep in mind that once a niche such as that occupied by stem borers becomes vacant due to the effect of the transgene on the target species, the possibility exists that other Lepidoptera species could become secondary pests. It may however also be the case that the numbers of non-target pests decrease or are not affected at all (Peacock *et al.*, 1998). Species that may possibly occupy such a vacant niche once *B. fusca* and *C. partellus* becomes locally extinct in an area could be *S. calamistis*, *H. armigera*, *A. loreyi* and *E. gayneri*. As with *E. gayneri*, the ecosystem functions of *A. segetum* and *S. exigua* is not known and it is not possible to speculate on the effect that possible local extinctions, or, on the other hand, possible resistance development, could have in the ecosystem. It is critical to consider these species with unknown functions that are directly exposed to Bt maize, so that these species are not inadvertently overlooked because of lack of knowledge.

5.4 Experimental end point for the ecological model

An appropriate experimental end point for initial testing is generational relative fitness or some component of relative fitness (Andow & Hilbeck, 2004b). Generational relative fitness is the relative lifetime survival and reproduction of the non-target species. Thus, survival experiments should last at least through one full generation, including all the immature stages of the non-target species (Andow & Hilbeck, 2004a, b). The duration of the test should correspond to the time the non-target species would be exposed to the transgenic plant or plant parts. If the transgenic plant were to adversely affect non-target

species in the environment, its effects would come through some component of relative fitness. The result from such initial testing would guide the design of further ecologically realistic experiments (Birch *et al.*, 2004), as was done with *S. calamistis* (Chapter 4) and *B. fusca* (Van Rensburg, 2001).

Studies by Van Rensburg (1999b; 2001) and Singh *et al.* (2005) has shown that the generational fitness of the target pests *B. fusca* and *C. partellus* feeding on Bt maize (event MON810) is extremely low with no survival to the adult stage being reported. Appropriate methodologies and protocols to assess risk should be developed for high priority species (Birch *et al.* 2004), identified in this study. Conventional ecotoxicology methodologies are suggested by Birch *et al.* (2004) to assess effects of exposure to the transgene products. A “whole plant” methodology is also required to evaluate the effects of the whole transgenic plant, not just the transgene product (Birch *et al.*, 2004), such as that used for *S. calamistis* in this study (Chapter 3). Evaluation of the fitness of *S. calamistis* on different Bt maize varieties (all with event MON810) showed that no survival occurred on Bt maize (Chapter 3).

Table 5.1 Selection matrix for prioritizing non-target Lepidoptera species associated with maize in South Africa. *

Species	Maximum potential exposure				Possible adverse effect		
	Occurrence	Abundance	Presence	Linkage	Significance	Damage	**Rank
<i>Sesamia calamistis</i>	Occasional	Low - medium	Anytime	Sometimes – strong	Potential pest	High	1
<i>Helicoverpa armigera</i>	Certain	Abundant	Post-flowering	Always - strong	Significant potential pest	Low	1
<i>Acantholeucania loreyi</i>	Occasional	Medium – abundant	Pre- and post-flowering, also on tillers	Sometimes – strong	Potential pest	Low	1
<i>Eublemma gayneri</i>	Sporadic	Low	Post-flowering	Sometimes	-	Very low	2
<i>Agrotis segetum</i>	Occasional	Medium	Seedling	Always	Potential pest	Sometimes	2
<i>Spodoptera exigua</i>	Occasional	Low	Seedling	Sometimes – weak	Potential pest	Sometimes	2

* Based on a selection matrix suggested by Birch *et al.* 2004.

** Species that were considered most important were ranked 1.

5.5 Resistance risks of target and non-target species

The continuous use of transgenic crops may result in an increased risk that insect species directly exposed to Bt maize may evolve resistance to Bt proteins (Ferré & Van Rie, 2002; Gould, 1998; Höfte & Whiteley, 1989). Development of insecticide resistance is a common response among insects to the selection pressure imposed by chemicals. If species have the ability to become resistant to insecticides the possibility may also exist that species can develop resistance to transgenic crops (Sharma & Pampapathy, 2006)

Fitt *et al.* (2004) suggested compiling a list of target and non-target species associated with the transgenic plant that are susceptible to the transgene and that could be at risk to develop resistance. Of the three stem borer species of maize in South Africa *B. fusca* and *C. partellus* are the most dominant while *S. calamistis* occur at very low levels. The former two species therefore present the most likely risk for resistance development in South Africa. The risk of resistance development differs between species based on geographical area and cultivation practices. In South Africa *B. fusca* occurs mainly on the Highveld region where it has three distinct generations per annum followed by a facultative diapause (Van Rensburg, 1985). *Busseola fusca* is less abundant and important in low-lying warmer areas and moth flight patterns are erratic (Van Rensburg, 1997). *Chilo partellus* is an exotic species which is absent from the cool, eastern and southern parts of the Highveld region of South Africa but occurs commonly in the warmer northern and north-western areas (Van Rensburg & Bate, 1986). It does not go into a rest phase and larval activity occurs throughout the year in the sub-tropical areas (Van Rensburg, 2000). *Sesamia calamistis* occurs mainly in coastal areas but is becoming increasingly important in the higher altitude and semi-arid areas of the country (Van den Berg & Drinkwater, 2000). It can therefore be concluded that the species most likely to develop resistance in the Highveld region will be *B. fusca* while *C. partellus* will be the species most at risk in low-altitude subtropical as well as arid-areas.

The non-target species *S. calamistis*, *H. armigera*, *A. loreyi*, *S. exigua* and *A. segetum* are however not excluded from the risk of resistance development. In order to develop a

resistance management strategy the history of resistance development of key pests (Fitt *et al.*, 2004) and possibly also key non-target pests need to be studied. Knowledge of the past history of pesticide resistance can be instructive in assessing the resistance risk (Fitt *et al.*, 2004). No evidence of pesticide resistance in stem borers has been reported in South Africa. No resistance to Bt in any stem borer has as yet been reported (Smale & De Groote, 2003). However, alleles conferring resistance of European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), to Cry1Ab were reported to be more common in dietary bioassays than under field conditions in which F₂-screenings were done with this species on maize (Tabashnik *et al.*, 2003). Van Rensburg (2001) reported the survival of *B. fusca* on Bt maize during the 1998/99 cropping season in commercial maize fields. This phenomenon was not ascribed to resistance but to feeding of larvae on plant tissues that contained sub-lethal concentrations of Bt-toxin. This evidence indicates that *B. fusca* has the potential to survive on Bt maize. Future research should be directed towards evaluation of the generational relative fitness of the F₂-generation of *B. fusca* larvae that survived on Bt.

Helicoverpa armigera and *A. loreyi* feed on reproductive structures that have been reported not to be lethal to *B. fusca* (Van Rensburg, 2001). Because of the uncertainties regarding expression of Bt toxin in different plant parts (Andow & Hilbeck, 2004) it is not known what the risk of resistance development may be of species that feed on different plant parts. *Helicoverpa armigera* has the ability to survive and develop resistance to Bt cotton expressing Cry1Ac in laboratory strains (Akhurst *et al.*, 2003; Tabashnik *et al.*, 2003). Resistance to Cry1Ab was also detected (Akhurst *et al.*, 2003). Dowd (2001) reported nearly 50% infestation of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) on Bt maize hybrids that expressed high levels of the protein in silks and kernels. Because of the continuous and indiscriminate use of insecticides against *H. armigera*, it has developed high levels of resistance to conventional insecticides (Sharma & Pampapthy, 2006). Resistance to insecticides was also detected in field strains of *H. armigera* in South Africa (Van Jaarsveld, Basson & Marais, 1998). This evidence suggests that it may also be possible for *H. armigera* to develop resistance against Bt maize. *Helicoverpa armigera* and *A. loreyi* have been shown to survive on Bt maize

under field conditions in this study (Chapter 4). Together with its history of developing resistance to insecticides and reports of survival on artificial diets containing Bt toxin there seem to be a strong possibility that it may develop resistance to Bt toxin on maize.

Aspects of population ecology, behavior and the structure of cropping systems that may affect potential exposure of species collected on maize during this study is summarized in Table 5.2. From this information it is possible to recognize information gaps and determine research questions that must be addressed.

No information exists on *E. gayneri* that can be used in risk assessment of this “value unknown” species (Table 5.2). Except for the stem borer species, little information is available on the host plant range of the species recorded on maize. The polyphagous nature of stem borers (Le Ru *et al.*, 2006) or the mere presence of certain wild host plants in the maize agro-ecosystem will not necessarily reduce the risk of resistance development and cannot be used with confidence as a factor in risk assessment. The interaction between populations of insects and their wild hosts are important. Gene flow between the maize and wild hosts in the area does not always happen since populations in the wild may constitute separate sub-populations that do not move to maize. The wild ecosystem system cannot always be viewed as a refuge that will sustain the maize attacking population. Bourget *et al.* (2000) observed that *O. nubilalis* populations on non-maize plants may constitute separate sub-populations. Sezonlin *et al.* (2005) reported that *B. fusca* occurred in negligible levels in wild host plants in East Africa.

The attributes of different species (Table 5.2) provide the opportunity to develop testable hypotheses that could shed light on aspects of insect biology that may contribute to resistance development or the management of resistance, should it develop. For example larval development may be delayed by feeding on Bt crops (Eizaguirre *et al.*, 2005) and this delay may affect the rate of Bt resistance development. Gene flow studies on *O. nubilalis* done by Bourguet *et al.* (2000) pointed out that those resistant individuals that feed on Bt maize may develop more slowly than susceptible individuals. This temporal delay in development may lead susceptible *O. nubilalis* to complete mating before

resistant moths emerged (Bourguet, Bethenod, Pasteur & Viard, 2000). The effect of Bt maize on diapause induction and development of borers also may delay the phenology of flights of adults emerging from Bt fields. This delay could decrease the efficacy of non-Bt refuges as adults from Bt fields would have less probability of mating with adults from non-Bt fields than with adults from the same field (Eizaguirre *et al.*, 2005). Since the high dose-refuge approach is used to delay resistance development of stem borers in South Africa, the findings on delayed diapause in other species indicate that this topic should also be studied for *B. fusca*.

Another concern is the growth of Bt maize and other Bt crops such as cotton in the same region. This could lead to an increase in the rate of Bt resistance development in *H. armigera*. Exposure to *Cry1Ab* in ears of Bt maize would add to selection pressure in a Bt cotton system that would likely express *Cry1Ac* in cotton-producing regions. *Cry1Ab* and *Cry1Ac* are similar in mode of action and are widely reported to confer cross-resistance (Fitt *et al.*, 2004). Fertilization of non-Bt maize by Bt pollen could often yield intermediate toxin levels in ears of non-Bt maize (Fitt *et al.*, 2004). Polyphagy and mobility will operate to reduce selection pressure of Bt maize on *H. armigera*, although localized intense selection may occur (Fitt *et al.*, 2004).

Using the information on history of resistance, exposure, toxin dose and the frequency of resistance, insect species can be ranked according to their potential resistance risk, allowing identification of the weak link in the pest management system that may be exploited for pre-emptive resistance management (Fitt *et al.*, 2004). This includes identifying species with no or low resistance risk, so that further analysis is not necessary, and identifying species with high risk which must be considered further (Fitt *et al.*, 2004). *Busseola fusca* is the weak link in the system and wherever *B. fusca* dominates the pest fauna, resistance management strategies should be implemented (Fitt *et al.*, 2004). The high dose strategy, combined with the use of refuges, is widely agreed to be the best technical approach for managing resistance, and evidence is accumulating that “separate” refuges are more effective at conserving pest susceptibility than “mixed” refuges (Cannon, 2000). In South Africa the high-dose refuge strategy is recommended. This

refuge strategy can only involve non-Bt maize. Research on the wild hosts of stem borers in east Africa showed that, in the case of *B. fusca*, these plants may not be as important as considered before in maintaining sustainable populations of this species (Sezonlin *et al.*, 2005).

Table 5.2. Ecological/behavioural attributes of lepidopterous species and the efficacy of Bt maize against each species, information used to make preliminary resistance risk assessment. *

Attribute	<i>Busseola fusca</i>	<i>Chilo partellus</i>	<i>Sesamia calamistis</i>	<i>Helicoverpa armigera</i>	<i>Acantholeucania loreyi</i>	<i>Eublemma gayneri</i>	<i>Spodoptera exigua</i>	<i>Agrotis segetum</i>
Toxicity of current Bt plant ****	High	High	High	Low	Low	Medium	High?	High?
Other Bt crops	None	None	None	Yes	None	?	?	?
Diapause	Yes, 3-6 months during winter	No, larvae becomes quiescent	No	Yes, in pupal stage	?	?	?	No
Larval dispersal	Larvae disperse one to several meters	Larvae disperse one to several meters	Larvae disperse one to several meters	?	?	?	?	Larvae crawl several meters
Adult dispersal	?	?	?	?	?	?	250 – 750km***	?
Generations per year **	3	5	5	5 – 6	5	?	?	?
Generations in maize **	3	5?	5?	?	?	?	?	?
Duration of life cycle (days) **	40 – 65	25 – 50	40 - 70	40 – 50	25 - 65	?	21	42 – 175
Abundance in wild hosts	?	?	?	?	?	?	?	?
Fecundity **	1000	500	1000	1600	?	?	1500	1000
Egg batch size **	10 – 80	50 – 100	20 - 100	Single eggs	10 - 100	?	100 – 300	Singly or in groups

(Compiled on the basis of experts opinions of: J.B.J. van Rensburg, T.W. Drinkwater, H. du Plessis and J. van den Berg)

* Based on Fitt *et al.* (2004); ** May vary according to environmental conditions; *** Grist, 1975; **** Based on observed effect on MON 810 in South Africa.

5.6 Post-release monitoring

McGeoch & Rhodes (2006) indicated that the details regarding the two different phases of the ecological risk assessment process, i.e. *a priori* (permit application to general release) and *a posteriori* (ongoing production and monitoring), has not been outlined in South Africa.

Since Bt maize has already been released in South Africa this report largely contributes to focusing post-release monitoring of potential ecological impact. According to Fitt *et al.* (2004) the goals for monitoring should be: i) to detect an increase in resistance in a population early enough that remedial actions can be taken; ii) to document the failure of resistance management; iii) to determine if the needs of farmers for adequate pest control are being met; and iv) to evaluate the effectiveness of the implementation of resistance management. These goals however only address target species and regulatory issues. Monitoring should also be conducted of population levels of non-target primary consumers that are directly exposed to Bt toxin under field conditions since these species may become resistant to the toxin or become secondary pests once niches are vacated by target species.

5.7 Conclusions

The large scale availability of Bt maize in South Africa provides ideal circumstances for assessing the potential effects of transgenic crops on biodiversity. These studies conducted during the two cropping seasons of 2004/5 and 2005/6 in South Africa's main maize production area provided base line data on diversity and infestation levels of Lepidoptera that feed on maize in South Africa. The most important non-target Lepidoptera species that are directly exposed to Bt toxin were identified as well as species at risk of evolving resistance. Further research is necessary on the effect of Bt maize on the generational fitness of species that were assigned high priority status. Since the majority of species as well as the production systems evaluated in this study is

representative of the southern African region, information from this study will be useful in guidance of pre-release risk assessments in countries where release of Bt maize is considered.

5.8 References

Akhurst, R.J., James, W., Bird, L.J. & Beard, C. 2003. Resistance to the Cry1Ac δ -endotoxin of *Bacillus thuringiensis* in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 96: 1290 – 1299.

Andow, D.A. & Hilbeck, A. 2004a. Environmental risk assessment of genetically modified organisms: Vol. 1. A case study of Bt maize in Kenya. CAB International. Wallingford. UK.

Andow, D.A. & Hilbeck, A. 2004b. Science-based risk assessment for non-target effects of transgenic crops. *BioScience* 54: 637 – 649.

Barton, J.E. & Dracup, M. 2000. Genetically modified crops and the environment. *Agronomy Journal* 92: 797 – 803.

Birch, A.N.E., Wheatley, R., Anyango, B., Arpaia, S., Capalbo, D., Getu Degaga, E., Fontes, E., Kalama, P., Lelmen, E., Lovei, G., Melo, I.S., Muyekho, F., Ngi-Song, A., Ochieno, D., Ogwang, J., Pitelli, R., Schuler, T., Sétamou, M., Sithanantham, S., Smith, J., Van Son, N., Songa, J., Sujii, E., Tan, T.Q., Wan, F.H. & Hilbeck, A. 2004. Biodiversity on non-target impacts: a case study of Bt maize in Kenya. (eds.: Hilbeck & Andow). Environmental risk assessment of genetically modified organisms: Vol. 1. A case study of Bt maize in Kenya. CAB International. Wallingford. UK.

Bourguet, D., Bethenod, M.T., Pasteur, N. & Viard, F. 2000. Gene flow in the European corn borer *Ostrinia nubilalis*: implications for the sustainability of transgenic insecticidal maize. *Proceedings of the Royal Society of London* 267: 117 – 122.

Cannon, R.J.C. 2000. Bt transgenic crops: Risks and benefits. *Integrated Pest Management Reviews* 5: 151 – 173.

Dowd, P.F. 2001. Biotic and abiotic factors limiting efficacy of Bt corn in indirectly reducing mycotoxin levels in commercial fields. *Journal of Economic Entomology* 94: 1067 – 1074.

Drinkwater, T.W. 1999. Other soil insects in maize. (eds.: McDonald & Van Rensburg). Protection of grain crops: Principles & practices. Third edition. ARC-Grain Crops Institute. Potchefstroom. South Africa.

Drinkwater, T.W. & Van Rensburg, J.B.J. 1992. Association of the common cutworm, *Agrotis segetum* (Lepidoptera: Noctuidae), with winter weeds and volunteer maize. *Phytophylactica* 24: 25 – 28.

Dutton, A., Romeis, J. & Bigler, F. 2003. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. *BioControl* 48: 611 – 636.

Eizaguirre, M., Tort, S., López, C. & Albajes, R. 2005. Effects of sublethal concentrations of *Bacillus thuringiensis* on larval development of *Sesamia nonagrioides*. *Journal of Economic Entomology* 98: 464 – 470.

Ferré, J. & Van Rie, J. 2002. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* 47: 501 – 533.

Fitt, G.P., Andow, D.A., Carrière, Y., Moar, W.J., Schuler, T.H., Omoto, C., Kanya, J., Okech, M.A., Arma, P. & Maniania, N.K. 2004. Resistance risks and management associated with Bt maize in Kenya. (eds.: Hilbeck & Andow). Environmental risk assessment of genetically modified organisms: Vol. 1. A case study of Bt maize in Kenya. CAB International. Wallingford. UK.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology. *Annual Review of Entomology* 43: 701 – 726.

Grist, D.H. 1975. Rice. Tropical agriculture series. Fifth edition. Longman Group Limited. London. Pp 318 – 322.

Hilbeck, A., Andow, D.A., Apraia, S., Birch, A.N.E., Fontes, E.M.G., Lövei, G.L., Sujii, E.R., Wheatley, R.E. & Underwood, E. 2006. Methodology to support non-target and biodiversity risk assessment. (eds.: Hilbeck, Andow & Fontes). Environmental risk assessment of genetically modified organisms: Vol. 2. Methodologies for assessing Bt cotton in Brazil. CAB International. Wallingford. UK.

Hilbeck, A., Andow, D.A. & Fontes, E.M.G. 2006. Environmental risk assessment of genetically modified organisms. Vol. 2. Methodologies for assessing Bt cotton in Brazil. CAB International. Wallingford. UK.

Höfte, H. & Whiteley, H.R. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Reviews* 53: 242 – 255.

Kroon, D.M. 1999. Lepidoptera of Southern Africa. Host-plants and other associations. A Catalogue. Lepidopterists' Society of Africa. The Honorary Secretary. Jukskei Park. South Africa.

Luna, S.V., Figueroa, J.M., Baltazar, B.M., Gomez, R.L., Townsend, R. & Schoper, J.B. 2001. Maize pollen longevity and distance isolation requirements for effective pollen control. *Crop Science* 41: 1551 – 1557.

McGeoch, M.A. & Rhodes, J.I. 2006. Ecological risk assessment of genetically modified organisms in South Africa: an assessment of the current policy framework. *C.I.B. Occasional Paper* No. 2. Center for Invasion Biology, Stellenbosch University, Stellenbosch, South Africa. Pp. 19.

Paterniani, E. & Stort, A.C. 1974. Effective maize pollen dispersal in the field. *Euphytica* 23: 129 – 134.

Peacock, J.W., Schweitzer, D.F., Carter, J.L. & Dubois, N.R. 1998. Laboratory assessment of the effects of *Bacillus thuringiensis* on native Lepidoptera. *Environmental Entomology* 27: 450 – 457.

Romeis, J., Sharma, H.C., Sharma, K.K., Das, S. & Sarmah, B.K. 2004. The potential of transgenic chickpeas for pest control and possible effects on non-target arthropods. *Crop Protection* 23: 923 – 938.

Schoper, J.B., Lambert, R.J. & Vasilas, B.L. 1987. Pollen viability, pollen shedding, and combining ability for tassel heat tolerance in maize. *Crop Science* 27: 27 – 31.

Sezonlin, M., Dupas, S., Le Rü, B., Le Gall, P., Moyal, P., Calatayud, P.A., Giffard, I., Faure, N. & Silvain, J.F. 2005. Phylogeography and population genetics of the maize stalk borer *Busseola fusca* (Lepidoptera, Noctuidae) in sub-Saharan Africa. *Molecular Ecology*, 10.1111/j.1365-294X.2005.02761.x.

Sharma, H.C. & Pampapathy, G. 2006. Influence of transgenic cotton on the relative abundance and damage by target and non-target insect pests under different protection regimes in India. *Crop Protection* 25: 800 – 813.

Singh, R., Channappa, R.K., Deebe, F., Nagaraj, N.J., Sukaveneswaran, M.K. & Manjunath, T.M. 2005. Tolerance of Bt corn (MON 810) to maize stemborer, *Chilo partellus* (Lepidoptera: Pyralidae). *Plant Cell Reports* 24: 556 – 560.

Smale, M. & De Groote, H. 2003. Diagnostic research to enable adoption of transgenic crop varieties by smallholder farmers in Sub-Saharan Africa. *African Journal of Biotechnology* 2: 586 – 595.

Tabashnik, B.E., Carrière, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M. & Zhao, J. 2003. Insect resistance to transgenic Bt crops: Lessons from the laboratory and field. *Journal of Economic Entomology* 96: 1031 – 1038.

Van den Berg, J. & Drinkwater, T.W. 2000. Pink stem borer. *Crop Protection Series no. 20*. ARC-Grain Crops Institute. Potchefstroom. South Africa.

Van Jaarsveld, M.J., Basson, N.C.J. & Marais, P. 1998. Synthetic pyrethroid resistance in field strains of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in South Africa. *African Plant Protection* 4: 15 – 18.

Van Rensburg, G.D.J. 1994. Host plant preference of the common cutworm, *Agrotis segetum*. South African Society for Crop Production. 18 – 20 January 1994. Cedara.

Van Rensburg, J.B.J. & Bate, R. 1986. Preliminary studies on the relative abundance and distribution of the stalk borer *Busseola fusca* and *Chilo partellus*. (eds. Fourie & Du Plessis) Proceedings of the seventh South African maize breeding symposium. Department of Agriculture and Water supply technical communication. Summer Grain Centre, Grain Crop Institute. Potchefstroom.

Van Rensburg, J.B.J. 1997. Seasonal moth flight activity of the maize stalk borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in small farming areas of South Africa. *Applied Plant Science* 11: 20 – 23.

Van Rensburg, J.B.J., Walters, M.C. & Giliomee, J.H. 1985. Geographical variation in the seasonal moth flight activity of the maize stalk borer, *Busseola fusca* (Fuller), in South Africa. *South African Journal of Plant and Soil* 2: 123 – 126.

Van Rensburg, J.B.J. 1999a. Evaluation of Bt-transgenic maize for resistance to the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) in South Africa. *South African Journal of Plant and Soil* 16: 38 – 43.

Van Rensburg, J.B.J. 1999b. The armyworm complex: *Spodoptera exempta* and *Spodoptera exigua*. (eds.: McDonald, A.H. & Van Rensburg, J.B.J.). Protection of grain crops: Principles & Practices. Third edition. ARC-Grain Crops Institute. Potchefstroom. South Africa.

Van Rensburg, J.B.J. 2000. The Chilo borer in maize. *Crop Protection Series no. 22*. ARC-Grain Crops Institute. Potchefstroom. South Africa.

Van Rensburg, J.B.J. 2001. Larval mortality and injury patterns of the African stalk borer, *Busseola fusca* (Fuller) on various plant parts of Bt-transgenic maize. *South African Journal of Plant and Soil* 18: 62 – 68.

CHAPTER 6: CONCLUSIONS

The advent of genetically modified organisms (GMOs), and in particular GM plants with insect resistance provided new options for pest management and a unique challenge for pest management practitioners. This is especially true for Bt maize since the introduction of these crop varieties into an ecosystem could have both negative and positive effects associated with it. In the case of Bt maize increased food security resulting from reduced pest damage as well as reduced insecticide use are some of the reported benefits (Sharma *et al.*, 2000; Mugo *et al.*, 2002). A great advantage of Bt maize is that it provides resistance against target species and reportedly does not affect non-target organisms (Mugo *et al.*, 2002; Meeusen & Warren, 1989; Cannon, 2000). Another potential advantage is a decrease in the numbers of insecticide applications which are often broad spectrum and which has a negative effect on many non-target insects (Meeusen & Warren, 1989; Cannon, 2000; Huang *et al.*, 2003).

There are however also many concerns regarding the use of Bt maize technology. The most important of these are the effects on non-target organisms and resistance development (Barton & Dracup, 2000; Cannon, 2000; Dutton *et al.*, 2003; Gould, 1998; Levidow, 2003; Meeusen & Warren, 1989; Tabashnik *et al.*, 2003). It has been reported that some GMOs used in agriculture also affects biodiversity in natural ecosystems (Dutton *et al.*, 2003; Barton & Dracup, 2000; Wisniewski *et al.*, 2002). Since Bt maize have been developed to be resistant to the target pest and not to produce higher yields *per se*, pest control benefits will only be recognized if the target species is present in the maize field.

The diversity of Lepidoptera species on maize were assessed during this study and 15 species were recorded. Eleven species were recorded to feed on non-Bt maize while pupae of four other species were also found on maize. Six of these species were also recorded to feed on Bt maize. Species were divided into different guilds based on the different plant tissues they were feeding on. The following guilds were recognized:

seedling and leaf feeders; stem borers; two post-flowering guilds that feed on maize tassels and ears (including silk) respectively; and a visitors guild. The species were recorded to feed on maize were stem borers (*Busseola fusca* (Lepidoptera: Noctuidae), *Chilo partellus* (Lepidoptera: Pyralidae) and *Sesamia calamistis* (Lepidoptera: Noctuidae)) while two can be regarded as leaf feeders (*Acantholeucania loreyi* (Lepidoptera: Noctuidae) and *Spodoptera exigua* (Lepidoptera: Noctuidae)). *Agrotis segetum* (Lepidoptera: Noctuidae) is a pest of maize seedlings while *Helicoverpa armigera* (Lepidoptera: Noctuidae) usually attacks plants during the reproductive stages when it feeds on maize ears and silks. The stem borers can however also belong to both the leaf feeding- and post-flowering guilds since they feed on maize plants from the early whorl stage onwards and also damage the stem and ears during the post-flowering stages. The other four species that were recorded were assigned to the post-flowering guild. These species were *Eublemma gayneri* (Lepidoptera: Noctuidae), *Nola phaeocraspis* (Lepidoptera: Nolidae), *Chlorissa stibolepidae* (Lepidoptera: Geometridae) and *Chrysodeixis acuta* (Lepidoptera: Noctuidae). Only *B. fusca*, *H. armigera*, *A. loreyi*, *E. gayneri*, *A. segetum* and *N. phaeocraspis* were recorded on Bt maize.

The incidences of Lepidoptera-infested plants and infestation levels were generally lower in Bt fields than in non-Bt fields, possibly indicating that some of the non-target species may be affected by Bt maize. Some of the Lepidoptera species recorded during this study were first-reports on maize. Little is known of the biology of species like *A. loreyi*, *E. gayneri* and *N. phaeocraspis*. Further research should be directed at determining the effects of Bt maize on these non-target species since Bt maize may affect these species that provide beneficial ecosystem services. Although Bt maize (event MON810) specifically targets *B. fusca* and *C. partellus* in South Africa, there are other stem borer species as well as many other lepidopteran species that are directly or indirectly exposed to Bt toxin.

During the study of succession of different species and guild structure it was observed that the incidence of infested plants increased significantly during the post-flowering period on both Bt- and non-Bt maize fields. During the succession study Lepidoptera

damage was recorded on Bt- and non-Bt maize under field conditions. *Helicoverpa armigera* and *A. loreyi* occurred in significant numbers on both Bt- and non-Bt maize, however, the incidence of damaged plants was always lower in Bt- than non-Bt fields. This indicates that Bt toxin in the reproductive structures of maize possibly has a negative effect on these two species. Since significant survival of *H. armigera* and *A. loreyi* was observed on Bt maize these species have the potential to become secondary pests. Results showed that *B. fusca* larvae were able to survive on Bt maize at low infestation levels. *Chilo partellus* and *S. calamistis* were highly susceptible and no survival of these two species was observed on Bt maize under field conditions. In terms of resistance development the focus should therefore be on *B. fusca*.

The high susceptibility of *S. calamistis* to Bt toxin was confirmed in laboratory and greenhouse experiments conducted during this study. It was observed that the unique feeding behaviour of *S. calamistis* larvae, which do not feed inside plant whorls but enter directly into the stem after egg hatch, did not result in larvae escaping exposure to Bt toxin.

Since South Africa is the only country in Africa where Bt maize has been commercialized, information on ecological impacts of this crop is hard to find. The information compiled during this study was subsequently used in an ecological model to identify species for future studies and risk assessment of Bt maize in South Africa. From this study five important non-target Lepidoptera species were identified (*S. calamistis*, *H. armigera*, *A. loreyi*, *A. segetum* and *S. exigua*). The most important non-target species with the highest maximum potential exposure to Bt toxin were *S. calamistis*, *H. armigera* and *A. loreyi*. *Eublemma gayneri* can be considered to be a “value unknown” species in the wild. *Agrotis segetum* and *S. exigua* were considered of less importance but can be considered in pre- or post-release monitoring. In South Africa these priority species can be used in post-release monitoring surveys since Bt maize is already commercialized. Knowledge about these species can be used in pre-release risk assessments in other African countries where release of Bt maize is considered.

The long-term use of transgenic crops could directly or indirectly affect biodiversity. Finally, it needs to be stressed that sustainability of transgenic crops is about more than just controlling the target species. It is also important to know what impacts Bt maize has on the rest of the agroecosystem. While there have been studies of the farm-level economic impacts of GM crops in South Africa, no research has been done on the environmental impact on Lepidoptera species in the country. Future research should be directed at assessing the impact of Bt maize on the priority species identified during this study.

6.1 References

Barton, J.E. & Dracup, M. 2000. Genetically modified crops and the environment. *Agronomy Journal* 92: 797 – 803.

Cannon, R.J.C. 2000. Bt transgenic crops: Risks and benefits. *Integrated Pest Management Reviews* 5: 151 – 173.

Dutton, A., Romeis, J. & Bigler, F. 2003. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. *BioControl* 48: 611 – 636.

Huang, J., Hu, R., Pray, C., Qiao, F. & Rozelle, S. 2003. Biotechnology as an alternative to chemical pesticides: a case study of Bt cotton in China. *Agricultural Economics* 29: 55 – 67.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology. *Annual Review of Entomology* 43: 701 – 726.

Levidow, L. 2003. Precautionary risk assessment of Bt maize: what uncertainties? *Journal of Invertebrate Pathology* 83: 113 – 117.

Meeusen, R.L. & Warren, G. 1989. Insect control with genetically engineered crops. *Annual Review of Entomology* 34: 373 – 381.

Mugo, S., Songa, J., DeGroote, H. & Hoisington, D. 2002. Insect resistant maize for Africa (IRMA) project: An overview. *Syngenta Symposium*, June 25, Washington DC.

Sharma, H.C., Sharma, K.K., Seetharama, N. & Ortiz, R. 2000. Prospects for using transgenic resistance to insects in crop improvement. *Electronic Journal of Biotechnology* 3: 76 – 95.

Tabashnik, B.E., Carrière, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M. & Zhao, J. 2003. Insect resistance to transgenic Bt crops: Lessons from the laboratory and field. *Journal of Economic Entomology* 96: 1031 – 1038.

Wisniewski, J., Frangne, N., Massonneau, A. & Dumas, C. 2002. Between myth and reality: genetically modified maize, an example of a sizeable scientific controversy. *Biochimie* 84: 1095 – 1103.