

The response of lepidopteran pests to commercialised Bt maize in South Africa

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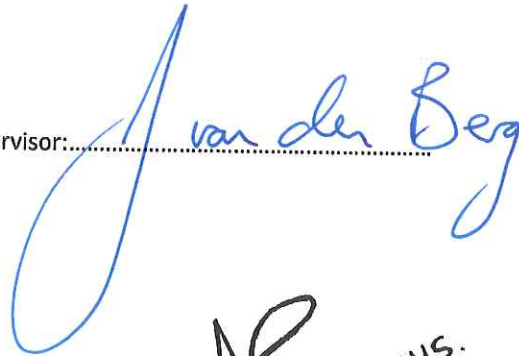
Declaration

I declare that the work presented in this Masters dissertation is my own work, that it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been acknowledged by complete reference.

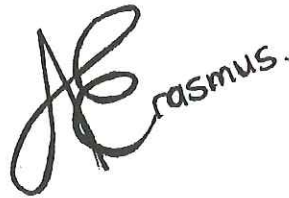
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ABSTRACT

Bt maize expressing Cry1Ab was approved for release in South Africa for control of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in 1998. During 2012, a stacked Bt maize event, expressing Cry2Ab2 + Cry1A.105, was also approved for control of these abovementioned pests. The aim of this study was to determine the effects of two Bt maize events expressing Cry1Ab (MON810 and Bt11) and a Bt maize event expressing Cry2Ab2 + Cry1A.105 (MON89034) on selected lepidopteran non-target pest species and certain lepidopteran stem borer species in South Africa. Results from previous studies and this study will provide information regarding efficacy of Bt maize against pests which have not been evaluated yet. Insects with significance in maize agro-ecosystems in South Africa as well as the rest of Africa, were prioritised and laboratory bioassays were conducted to evaluate the effect of Bt maize against these selected target and non-target pest species. Studies were conducted on three stem borers *C. partellus*, *Eldana saccharina* and *Sesamia calamistis* and three non-target lepidopteran pest species *Agrotis segetum*, *Helicoverpa armigera* and *Spodoptera exempta*. Results showed that MON810 maize was not effective against *A. segetum* larvae feeding on maize seedlings. Differential levels of survival were observed between two *A. segetum* populations on MON89034 with a population from Polokwane showing survival on the stacked maize event. No *S. exempta* 1st instar larvae survived on MON810 and MON89034 but 3rd instar larvae survived on MON810 maize. *Helicoverpa armigera* larvae survived on ears of MON810 maize plants but not on events MON89034 or Bt 11. Results further indicated that *C. partellus* larvae were highly susceptible to these three Bt maize events. Larval survival of *S. calamistis* was recorded for larvae feeding on MON810 and Bt11 maize ears but not on MON89034 maize ears. Bt maize during the vegetative growth stages therefore effectively controlled *C. partellus*, *S. calamistis* and *E. saccharina* but the latter two species was not effectively controlled when feeding on ear tissue. This study provides important information on the effects of Bt maize on the most important non-target pest species of maize in sub-Saharan Africa.

Key words: Bt maize, genetically modified, pests, stacked maize, stem borers.

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CHAPTER 1: Introduction and literature review

1.1.1 Introduction

Throughout the world there is a continuous need for increased food production, especially in developing regions such as Asia, Africa and Latin America. Insect pests are one of the largest constraints and can cause devastation when it comes to yield losses (Sharma *et al.*, 2004). The application of insecticides can be an effective way of controlling insect pests, however, large scale applications of insecticides can leave toxic residues on the crop or crop products and it has adverse effects on non-target organisms (Sharma *et al.*, 2004; Isman, 2006).

These above mentioned losses due to insect pests can be minimised effectively through host plant resistance to insects by means of biotechnological approaches (Sharma *et al.*, 2004). A new era of targeted plant breeding has opened since the ability to isolate and manipulate single genes through recombinant DNA technology, as well as the ability to insert specific genes into a chosen variety. Since the early 1980's there has been significant progress in introducing foreign genes into plants, providing opportunities to modify crops to reduce yield losses (Sharma *et al.*, 2002).

1.1.2 How does Bt work?

Bacillus thuringiensis is a gram-positive bacterium, typically found in soil, which can be distinguished from the closely related species *B. cereus* and *B. anthracis* by means of large crystalline parasporal inclusions that appear during sporulation, where these inclusions contain crystal proteins that exhibit highly specific insecticidal activity (Aronson *et al.*, 1986; Whiteley and Schnepf, 1986; Höfte and Whiteley, 1989; Wasano *et al.*, 1997; Schnepf *et al.*, 1998). Ishiwata discovered 'sotto bacillus' in 1901, where it was a pathogen of the silkworm *Bombyx mori* (L.) (Lepidoptera: Bombycidae), and since then many Lepidoptera-specific *B. thuringiensis* strains have been isolated and characterised (Wasano *et al.*, 1997).

These insecticidal crystal proteins (ICP's or Cry proteins) consist of smaller subunits that are called protoxin, which can be activated by enzymes within the digestive tract of the larvae (Hofmann *et al.*, 1988; Van Rie *et al.*, 1989). When this toxin is ingested by susceptible insect larvae, the toxins dissolve in the intestinal lumen and it is proteolytically processed to toxic polypeptides, where this activated toxin interacts with the midgut epithelium cells to the extent that they swell and lyse (Van Rie *et al.*, 1989; Höfte and Whiteley, 1989). This causes the permeability of the mid-gut membrane to be altered (Hofmann *et al.*, 1988; Höfte and Whiteley, 1989). This proliferation of the midgut epithelium allows for bacteria to enter the haemolymph, leading to cessation of feeding, septicemia and finally death (Gill *et al.*, 1992).

1.1.3 Bt crops globally and in South Africa

Globally, since the commercialization of genetically modified (GM) crops in 1996, there has been a 100 fold increase in the area being planted with these GM crops until 2012, where it was 1.7 million hectares in 1996 and 170.3 million hectares in 2012 (James, 2004; James, 2012). According to James (2004), there were fourteen biotech mega-countries in 2004, growing 50 000 hectares or more (James, 2004). In 2012, there were 18 biotech mega-countries, which are listed below in descending order of area planted with GM crops: U.S.A., Brazil, Argentina, Canada, India, China, Paraguay, South Africa, Pakistan, Uruguay, Bolivia, Philippines, Australia, Burkina Faso, Myanmar, Mexico, Spain and Chile (James, 2012).

In countries such as U.S.A., Argentina and Canada, large scale cultivation of Bt crops began during 1996. Farmers in South Africa only began cultivating Bt maize and Bt cotton for control of lepidopteran pests in 1998 (Van Rensburg, 1999; James, 2008; Van Wyk *et al.*, 2008). In South Africa there was a 26% increase in the combined area of GM maize, soybean and cotton being cultivated from 2011 (2.3 million hectares) to 2012 (2.9 million hectares) (James, 2011; James, 2012). In 2014, South Africa cultivated 2.7 million hectares of the global area (181.4 million hectares) of GM maize (James, 2014).

1.1.4 Resistance development to Bt maize

Poor refuge compliance and failure to meet high-dose requirements were put forward as the main contributors to resistance development in four lepidopteran and one coleopteran species resistant to Bt crops (Tabashnik, *et al.*, 2013). These are *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) in Puerto Rico to Bt maize (Storer *et al.*, 2010, 2012), *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in South Africa to Bt maize (Van Rensburg, 2007), *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) to Bt cotton (*Gossypium hirsutum*) in the south-eastern United States (Luttrell *et al.*, 2004; Ali *et al.*, 2006), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) to Bt cotton in India (Dhuria and Gujar, 2011), and more recently *Diabrotica virgifera virgifera* (LeConte) (Coleoptera: Chrysomelidae) to Bt maize in the U.S.A. (Gassmann *et al.*, 2011).

1.1.5 Bt maize in South Africa

Bt maize was initially developed to control two North American lepidopteran stem borer species. These stem borers are *Diatraea grandiosella* (Dyar) (Lepidoptera: Crambidae) (Archer *et al.*, 2001) and *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) (Ostlie *et al.*, 1997). In South Africa, the target pests of Bt maize are the lepidopteran stem borers *Busseola fusca*, *Chilo partellus*, and *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) (Van Wyk *et al.*, 2008; Erasmus *et al.*, 2010). These pests were effectively controlled by the Cry1Ab toxin expressed by the MON810 and Bt11 events (Van Rensburg, 1999), until there was resistance development by *B. fusca* for the Cry1Ab toxin (Van Rensburg, 2007). Monsanto secured cultivation approval for MON89034 (YieldGard II) at the end of 2010 in South Africa. MON89034 is a stacked Bt event expressing two crystal proteins, which are Cry2Ab2 and Cry1A.105 (Monsanto, 2010).

While the efficacy of the above mentioned Bt maize events against *B. fusca*, *C. partellus* and *S. calamistis* have been evaluated and reported on (Van Rensburg, 1999; Van Wyk, 2008), little or no information is available on efficacy against other lepidopteran species that also attack maize but that are not considered target pests

in South Africa. The following lepidopteran pests that also attack maize are *Agrotis segetum* (Denis and Schiffermüller) (Lepidoptera: Noctuidae), *C. partellus*, *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), *S. calamistis* and *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae). These species are all exposed to Bt maize to a more or a lesser extent. If Bt maize has an effect on these species, it could affect their pest status, and change pest dynamics in maize ecosystems.

1.2 *Agrotis segetum* (Lepidoptera: Noctuidae) - Common cutworm

There are several *Agrotis* cutworm species that occur in South Africa. These are Black cutworm (*Agrotis ipsilon*), Grey cutworm (*Agrotis subalba*), Brown cutworm (*Agrotis longidentifera*), spiny cutworm (*Agrotis spinifera*) and the common cutworm (*Agrotis segetum*). *Agrotis segetum* occurs throughout South Africa and is an important pest of crops such as cotton, sorghum, sunflower and maize. This species is the most common and it is economically important in maize production areas (Anneck and Moran, 1982; Du Plessis, 2000; Erasmus *et al.* 2010).



Figure 1.1. *Agrotis segetum* larva.

Agrotis segetum larvae (Fig 1.1) can cause severe damage to seedlings. They move from one seedling to another, cutting and destroying the stems of seedlings near ground level, leading to the death of the plant (Annecke and Moran, 1982). One larva can destroy numerous plants in one night (Drinkwater, 1980). If pest outbreaks occur, replanting of the crop often has to be done (Annecke and Moran, 1982). These larvae are active at night and during the day they can be found near the soil surface in the vicinity of dead seedlings (Annecke and Moran, 1982). Damage to seedlings in some cases occur beneath the soil surface, because if the soil is dry, the larvae tend to damage the seedlings below the soil surface (Du Plessis, 2000). The moths lay their eggs on weeds during autumn and winter, and the larvae that emerge overwinter on weeds, and other volunteer crop plants serve as a food source during spring and therefore a high number of winter weeds may enhance the chances for a cutworm infestation on crops (Drinkwater, 1980).

The first two instars of *A. segetum* feed on plant material on the soil surface, whereas the older larvae shelter in the soil during day and emerge at night to feed (Drinkwater and Van Rensburg, 1992; Du Plessis, 2000). The larvae which can be of different sizes, overwinter in the soil until the arrival of spring. These larvae will start developing into pupae in pupal cells during August and September. First-generation moths in the new season will emerge from these pupae approximately two weeks after pupa formation, and these moths will lay their eggs on leaves of weeds and volunteer crop plants in the field (Smit, 1964).

The adults lay eggs singly or in groups on the soil surface or lower plant parts. Hatching time of eggs and the duration of the development period of larval stages are influenced by environmental conditions. Lower temperatures can cause the developmental period to be extended. Larvae moult five times and the last larval instar is followed by a pupal stage after which moths emerge. The life cycle takes approximately 50 days to complete during the summer (Annecke and Moran, 1982; Du Plessis, 2000). The larvae are thick, usually darkish grey or brown in colour with a waxy appearance. Cutworm moths can be identified by characteristic markings on the wing. The common cutworm has brown to grey-black fore wings, and pale whitish hind wings (Drinkwater, 1980).

The damage of the common cutworm is not only done to maize seedlings, plants in the four leaf stage or older can also be damaged (Drinkwater, 1980). This damage can be seen in older plants as neat and clean round holes into the stem, just below the soil surface. The damage caused by the common cutworm can easily be distinguished from that of the black maize beetle, *Heteronychus arator* (Fabricius) (Coleoptera: Scarabaeidae) or the false wire worm, *Somaticus angulatus* (Fahraeus) (Coleoptera: Tenebrionidae), where these last two mentioned pests cause damage where the edges of damaged seedlings are ragged and unravelled (Drinkwater 1980; Drinkwater and Walters, 1979). However, the damage that can be seen above ground is similar to that of the black maize beetle and false wire worm. The central whorl leaf wilts first, followed by the wilting of the entire plant (Drinkwater, 1980).

Increased cutworm populations during spring as a result of increased winter weed populations can be seen if there were abundant autumn rains. A spring cultivation of fields at least 35 days prior to seeding is generally suggested in order to reduce the local cutworm populations by starvation (Drinkwater and Van Rensburg, 1992).

1.3 *Helicoverpa armigera* (Lepidoptera: Noctuidae) - African bollworm

The African bollworm is distributed all over Africa, southern Europe, the near and Middle East, India, Central and Southeast Asia, Japan, the Philippines, Indonesia, New Guinea, eastern Australia, New Zealand, Fiji, and some other Pacific islands, however, there is a probability that it is not indigenous to southern Africa (Annecke and Moran, 1982; Reed and Pawar, 1982). It was also recently recorded in Brazil (Tay *et al.*, 2013).

Helicoverpa armigera is generally regarded as the most important pest of agricultural crops throughout the world because of its wide host range (Van Hamburg and Guest, 1997). This wide host range of agricultural crops include: cotton, maize, chickpea, pigeonpea, sorghum, sunflower, soybean, tobacco, citrus and groundnut (Annecke and Moran, 1982; Fitt, 1989; Van Hamburg and Guest, 1996, Cunningham *et al.*, 1999). According to Fitt *et al.* (2004), *H. armigera* is the main non-target pest of concern for resistance development against Bt maize since it is consistently

associated with maize, even though it is regarded as a minor and sporadic pest. *Helicoverpa armigera* is an economic pest of maize wherever maize is grown in South Africa (Matthee, 1974). According to Van Hamburg and Guest (1996), this species is also the most important pest species of the bollworm complex on cotton, which consists of the African bollworm (*H. armigera*), the red bollworm (*Diparopsis castanea*) (Hampson) (Lepidoptera: Noctuidae) and the spiny bollworm complex (*Earias* spp.) (Lepidoptera: Noctuidae).

The African bollworm was previously known as the American bollworm or *Heliothis armigera*, however this species does not occur in North-America, the name was subsequently changed to the African bollworm (Du Plessis and Van den Berg, 1999). *Helicoverpa armigera* (Fig. 1.2) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) look very similar, and *H. zea* can be found in the Americas, and therefore confusion arose in the naming of the pest (Reed and Pawar, 1982).



Figure 1.2. *Helicoverpa armigera* larva.

The moth of the African bollworm has brownish, yellowish-brown or greyish brown fore wings, with darker brown markings, where the hind wings are pale, greyish-white, with dark veins. The hindwings have a broad dusky apical band with two

distinct pale spots (Annecke and Moran, 1982). The eggs are almost spherical, and up to 0.5mm in diameter (Annecke and Moran, 1982), yellow to white in colour (Du Plessis and Van den Berg, 1999), turning brown before they hatch (Annecke and Moran, 1982). There are six to seven larval instars during the life cycle (Sigsgaard *et al.*, 2002; Annecke and Moran, 1982), where the first two larval instars are of a yellow to a reddish-brown colour and the later larval instars have the characteristic pattern of the three longitudinal dark bands separated by pale ones, although these patterns are variable. The larvae may reach a length of 40mm and has three pairs of thoracic legs and leg-like protuberances on each of the third to sixth abdominal segments as well as on the last segment. The pupa is dark brown (Annecke and Moran, 1982).

Eggs are laid singly near or on the maize ears. One female can produce more than a 1000 eggs during her lifespan, and these eggs can hatch within three to five days after oviposition (Du Plessis and Van den Berg, 1999; Rajapakse and Walter, 2007). Egg cannibalism may occur after larvae have emerged (Sigsgaard *et al.*, 2002), or the larvae will consume the shell of its own egg and then go in search of a viable food source (Annecke and Moran, 1982).

Helicoverpa armigera larvae largely feed on maize ears (Fitt *et al.*, 2004). Larvae usually feed on the silks of the maize ears while the ears are still young, and the damage can be of such a degree that poor pollination occurs. Husk leaves, providing cover for young ears, can be damaged by the larvae, and if there is heavy rainfall, water may enter the ears. This may lead to fungal growth that can cause kernels to become discoloured and prone to ear rot infection (Du Plessis and Van den Berg, 1999).

1.4 *Spodoptera exempta* (Lepidoptera: Noctuidae) - African armyworm

Larvae of the African armyworm feed almost only on plants of the families Poaceae and Cyperaceae (Brown and Dewhurst, 1975), however, under field conditions it is unlikely for first instar larvae to feed on maize, they only begin feeding on maize at a later instar (Odiyo, 1979). These larvae may sporadically reach very high densities.

These high densities of armyworm can cause severe damage to crops such as maize, sorghum, millet and rice, as well as to pastures and rangeland grasses. Larvae in their early instars prefer grasses in the genus *Cynodon* (Odiyo, 1979; Janssen, 1994). This pest has a very wide distribution and occurs in sub-Saharan Africa, eastern Africa, southwest Arabia, South East Asia, Australasia and Hawaii (Brown and Dewhurst, 1975). The moths can migrate tens and often hundreds of kilometres between the emergence site and the locality of the next oviposition for the next generation (Brown and Swaine, 1966; Haggis, 1984).

Rose (1979) proposed two possible mechanisms for African armyworm outbreaks. The first being the importance of moths emerging from known outbreaks, where very dense populations of gregarious-phase larvae (mostly black larvae with yellow stripes) provide the means to large numbers of moths to emerge over a short period (Brown and Swaine, 1966). It is possible for the moths that migrate to be carried down-wind, and if there are further generations, they are likely to become crowded and cause further outbreaks (Rose, 1979). The second mechanism is the significance of moths developing from low-density populations. If the first outbreak of the season cannot be ascribed to moths from previously known outbreak centers, there is a possibility that they can come from inconspicuous larvae, distributed in seasonally green grasses at low densities, or from denser populations where temperatures are favourable most of the year (Rose, 1979).

The moths have brown fore wings with paler markings, and the hind wings are whitish (Annecke and Moran, 1982). Armyworm moths are very fecund. Eggs are usually laid in clusters on leaves of host plants, with each cluster having two to three layers and containing as much as 400 eggs. A single moth may lay more than 600 eggs. The eggs are white directly after oviposition and turn brown before the larvae emerge, which is usually three to four days at higher temperatures and up to seven days at lower temperatures (Annecke and Moran, 1982).

Spodoptera exempta have two distinct forms in the larval stage (Rose, 1979). During the gregarious phase larvae have a darker appearance, after which they ultimately become black with yellow stripes (Fig. 1.3). Larvae in the solitary phase remain green or a pinkish green until pupation. If the gregarious phase larvae are at

high densities, they will march in one direction (Rose, 1979), hence the name armyworm (Cheke and Tucker, 1995). There are no phase differences between the moths of *S. exempta* (Rose, 1979). There are five to six larval instars and larval development can be completed in two to three weeks at higher temperatures and considerably longer in colder weather (Annecke and Moran, 1982). Pupation occurs in the soil or in protected areas under stones or plant debris, and the pupal stage varies in duration, according to temperatures, the average being ten days in summer (Annecke and Moran, 1982). The life cycle of *S. exempta* is very short and can be completed within a month in the field. Larvae usually appear without any notice, from synchronized breeding of adult moths (Brown and Swaine, 1966).

Brown and Odiyo (1968) performed a trial in which they placed a piece of cut maize leaf (2.5cm by 7.5cm) in a petri dish and they estimated the average amount of leaf eaten by one larva during each successive 24 hour period after emergence. It was concluded that two larvae will be able to destroy a ten day old maize plant with six to seven open leaves within their life cycle. One larva can consume up to 200mg of maize leaves (dry mass) in the course of the sixth instar (Brown and Odiyo 1968).



Figure 1.3. *Spodoptera exempta* larva.

According to Fitt *et al.* (2004), *S. exempta* as well as other *Spodoptera* spp., are not effectively controlled by Cry1A protein, making the threat of resistance development insignificant. TC1507 maize, expressing the Cry1F protein was approved in Puerto Rico in 2003 for control of *S. frugiperda* (Storer *et al.*, 2010, 2012). TC1507 maize can control important lepidopteran pests, including *S. frugiperda* (Storer *et al.*, 2010).

1.5 *Chilo partellus* (Lepidoptera: Crambidae) - Spotted stem borer

Chilo partellus is indigenous to Asia and became established in eastern Africa in the early 1930's (Mohamed *et al.*, 2004). It is very likely that this pest is indigenous to western Asia, and there it is a pest of rice, maize, sugarcane and sorghum. It only later on immigrated to southern Africa, where it was first recorded in the 1950's in the Springbok flats (Annecke and Moran, 1982). Since then it has spread to Gauteng province, North West province, Northern Cape province, Mpumalanga province and KwaZulu-Natal province (Annecke and Moran, 1982; Van Rensburg, 1999; Kfir *et al.*, 2002). In South Africa, *C. partellus* is an important pest of maize and sorghum (Kfir *et al.*, 2002), however they have several gramineous and other wild host plants (Mohamed *et al.*, 2004; Koji *et al.*, 2007; Mohamed *et al.*, 2007).

The larvae of the spotted stem borer (Fig. 1.4) as well as the other stem borers feed and tunnel through the stem and this can result in crop losses as a consequence of destruction of stem tissue (Kfir *et al.*, 2002). This damage also causes problems with translocation of metabolites and nutrients that result in abnormalities of the seeds, it can cause stems to break with the slightest of winds and restricts plant growth (Matthee, 1974; Kfir *et al.*, 2002).

Female moths can lay up to 500 eggs (Annecke and Moran, 1982; Ofomata *et al.*, 2000), in batches of varying sizes (Annecke and Moran, 1982). The female lays most of the eggs in the first three oviposition nights (Päts and Ekblom, 1994), with about two thirds of the eggs on the upper surface of the leaf, and the other third on the underside of the leaf (Annecke and Moran, 1982). The first instar larvae can spin silk threads from which they can suspend themselves, and by using wind they can disperse from the oviposition plant to adjacent plants (Päts and Ekblom, 1994).

The moths are very ordinary in appearance with a pale brown colouration, 15mm in length, the female being a little larger than the male (Annecke and Moran, 1982). Bate and Van Rensburg (1990) reported that the spotted stem borer has two flight activity periods in the in the North-West Province, the first occurs during September to December and the second from January to May. More adults are present during the second flight period. The generations can overlap with all the stages being present throughout the summer, owing to staggered pupation (Annecke and Moran, 1982; Van den Berg and Van der Westhuizen, 1995). In the winter or dry season the spotted stem borer remains as larvae, in a state of suspended development in crop residues in the field. Larvae pupate as soon as conditions become favourable, while others may take as long as a month to respond to the onset of favourable conditions (Annecke and Moran, 1982; Kfir *et al.*, 2002).



Figure 1.4. *Chilo partellus* larva.

Van Rensburg (1999) conducted a study on *C. partellus* to evaluate the efficacy of Cry1Ab (MON810) Bt maize to control this species. Results showed that *C. partellus* was highly susceptible to Bt maize expressing Cry1Ab proteins.

Even though, *C. partellus* is not indigenous to Africa, egg and larval parasitoids are known to occur in South Africa. It is however apparent that they are incapable of limiting the annual summer increase in population numbers of this species (Kfir *et al.*, 2002). An example of such parasitoid is *Dentichasmias busseolae* (Heinrich) (Hymenoptera: Ichneumonidae), which parasitizes larvae and emerges from the pupa. This species is an important controlling factor, especially late in the season. According to Kfir *et al.* (2002), *Cotesia* spp. (Hymenoptera: Braconidae) was also introduced to control the spotted stem borer in South Africa but with no establishment taking place.

1.6 *Eldana saccharina* (Lepidoptera: Pyralidae) - African sugarcane borer

The African sugarcane borer, *Eldana saccharina*, is indigenous to Africa and surrounding islands where it feeds on a diversity of host plants (Carnegie, 1974; Conlong, 1997; Mazodze and Conlong, 2003; Assefa *et al.*, 2006). Wetland sedges (Cyperaceae and Typhaceae) make up a large proportion of its natural host plants (Mazodze and Conlong, 2003). In certain parts of Africa this indigenous insect is a recognised and major pest of sugarcane and, to a lesser extent, maize and sorghum, rice and millet (Mazodze and Conlong, 2003; Keeping *et al.*, 2007). Even though this lepidopteran pest was described more than a century ago from sugarcane in West Africa, it was not recorded in South Africa until about the 1940's when it made its appearance in the Umfolozi area in Zululand (Atkinson, 1979).

Eldana saccharina does not attack maize in South Africa and is therefore not a target pest of Bt maize in the country. According to Muhammad and Underwood (2004), *E. saccharina* is a pest of maize in western Kenya and also in other regions of southern Africa (Mazodze and Conlong, 2003). In such countries, this species will in the future be regarded as a target pest of Bt maize, once GM varieties of this crop is approved for cultivation.

The moth is light brown with a wingspan of up to 30-35mm. The wings are resting in a folded position across the back of the moth. Mating of moths occurs shortly after sunset, and oviposition by the female begins in approximately 24 hours after mating.

The female may fly 200m or more to find a viable site for oviposition, however, eggs are usually laid closer to the adult's emergence site (Carnegie, 1974).



Figure 1.5. *Eldana saccharina* larva.

Eldana saccharina lays its eggs on dry maize leaves and plant residue material on the soil but also on the hairy margins of the leaves (Carnegie, 1974; Ajala *et al.*, 2001). The eggs are laid in batches, each batch containing about 20 eggs. The female can lay up to 450 eggs, which hatch after eight to ten days (Carnegie, 1974). In West Africa larvae of *E. saccharina* (Fig. 1.5) usually attack maize at the flowering stage, resulting in stem tunnelling, breakage and ear damage (Bosque-Pérez and Mareck, 1991; Ajala *et al.*, 2001). The larvae do not enter the maize stem immediately after emergence, and prefer to initially feed on leaves. After a certain period the larvae are sufficiently vigorous to enter the plant tissue, and the rest of its immature life is spent as a borer inside the maize stem. The larval period may range from 20 days in the summer to 60 days in the winter. Male larvae moult 5 or 6 times, and the female larvae moult 6 or 7 times, before entering the pupal stage (Carnegie, 1974).

1.7 *Sesamia calamistis* (Lepidoptera: Noctuidae) - Pink stem borer

According to Van den Berg and Van Wyk (2007), *S. calamistis* was initially not a target pest of Bt maize in South Africa. It was only listed as a target pest after their study which showed that plant tissue of the MON810 event was toxic to *S. calamistis*. The inclusion of *S. calamistis* as a test species for determining the effect of Bt maize on non-target Lepidoptera species for risk assessment was therefore suggested by Van Wyk *et al.* (2007).

Sesamia calamistis has a very wide distribution, probably the widest distribution of all the stem borer species in Africa. It occurs throughout sub-Saharan Africa below 2400 m above sea level (Muhammad and Underwood, 2004). The pink stem borer also occurs in the Indian Ocean islands of Madagascar, Mauritius and Reunion (Annecke and Moran, 1982). Ajala *et al.* (2001) described it as an economically important pest in West Africa. According to Kfir *et al.* (2002), it is of importance and widely distributed in sub-Saharan Africa, alongside other lepidopterous stem borers such as *B. fusca* and *C. partellus*. This species has a very wide host range, including a variety of hosts in the Poaceae and it also can be found on cultivated crops such as maize, rice, wheat and sugar cane (Matthee, 1974; Annecke and Moran, 1982).

Sesamia calamistis is a very variable species, and this variability includes variations in colour of the wings and wing markings, however the fore wings has a pale brown colour and the hind wings are white (Matthee, 1974; Annecke and Moran, 1982). The pink stem borer lays its eggs between the leaf sheaths and stems of young maize plants in batches of 100 or more at about three weeks after seedling emergence. Larvae of *S. calamistis* (Fig 1.6) emerge approximately a week later (Annecke and Moran, 1982; Ajala *et al.*, 2001). When the larvae emerge, they penetrate either the whorl but more frequently the stem resulting in leaf or stem damage and also deadheart formation (Ajala *et al.*, 2001). During summer months the larval period can be as long as three to six weeks, while the larvae pass through six to seven larval instars (Annecke and Moran, 1982). The dorsal surface of the larval body is a pronounced pink. The pupal stage lasts about two to three weeks and the whole life cycle lasts approximately six to eight weeks (Annecke and Moran, 1982).



Figure 1.6. *Sesamia calamistis* larva.

1.8. Objectives

The aim of this study was to evaluate the efficacy of currently available Bt maize events (MON810, Bt11 and MON89034) against lepidopteran pests of maize.

The specific objectives of this study were to:

- evaluate the efficacy of currently available Bt maize events against *Agrotis segetum* (Lepidoptera: Noctuidae), *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Spodoptera exempta* (Lepidoptera: Noctuidae).
- evaluate the efficacy of currently available Bt maize events against the lepidopterous stem borers *Chilo partellus* (Lepidoptera: Crambidae), *Eldana saccharina* (Lepidoptera: Pyralidae) and *Sesamia calamistis* (Lepidoptera: Noctuidae).

CHAPTER 2: The efficacy of Bt maize against *Agrotis segetum*, *Helicoverpa armigera* and *Spodoptera exempta*

2.1. Abstract

In South Africa, cultivation of maize expressing Cry1Ab insecticidal protein was approved in 1998 for control of two lepidopteran stem borers. These species were *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). In 2012, a stacked Bt maize event expressing Cry2Ab2 + Cry1A.105 was approved for control of these lepidopteran pests of maize in South Africa. Due to reported lower toxicity of Bt maize to other noctuid species, the effect of Bt maize expressing Cry1Ab and Cry2Ab2 + Cry1A.105 was evaluated for two populations of *Agrotis segetum* (Denis and Schiffermüller) (Lepidoptera: Noctuidae), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae). *A. segetum*, *H. armigera* and *S. exempta* have characteristic larval behaviour, feeding on different maize plant tissues. This behaviour may result in reduced exposure to Bt toxin which may result in higher levels of survival. Two Bt maize events were used, MON810 (Cry1Ab) and MON89034 (Cry2Ab2 + Cry1A.105) in laboratory bioassays with *A. segetum* and *S. exempta*. Three Bt maize events, MON810, MON89034 and Bt11 (Cry1Ab) were used in a laboratory bioassay for *H. armigera*. All experiments included a non-Bt, isoline. Plant tissue in plastic containers were inoculated with larvae. Mean larval mass and percentage larval survival was recorded at 3 – 4 day intervals. At the end of the experiment, larval survival was observed on MON810 and MON89034 maize for *A. segetum*. However, larvae from a Potchefstroom population feeding on plant tissue of MON89034 maize had 4.0% survival at the end of the experiment. Larval survival of *H. armigera* feeding on MON810 and Bt11 maize was observed at the end of the experiment (day 24) with 7.0% on MON810 maize and 9.0% on Bt11 maize. No larvae survived longer than 10 days on MON89034 maize. *Spodoptera exempta* 1st instars feeding on MON810 and MON89034 maize survived for only 7 and 3 days respectively after the commencement of the experiment. No *S. exempta* 3rd instar larvae survived on MON89034 maize throughout the experiment, but 6.0%

survived on MON810 maize and entered the pupal stage. The stacked Bt event performed better against all of these pests, and it can be recommended that in certain countries in Africa get approval to cultivate Bt maize, MON89034 or another suitable stacked Bt maize event will effectively control these above mentioned pests.

2.2. Introduction

Agrotis segetum and *Helicoverpa armigera* have cosmopolitan distributions (Annecke and Moran, 1982; Reed and Pawar, 1982; Riley *et al.*, 1992; Svensson *et al.*, 1998). *Spodoptera exempta* is present in sub-Saharan Africa, eastern Africa, southwest Arabia and South East Asia (Brown and Dewhurst, 1975; Haggis, 1986). *Agrotis segetum* has a wide host range, including crops such as cotton, sorghum, sunflower and maize (Du Plessis, 2000; Annecke and Moran, 1982). *Helicoverpa armigera* has a very wide host range, consisting of many major crops (Annecke and Moran, 1982; Fitt, 1989; Van Hamburg and Guest, 1996). *Spodoptera exempta* can cause devastating damage to crops such as maize, sorghum, millet and rice, as well as to pastures and rangeland grasses (Odiyo, 1979; Janssen, 1994)

All these abovementioned species are damaging to maize in Africa (Odiyo, 1979; Fitt *et al.*, 2004). These three pests cause damage to different maize plant tissue. *Agrotis segetum* mainly cause damage to maize seedlings by severing seedlings at ground level or just beneath the soil surface (Drinkwater, 1980; Annecke and Moran, 1982). One larvae of *A. segetum* can damage numerous plants in one night (Drinkwater, 1980). Larvae of *H. armigera* mainly feed on the silks and kernels of young maize ears and feeding on the silks may lead to poor pollination (Du Plessis and Van den Berg, 1999; Fitt *et al.*, 2004). Larvae of *S. exempta* feed on the maize foliage and in some cases, the severity of damage they cause lead to replanting of the crop.

Dutton *et al.* (2003) reported that there are numerous transgenic maize varieties available for commercial use and these different maize varieties use different promoters and it has been shown that within these different transgenic maize hybrids toxin concentrations may vary between plant parts at any one given time.

The aims of this study were to evaluate the efficacy of currently available Bt maize events against *A. segetum*, *H. armigera* and *S. exempta*.

2.3. Material and methods

The Bt maize events that were used for this study were MON810 (expressing Cry1Ab toxin), Bt11 (expressing Cry1Ab toxin) and MON89034 (expressing Cry2Ab2 + Cry1A.105 toxin) as well as a non-Bt isolate.

2.3.1. *Agrotis segetum*

Studies were conducted on fourth instar larvae of *A. segetum* from two geographically separate populations to determine larval growth and survival on Bt and non-Bt maize. As suggested by Birch *et al.* (2004) the 'whole plant' methodology was used to evaluate the effect of the whole transgenic maize plant, or parts thereof, when consumed by the pest and not only the transgenic products.

Larvae were collected from maize fields from two different geographical regions i.e., the Potchefstroom area (26° 39' S, 27°07' E) in the North West Province and in the Polokwane area (23° 53' S, 29° 47' E) in the Limpopo Province. Larvae from both of these collection sites were kept separate. The larvae were reared on artificial diet (chickpea based agar diet, developed for *Chilo partellus*) and spinach until pupation. Offspring of these field-collected larvae were used in experiments. Larvae that were used in experiments were reared on spinach and starved for one day before the onset of the trial.

Fourth instar larval survival and growth were determined under laboratory conditions. There were 3 treatments with 10 replications per treatment, including MON810, MON89034 and a non-Bt isolate. Each replicate contained 5 falcon tubes, each falcon tube was inoculated with one larva. Eight to ten day old seedlings were placed in falcon tubes (15 × 2cm) along with very fine soil to reduce the accumulation of moisture. Falcon tubes were kept in an incubator at 26°C and 60% relative humidity and a 14L:10D photoperiod. Seedlings and soil were replaced with new seedlings

and soil every 3 – 4 days when larval survival and mass was recorded. The study was terminated when larvae that fed on non-Bt isoline reached the pupal stage.

2.3.2. *Helicoverpa armigera*

Larvae were collected from maize fields in Potchefstroom (26° 39 ` S, 27°07 ` E) and reared on artificial diet (chickpea based agar diet, developed for *Chilo partellus*) until pupation. The moths that emerged from pupae were allowed to lay eggs. First instar larvae were used in this laboratory bioassay. Larval growth was evaluated on Bt and non-Bt maize ear tips. The experimental lay-out was completely randomised.

Maize ear tips in the soft dough stage were collected from plants growing in the field and placed in sealable aerated plastic containers (5 × 4cm). There were 4 replicates per treatment. Each replicate consisted of 5 containers. Five 1st instar larvae were inoculated onto the silks of each maize ear tip by means of a camel hair brush in each container. The bottom of the containers was lined with filter paper to reduce the accumulation of moisture on the inside of the containers. Containers were kept in an incubator at 26°C and 60% relative humidity and a 14L:10D photoperiod. The first evaluation of larval survival and mass was done seven days after inoculation, followed by subsequent evaluations at 3 – 4 day intervals until larvae reached pupal stage. Maize ear tips and filter paper were replaced with fresh maize ear tips and filter paper each time larval survival and mass was determined. The study was terminated when larvae reached the pupal stage.

2.3.3. *Spodoptera exempta*

Larvae were collected from grass fields after an outbreak was reported in the Potchefstroom area (26° 44 ` S, 27°04 ` E). Larvae were reared on a non-Bt maize hybrid until pupation. Moths that emerged from the pupae were allowed to lay eggs for use in further studies. Two studies, one with 1st larvae and one with 3rd instar larvae, were conducted in the laboratory. Both studies were terminated when larvae that fed on the non-Bt isoline reached the pupal stage.

Experiment 1: First instar larvae

Maize leaves from 5-6 week old plants were placed in sealable aerated plastic containers (5 × 4cm). These leaves were collected from maize plants growing in the field. The bottom of the container was lined with filter paper to reduce the accumulation of moisture. There were 10 replicates per treatment. Each replicate consisted of 5 containers. Five 1st instar larvae were inoculated onto the maize leaves by means of a camel hair brush in each container. Containers were kept in an incubator at 26°C and 60% relative humidity and a 14L:10D photoperiod. Larvae were weighed at 3 – 4 day intervals until the pupal stage was reached. Both the maize leaves and filter paper were replaced upon determining larval mass and survival.

Experiment 2: Third instar larvae

Third instar larvae were obtained by rearing 1st instar larvae on non-Bt maize leaves to obtain larger larvae of uniform age to conduct the experiment. The same methods described above were used to determine larval survival and mass at 3 – 4 day intervals until the pupal stage was reached. There were 10 replicates of each treatment. Each replicate consisted of 5 containers. In this study one larva was inoculated onto the maize leaves in each container.

2.3.4 Data analyses

Data on larval mass and survival were analysed by means of Statistica. Data on larval survival was done by means of repeated measures ANOVA. One-way ANOVA's were also calculated using survival collected on the last day of the experiment, and data on larval mass, on the day that pre-pupae were observed for the first time.

2.4. Results

2.4.1. *Agrotis segetum*

Potchefstroom

There was a significant treatment x time interaction for larval survival ($F_{(16,216)} = 24.04$; $P \leq 0.0001$). On day 28 of the experiment, larval survival was 66.0% on non-Bt, 62.0% on MON810 and 4.0% on MON89034 (Fig. 2.1). Mean larval survival differed significantly between treatments on the last day of the experiment (Table 2.1).

Mean mass of larvae feeding on non-Bt and MON810 increased gradually, however, mean mass of larvae feeding MON89034 only increased slightly and remained constant throughout the experiment (Fig. 2.2). No significant difference was observed between non-Bt and Bt events ($F_{(2,20)} = 0.17$; $P = 0.85$). On day 24 of the experiment MON89034 differed significantly from the other treatments in the experiment (Table 2.1)

Table 2.1. Mean larval mass and survival of *Agrotis segetum* (Potchefstroom population) on Bt and non-Bt maize.

Treatment	Mean larval mass (mg) (day 24)	Mean larval survival (%) (day 28)
MON89034	0.102 a	4.0 a
Non-Bt	0.366 b	66.0 b
MON810	0.385 b	62.0 b
	$p \leq 0.001$	$p \leq 0.00001$

Means within columns followed by the same letter do not differ significantly at the indicated level of significance.

Polokwane

There was no significant treatment x time interaction for larval survival ($F_{(8,108)} = 1.03$; $P = 0.42$). Larval survival for the Polokwane population was 78.0% on non-Bt, 72.0%

on MON810 and 72.0% on MON89034 maize on day 14 (Fig. 2.3). No significant difference was observed between treatments on the last day of the experiment (Table 2.2).

Mean mass of larvae feeding on non-Bt increased more rapidly than on Bt events between the onset of the experiment and day 7 (Fig. 2.4). Larvae feeding on non-Bt reached the pre-pupae stage on day 7 of the experiment. A significant difference was observed in mean larval mass between non-Bt and Bt events ($F_{(2,27)} = 20.83$; $P \leq 0.0001$). Mean larval mass differed significantly on day 7 of the experiment (Table 2.2).

Table 2.2. Mean larval mass and survival of *Agrotis segetum* (Polokwane population) on Bt and non-Bt maize.

Treatment	Mean larval mass (mg) (day 7)	Mean larval survival (%) (day 14)
MON810	0.360 a	72.0 a
MON89034	0.375 a	72.0 a
Non-Bt	0.548 b	78.0 a
	$p < 0.00001$	$p = 0.749$

Means within columns followed by the same letter do not differ significantly at the indicated level of significance.

2.4.2 *Helicoverpa armigera*

There was a significant treatment x time interaction for larval survival ($F_{(18,72)} = 35.12$; $P \leq 0.0001$). Larval survival on all the Bt events showed a rapid decrease between the onset of the experiment and day 7 (Fig. 2.5). Percentage larval survival on Bt events after the first 10-day period were 26.0% (MON810), 27.0% (Bt11) and 2.0% (MON89034). Larval survival of non-Bt maize was 75.0% after 10 days. No survival was observed on MON89034 after 10 days. On day 24 of the experiment, larval survival on non-Bt maize was 47.0%, and 7.0% and 9.0% on MON810 and Bt11, respectively. Mean larval survival differed significantly between treatments on the

last day of the experiment (Table 2.3). Since no survival occurred on MON89034 on day 24 of the experiment, no statistical analyses was performed.

Mean mass of larvae feeding on non-Bt increased between day 7 and 14 and the same was observed for MON810 and Bt11 between day 10 and day 17 (Fig. 2.6). However, a significant difference was observed in mean larval mass between non-Bt and Bt events ($F_{(2,9)} = 7.79$; $P \leq 0.05$). Larval mass on MON89034 only had a slight increase between the onset of the trial and day 7. Larvae feeding on non-Bt, MON810 and Bt11 started changing into pre-pupae on day 17 of the experiment, explaining the decrease in mass from that stage until the end of the experiment. Mean larval mass differed significantly on day 17 of the experiment (Table 2.3). Since no survival occurred on MON89034 on day 17 of the experiment, no statistical analysis was performed.

Table 2.3. Mean larval mass and survival of *Helicoverpa armigera* on Bt and non-Bt maize.

Treatment	Mean larval mass (mg) (day 17)	Mean larval survival (%) (day 24)
Bt11	0.198 a	9.0 a
MON810	0.198 a	7.0 a
Non-Bt	0.308 b	47.0 b
MON89034	-	-
	$p < 0.00001$	$p < 0.00001$

Means within columns followed by the same letter do not differ significantly at the indicated level of significance.

2.4.3. *Spodoptera exempta*

1st instars

There was a significant treatment x time interaction for larval survival ($F_{(12,162)} = 35.18$; $P \leq 0.0001$). On day 3 of the experiment, 1st instar larval survival on non-Bt and MON810 maize was 42.4% and 3.2% respectively (Fig. 2.7). No larval survival occurred on MON89034 maize 3 days after the commencement of the experiment. Mean larval survival differed significantly between treatments on day 3 of the experiment (Table 2.4). Since no survival occurred on MON89034 on day 3 of the experiment, no statistical analysis was performed.

Larvae feeding on non-Bt maize had a rapid increase in mean larval mass between day 7 and day 14 of the experiment (Fig. 2.8). There was a significant difference in mean larval mass between non-Bt and MON 810 on day 3 of the experiment ($F_{(1,12)} = 35.15$; $P \leq 0.0001$) (Table 2.4). Since no survival occurred on MON89034 on day 3 of the experiment, no statistical analysis was performed.

Table 2.4. Mean larval mass and survival of *Spodoptera exempta* 1st instars on Bt and non-Bt maize.

Treatment	Mean larval mass (mg) (day 3)	Mean larval survival (%) (day 3)
MON810	0.375 a	14.73 a
Non-Bt	1.129 b	35.76 b
MON89034	-	-
	$p \leq 0.0001$	$p \leq 0.00001$

Means within columns followed by the same letter do not differ significantly at the indicated level of significance.

3rd instars

There was a significant treatment x time interaction for larval survival ($F_{(6,81)} = 3.19$; $P \leq 0.05$). On day 11 of the experiment, 3rd instar larval survival was 24.0% on non-Bt maize and 6.0% on MON810 maize. On day 11 of the experiment no survival of

3rd instar larvae were recorded on MON89034 maize (Fig. 2.9). Mean larval survival differed significantly between treatments on the last day of the experiment (Table 2.5). Since no survival occurred on MON89034 on day 11 of the experiment, no statistical analyses was performed.

Mean larval mass of larvae feeding on non-Bt had a rapid increase in the first three days of the experiment. Larvae feeding on MON89034 had a decrease in mean larval mass from the onset of the experiment (Fig. 2.10). There was a significant difference in mean larval mass between non-Bt and and the Bt events on day 3 of the experiment ($F_{(2,17)} = 10.94$; $P \leq 0.001$) (Table 2.5).

Table 2.5. Mean larval mass and survival of *Spodoptera exempta* 3rd instar larvae on Bt and non-Bt maize.

Treatment	Mean larval mass (mg) (day 3)	Mean larval survival (%) (day 11)
MON810	131.579 a	6.0 a
Non-Bt	273.960 b	24.0 b
MON89034	140.511 a	-
	$p \leq 0.001$	$p \leq 0.05$

Means within columns followed by the same letter do not differ significantly at the indicated level of significance.

2.5. Discussion

2.5.1. *Agrotis segetum*

According to Smit (1964), moths of *Agrotis segetum* lay their eggs on weeds and the larvae feed on the weeds until they reach the fourth instar. Blair (1975) also reported that larvae attacking maize seedlings are typically at their fourth or later instar of development. Therefore, first instar larvae were not used for the purposes of this study. Instead 1st instar larvae were reared on artificial diet and spinach to acquire larvae of uniform age and size (approximately at their fourth instar) to conduct the experiments.

Although there were no significant differences in percentage larval survival between non-Bt and Bt events of the Polokwane *A. segetum* population, there was a significant difference in mean larval mass. Mean larval mass of larvae feeding on non-Bt maize seedlings increased more rapidly and reached the pre-pupal stage earlier than larvae feeding on Bt events.

No significant difference in percentage larval survival for the Potchefstroom population of *A. segetum* was recorded between non-Bt and MON810, however, there was a significant difference between non-Bt and MON89034 expressing Cry2Ab2 + Cry1A.105. Not one of these Cry proteins being expressed by MON89034 target *A. segetum* (Monsanto, 2015).

According to Van Wyk *et al.* (2008), MON810 and Bt11 did not effectively control fourth instar larvae of *A. segetum*, although adverse effects on larval mass and fecundity were observed. For the results that were recorded in the Polokwane population, it seems that MON89034 will most likely also have no effect on *A. segetum*. Pilcher *et al.* (1997) reported that *A. ipsilon* feeding on Cry1Ab was not affected, even though high concentrations of Bt was expressed in the leaves.

Lambert *et al.* (1996) reported that Cry9Ca1 is the first insecticidal crystal protein with activity against cutworms. Cry9Ca1 is derived from *Bacillus thuringiensis* serovar *tolworthi* and it has insecticidal activity against a broad spectrum of lepidopteran pests, including members of the families Noctuidae, Plutellidae, Pyralidae and Sphingidae. Certain noctuid species such as *S. frugiperda* and *A. segetum* are insensitive to other insecticidal crystal proteins, therefore 12 000 isolates of *B. thuringiensis* were screened for activity against these members of the Noctuidae family (Lambert *et al.*, 1996). It was reported by Lambert *et al.* (1996) that Cry9Ca1 was very toxic to members of the Noctuidae family such as *S. exigua*, *S. frugiperda*, *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae) and *A. segetum*. Not only is *A. segetum* affected by Cry9Ca1, preliminary results showed activity against *A. ipsilon* as well. The discriminative spectrum of activity exhibited by Cry9Ca1 makes it a very appealing insecticidal crystal protein to control insect larvae either as sprays or through genetically engineered crop plants. De Maagd *et al.* (2003)

reported that *A. ipsilon* was the most susceptible to Cry9Ca, followed by Cry1Aa and Cry1Fb.

2.5.2. *Helicoverpa armigera*

Erasmus and Van den Berg (2014) reported that maize leaves are not a suitable food source for first instar larvae of *H. armigera*. Larvae of *H. armigera* show preference for feeding on maize ears, especially the silks. When silks are dried out, larvae penetrate the tips of the ears. Usually only one larva is found in a maize ear due to cannibalism (Matthee, 1974).

Results from this study indicate that the stacked Bt maize event, MON89034, has high enough concentrations of Cry proteins in the ears to control *H. armigera*. No larval survival was recorded on MON89034, 10 days after the commencement of the experiment. A significant difference was observed in percentage larval survival between non-Bt and MON810 (Cry1Ab) and Bt11 (Cry1Ab). Survival of *H. armigera* on these two Bt events occurred until the end of the experiment. A significant difference in mass was observed between larvae that fed on non-Bt and Bt events until the onset of the pre-pupal stage.

Erasmus and Van den Berg (2014) reported that larvae of *H. armigera* feeding on Bt maize ears had a significantly lower mean mass than larvae feeding on non-Bt maize ears and larval survival also differed significantly between non-Bt and the Bt event expressing Cry1Ab. A study conducted by Van Wyk *et al.* (2008) also indicated that larval survival of *H. armigera* does occur on Bt maize ears, expressing Cry1Ab, under field conditions, although the number of larvae found in Bt maize fields were always lower when compared to non-Bt maize fields.

Helicoverpa zea is not a target pest of Bt maize, but it occurs in the same maize ecosystems as *Ostrinia nubilalis*, a target pest of Bt maize in the U.S.A. (Pilcher *et al.*, 1997). Pilcher *et al.* (1997) reported that *H. zea* was not significantly affected by exposure to Bt maize when feeding on Bt maize silks expressing Cry1Ab. Fewer surviving larvae feeding on Bt maize silks were recorded. However, more surviving larvae of *H. zea* is found on Bt maize ears than the target pest *O. nubilalis*. Pilcher *et*

al. (1997) suggested that a higher concentration of Cry1Ab will be needed to control *H. zea* to the same extent as *O. nubilalis*.

A study by Archer *et al.* (2001) on damage caused to maize ears by *H. zea* on four Bt maize events (MON810, Bt176, Bt11 and CBH354), reported that none of these Bt maize hybrids provided consistent control against *H. zea* feeding on the kernels. Dowd (2001) indicated that even the feeding of *H. zea* was slowed down on Bt maize expressing high concentrations of Cry proteins in the ear and in the kernels. However, because the incidence of infestation is not affected, and larvae remained alive and eventually damaged an equivalent number of kernels as on non-Bt maize, the Bt maize is largely ineffective against *H. zea*.

With constitutive expression of Bt toxins through the entire plant for the duration of the growing season, the potential exists for Bt crops to have the highest selection pressure for resistance of any insecticide deployed to date (Storer *et al.*, 2003). Insecticidal activity of transgenic plants decrease significantly as the plants mature (Fitt *et al.*, 2004), especially in Bt maize expressing Cry1A (Brévault *et al.*, 2009). This makes it possible for some larvae of *H. armigera* to complete development later in the growing season (Van Wyk *et al.*, 2008).

2.5.3. *Spodoptera exempta*

According to Fitt *et al.* (2004), similar to other *Spodoptera* spp., Cry1A does not effectively control *S. exempta*, however the threat of resistance development is insignificant. In Puerto Rico, approval and cultivation of TC1507, expressing protein Cry1F, began in 2003. TC1507 maize, expressing the Cry1F protein was introduced in Puerto Rico in 1996 for experimental purposes, however, commercial cultivation of TC1507 began in 2003 (Storer *et al.*, 2010, 2012). TC1507 maize can control economically important lepidopteran pests, such as *S. frugiperda*, *O. nubilalis* and *D. grandiosella* (Storer *et al.*, 2010; Storer *et al.*, 2012). Reduced sensitivity or resistance against sprays and application products such as microbial Bt formulations are known. Storer *et al.* (2010) did a comparative study between populations collected in Puerto Rico and in the mainland United States and results showed that *S. frugiperda* from Puerto Rico had reduced sensitivity towards Cry1F. Results from

leaf disc bioassays conducted in the following season showed that Cry1F did not control *S. frugiperda*, nor did it inhibit feeding on any of the field collected populations from Puerto Rico. However, populations of *S. frugiperda* from mainland United States were highly susceptible to Cry1F (Storer *et al.*, 2012).

Results obtained from this study showed that Bt maize events MON810 and MON89034 can effectively control *S. exempta* 1st instar larvae within the first 7 days. However, 6.0% of 3rd instar larvae reached the pupal stage on day 11 of the experiment. The experiment with 3rd instar larvae of *S. exempta* was scientifically more representative of what would happen under field conditions, since larvae only migrate to feed on maize in their later instars. Due to this behavior of *S. exempta* there is a risk of resistance development on Bt maize only expressing Cry1Ab.

A study conducted by Dutton *et al.* (2002) showed that *S. littoralis* was only partially affected by Cry1Ab. Survival as well as larval growth was affected when larvae fed on Bt maize. Larvae feeding on Bt maize required more time to reach the second instar than larvae feeding on non-Bt maize. Chaufaux *et al.* (1997) also observed a strain of *S. littoralis* that was not susceptible to Cry1C and Cry1E, although showed susceptibility towards Cry1F.

Survival on non-Bt maize was unexpectedly low in both experiments. Two possible explanations can be that 1st instar larvae do not prefer to feed on maize and that larvae prefer to be aggregated or in high concentrations.

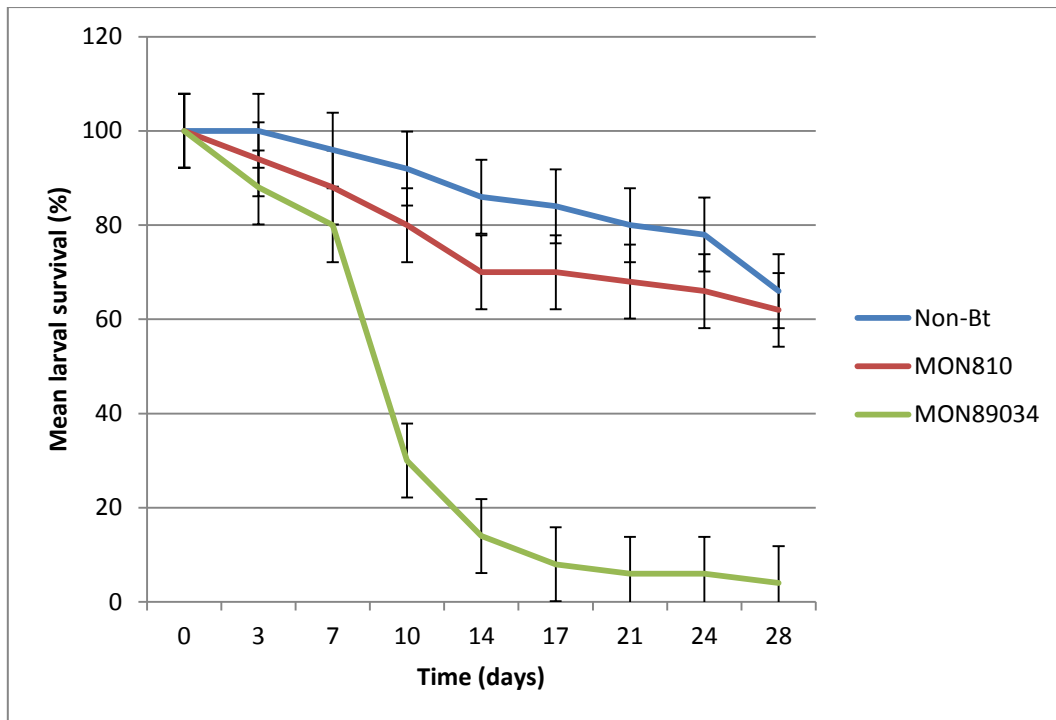


Figure 2.1. Mean percentage survival of *Agrotis segetum* (Potchefstroom population) larvae feeding on maize seedlings from 4th instar onwards (non-Bt, event MON810 and event MON89034) (Bars indicate SE).

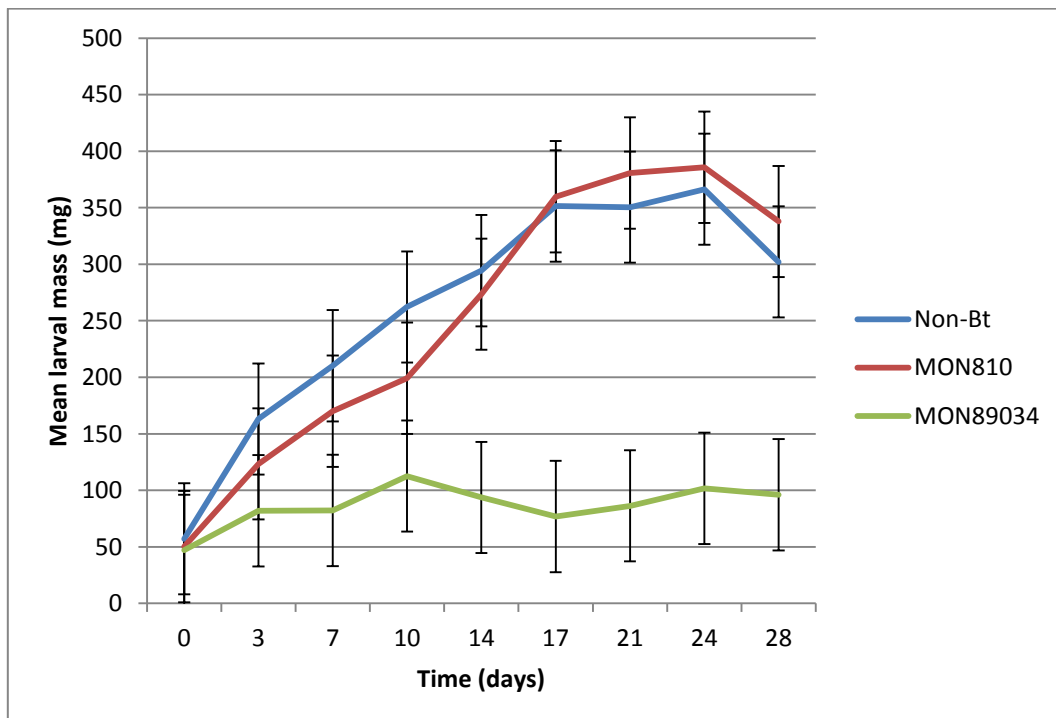


Figure 2.2. Mean larval mass of *Agrotis segetum* (Potchefstroom population) larvae feeding on maize seedlings from 4th instar onwards (non-Bt, event MON810 and event MON89034) (Bars indicate SE).

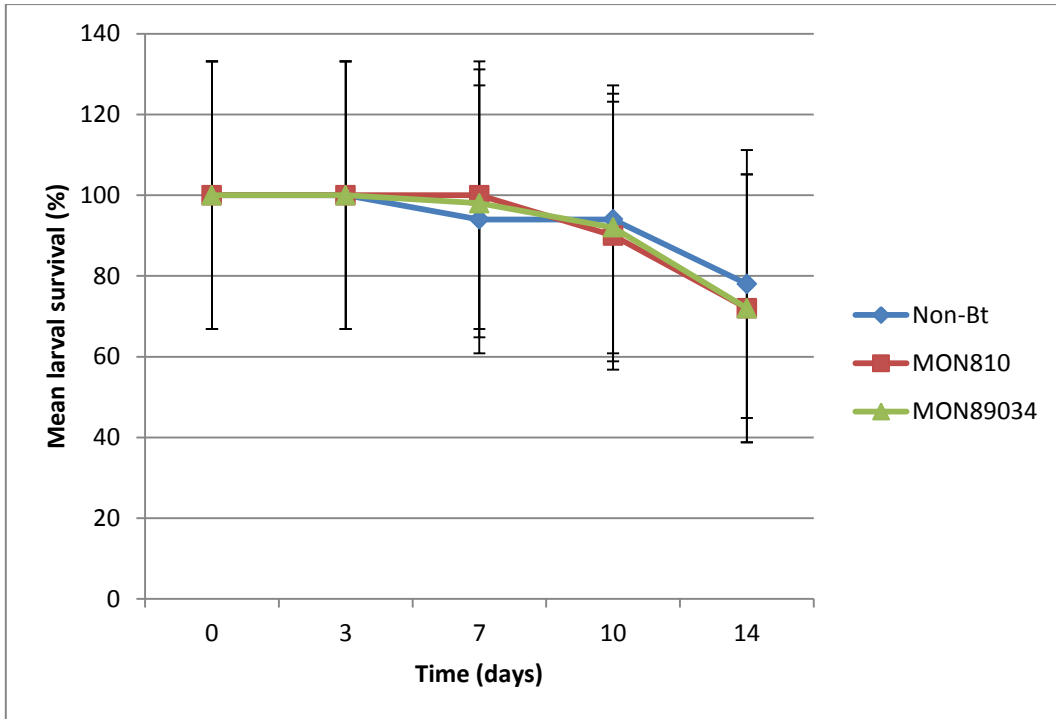


Figure 2.3. Mean percentage survival of *Agrotis segetum* (Polokwane population) larvae feeding on maize seedlings from 4th instar onwards (non-Bt, event MON810 and event MON89034) (Bars indicate SE).

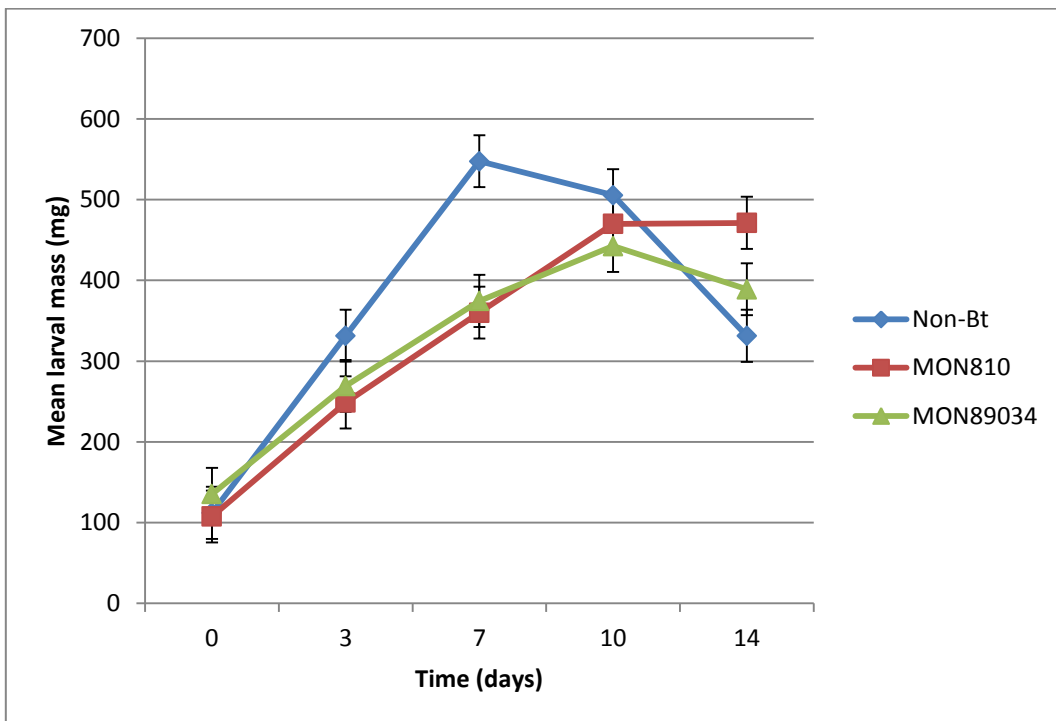


Figure 2.4. Mean larval mass of *Agrotis segetum* (Polokwane population) larvae feeding on maize seedlings from 4th instar onwards (non-Bt, event MON810 and event MON89034) (Bars indicate SE).

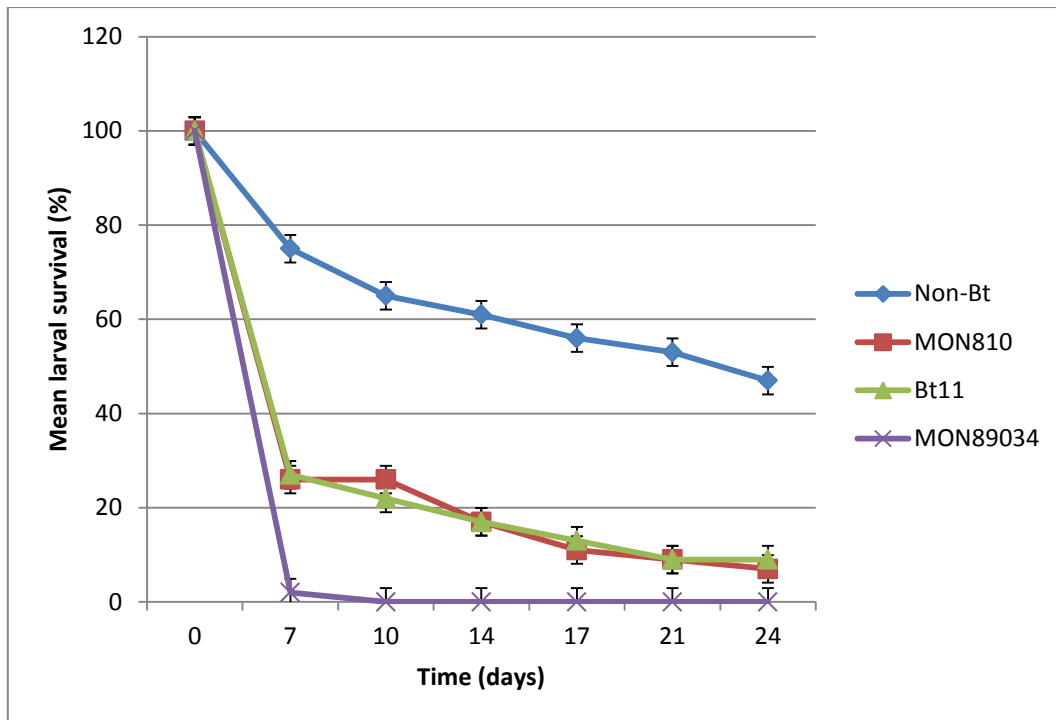


Figure 2.5. Mean percentage survival of *Helicoverpa armigera* larvae feeding on maize ears and silks from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

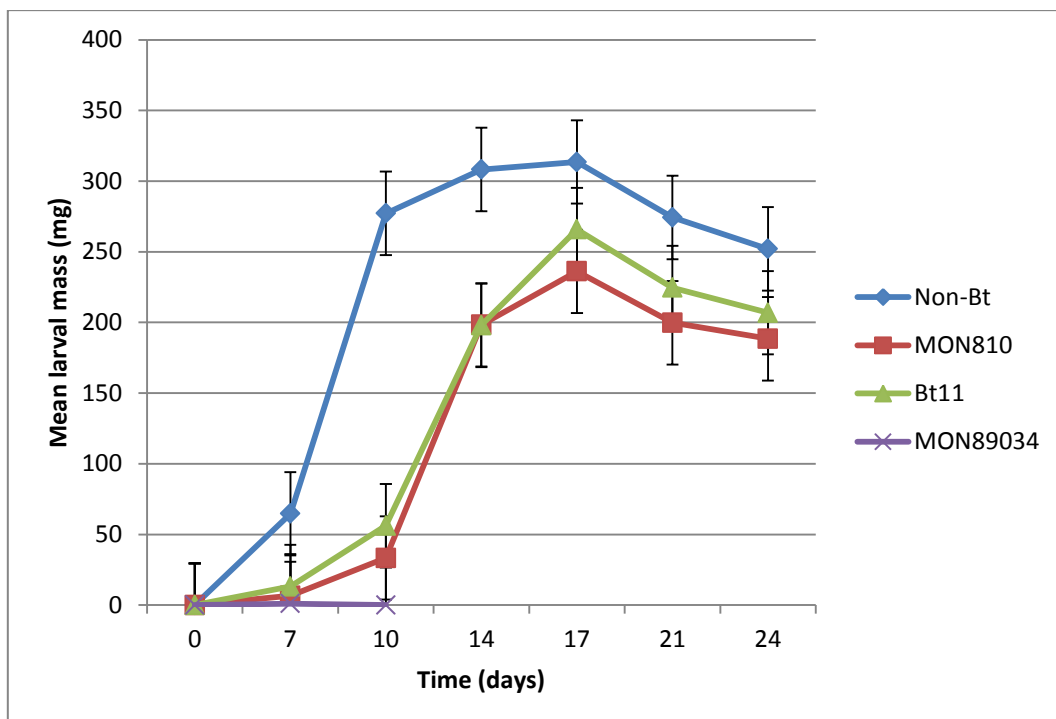


Figure 2.6. Mean larval mass of *Helicoverpa armigera* larvae feeding on maize ears and silks from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

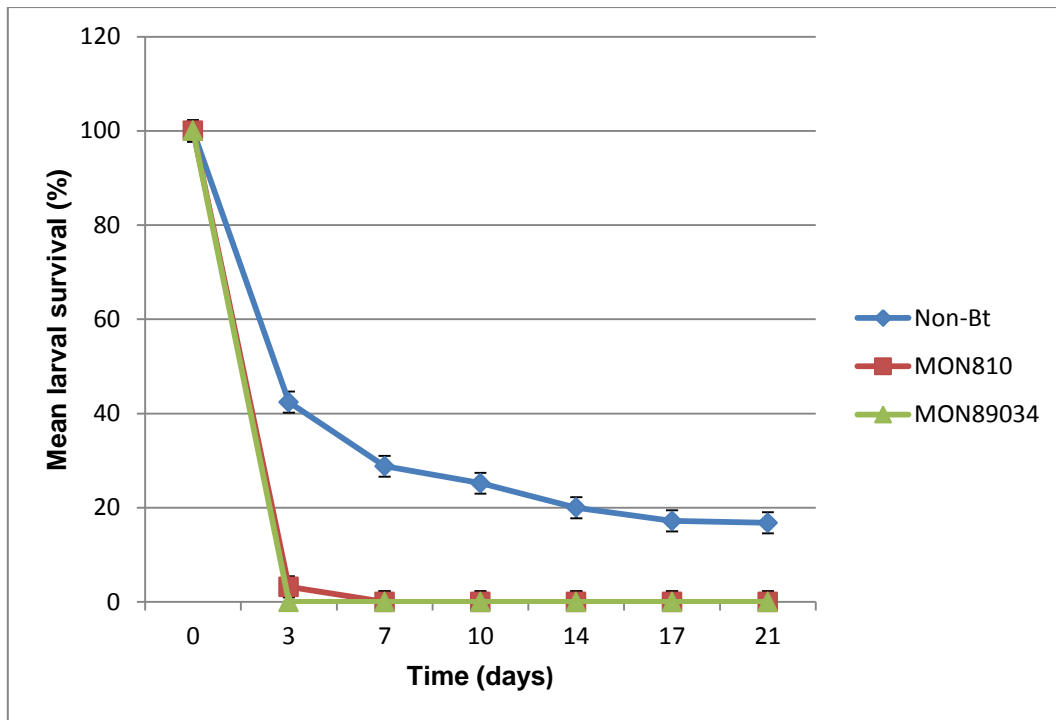


Figure 2.7. Mean percentage survival of *Spodoptera exempta* larvae feeding on maize leaves from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

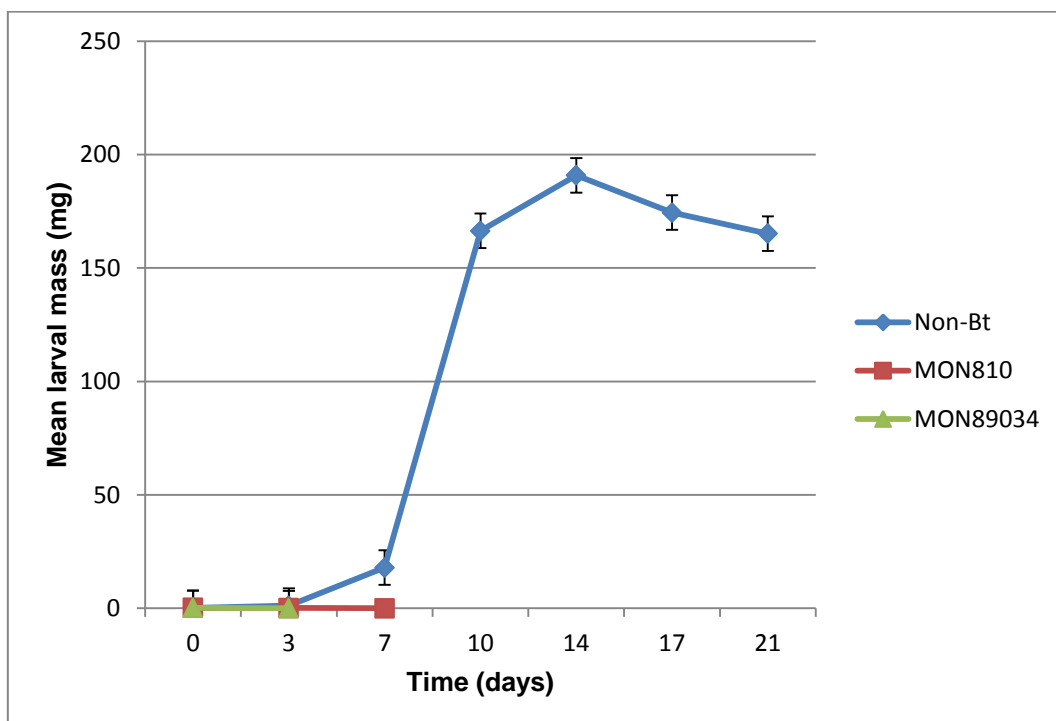


Figure 2.8. Mean larval mass of *Spodoptera exempta* larvae feeding on maize leaves from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

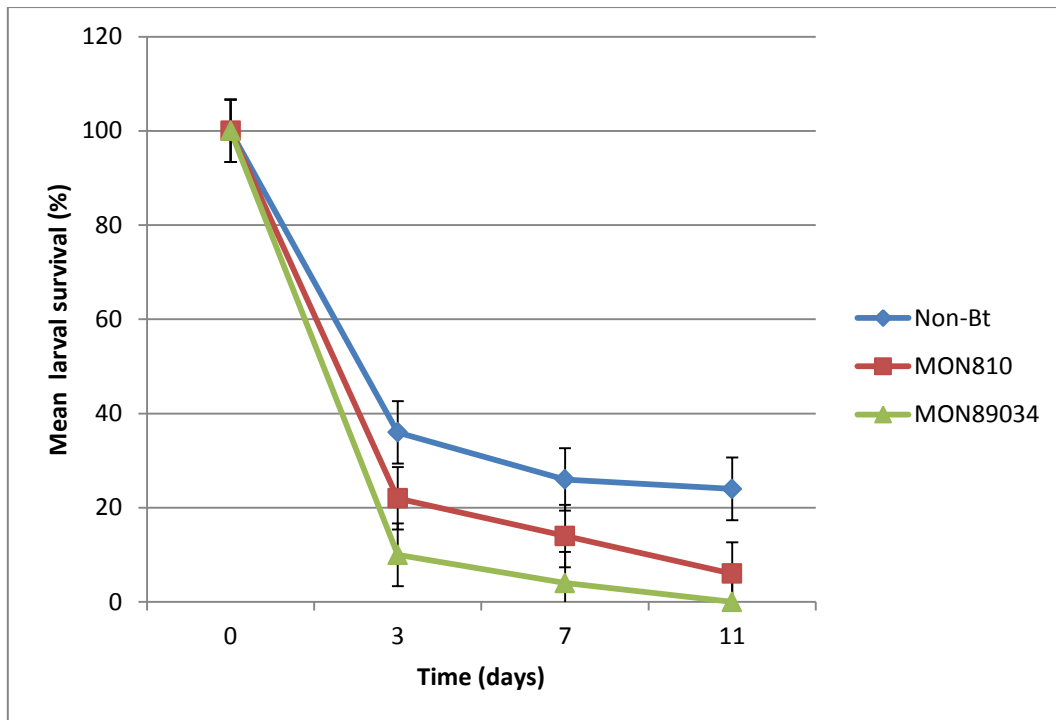


Figure 2.9. Mean percentage survival of *Spodoptera exempta* larvae feeding on maize leaves from 3rd instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

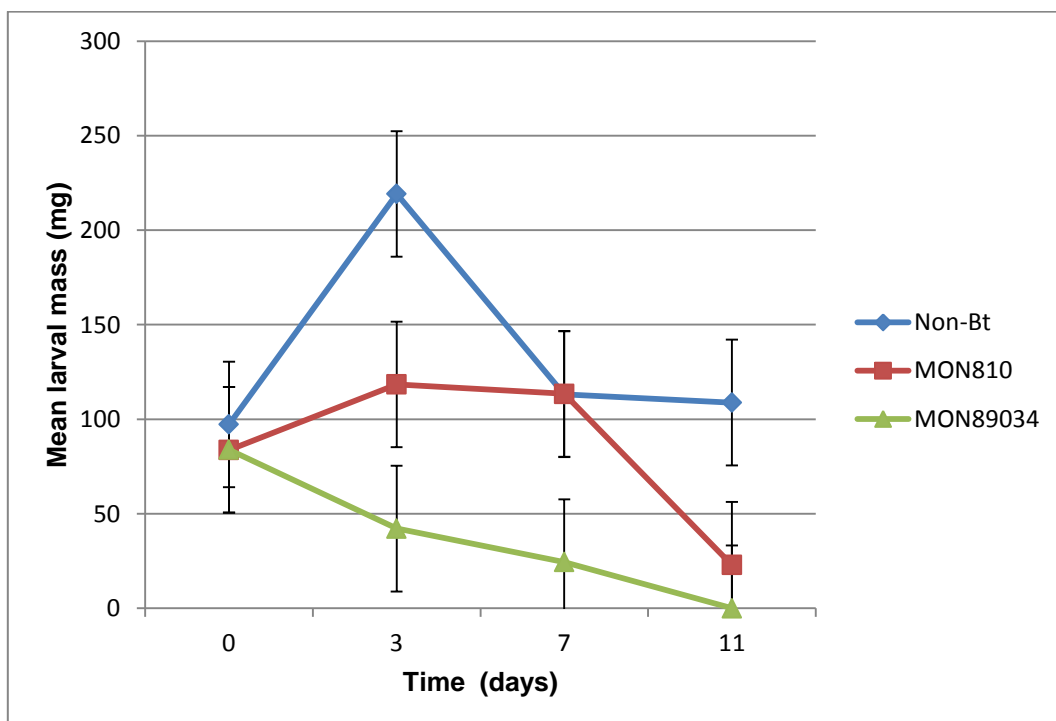


Figure 2.10. Mean larval mass of *Spodoptera exempta* larvae feeding on maize leaves from 3rd instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

CHAPTER 3: The efficacy of Bt maize against the lepidopterous stem borers *Chilo partellus*, *Eldana saccharina* and *Sesamia calamistis*

3.1. Abstract

The African maize stem borer, *Busseola fusca*, developed resistance to Bt maize expressing the Cry1Ab protein. *Busseola fusca* and three other stem borer species, *Chilo partellus*, *Eldana saccharina* and *Sesamia calamistis* limit the attainable maize yield throughout Africa. It is therefore important to screen these pest species to determine their susceptibility to Bt maize in South Africa. Since Bt maize will be approved for cultivation in many African countries over the next decade it is important to know which Bt maize events control these pest species and to assess their inherent tolerance to Bt maize. In this study, larval growth and survival were determined in laboratory bioassays with three Bt maize hybrids and a non-Bt, isoline hybrid for each pest species. Two experiments were conducted with each of *C. partellus*, *E. saccharina* and *S. calamistis*. The three Bt maize events that were used were MON810 (Cry1Ab), Bt11 (Cry1Ab) and MON89034 (Cry2Ab2 + Cry1A.105). Plastic containers with plant tissue were inoculated with 1st instar larvae and larval mass and survival was recorded at 3 – 4 day intervals. The *C. partellus* population from the Potchefstroom area did not survive on MON810 maize after 7 days. Survival on Bt11 maize was 0.4% on day 7 and no survivors were recorded on day 10. Fourteen days after the onset of the experiment no survival occurred on MON89034 maize. No *C. partellus* larvae from the Vaalharts area survived longer than 7 days on any of the Bt maize events. In the one experiment with *E. saccharina*, where larvae fed on maize ears, larvae survived up to the pupal stage on all the Bt maize events. When larvae fed on maize whorls, no larval survival was recorded on day 10 for larvae feeding on MON810 and Bt 11, however, on the same day 2.8% survival occurred on MON89034 maize. No *S. calamistis* larvae feeding on MON89034 maize ears survived longer than 21 days after the onset of the experiment. Survival of larvae feeding on maize ears of MON810 and Bt11 21 days after inoculation were 40.7% and 30.0%, respectively. When *S. calamistis* larvae fed on maize whorls, no survival was recorded on any of the Bt events 7 days after the onset of the experiment. In this study, larvae was effectively controlled when fed

maize whorls but not maize ears expressing Cry protein, possibly showing that Cry protein expression is lower in maize ears.

3.2. Introduction

The four most important stem borer species that infest maize in Africa are *Busseola fusca* (Lepidoptera: Noctuidae), *Chilo partellus* (Lepidoptera: Crambidae), *Eldana saccharina* (Lepidoptera: Pyralidae) and *Sesamia calamistis* (Lepidoptera: Noctuidae). *Sesamia calamistis* occurs throughout sub-Saharan Africa (Kfir *et al.*, 2002). *Chilo partellus* is mainly present in the eastern and southern parts of Africa, while *E. saccharina* occurs throughout southern, western and eastern Africa and on some of the neighbouring islands. All of these abovementioned stem borer species may seriously limit the potential attainable yield of maize (Kfir *et al.*, 2002).

Busseola fusca developed resistance to maize expressing Cry1Ab in South Africa (Van Rensburg, 2007). It is therefore necessary to screen other stem borer species such as *C. partellus*, *E. saccharina* and *S. calamistis* for their susceptibility to Bt maize events and to establish base-line data on their susceptibility. These three pests of maize come from three different lepidopteran families. Except for *B. fusca*, there are three other lepidopteran pests that have developed resistance to Bt crops. *Spodoptera frugiperda* (Lepidoptera: Noctuidae) developed resistance to Bt maize in Puerto Rico (Storer *et al.*, 2010, 2012), *Heliiothis zea* (Lepidoptera: Noctuidae) to Bt cotton in the south-eastern parts of the U.S.A. (Luttrell *et al.*, 2004; Ali *et al.*, 2006) and *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) to Bt cotton in India (Dhurua and Gujar, 2011).

Van Rensburg (2001) reported survival of *B. fusca* larvae feeding on different plant tissue other than maize whorl. According to Dutton *et al.* (2003), concentration of Cry protein varies throughout the different plant tissue and different Bt maize hybrids expressing the same Cry protein may sometimes express different concentrations of Cry protein in the same plant tissue, due to different promoters being used.

The aim of this study was to evaluate the efficacy of currently available Bt maize events against the lepidopterous stem borers *C. partellus*, *E. saccharina* and *S. calamistis*.

3.3 Material and methods

The Bt maize events that were used for this study were MON810 (expressing Cry1Ab toxin), Bt11 (expressing Cry1Ab toxin) and MON89034 (expressing Cry2Ab2 + Cry1A.105 toxin) as well as a non-Bt isolate.

3.3.1 *Chilo partellus*

Larvae were collected from maize fields from two different geographical regions i.e. the Potchefstroom area (26° 39 ` S, 27°07 ` E) in the North West province and in the Vaalharts irrigation scheme (27° 48 ` S, 24° 46 ` E) in the Northern Cape province. Larvae from both of these collection sites were kept separate. The larvae were reared on artificial diet (chickpea based agar diet) until pupation. Moths that emerged from the pupae were allowed to lay eggs. Offspring of these field collected larvae were used in this laboratory bioassay for each population. The experimental methods used in this study remained the same for both the populations. Larval survival and mass were determined on Bt and non-Bt maize whorl tissue. The experimental lay-outs were completely randomised.

Maize whorl tissue was placed in sealable aerated containers (5 × 4cm). Filter paper was placed at the bottom of each container to reduce accumulation of unnecessary moisture. There were 10 replicates, consisting of 5 containers per replicate. Five 1st instar larvae were inoculated in each container by means of a camel hair brush. Containers were kept in an incubator at 26°C and 60% relative humidity and a 14L:10D photoperiod. Larval survival and mass were determined seven days after inoculation, followed by assessments at 3 – 4 day intervals. Maize whorls and filter paper were replaced upon determining larval mass and survival. Larval survival was determined until the pupal stage was reached.

3.3.2 *Eldana saccharina*

Eggs were obtained from the South African Sugarcane Research Institute (SASRI) at Mount Edgecombe, KwaZulu-Natal. The larvae that emerged were used in laboratory bioassays. Two experiments were conducted with *E. saccharina*. The first experiment involved 1st instar larvae feeding on maize ear tips with 4 replicates per treatment. The second experiment involved 1st instar larvae feeding on maize whorls with 10 replicates per treatment. Each replicate consisted of 5 containers. Larval survival and mass were determined on Bt and non-Bt maize plant material. The experimental lay-outs were completely randomised.

Maize plant material was placed in sealable aerated containers (5 × 4cm). The bottom of the container was lined with filter paper to reduce the accumulation of moisture. Five 1st instar larvae were inoculated in each container by means of a camel hair brush. Containers were kept in an incubator at 26°C and 60% relative humidity and a 14L:10D photoperiod. Larval survival and mass was determined seven days after inoculation, followed by assessments 3 – 4 day intervals until pupation. Maize plant material and filter paper were replaced upon determining larval mass and survival.

3.3.3 *Sesamia calamistis*

Eggs were obtained from the South African Sugarcane Research Institute (SASRI) at Mount Edgecombe, KwaZulu-Natal. The larvae that emerged were used in laboratory bioassays. Two experiments were conducted with *S. calamistis*. The first experiment involved 1st instar larvae feeding on maize ear tips with 6 replicates per treatment. Each container was inoculated with 5 larvae. The second experiment involved 1st instar larvae feeding on maize whorl with 4 replicates per treatment. Each replicate consisted of 5 containers. Larval survival and mass were determined on Bt and non-Bt maize plant material. The experimental lay-outs were completely randomised.

Maize plant material was placed in sealable aerated containers (5 × 4cm). The bottom of the container was lined with filter paper to reduce the accumulation of

moisture. Five 1st instar larvae were inoculated in each container by means of a camel hair brush. Containers were kept in an incubator at 26°C and 60% relative humidity and a 14L:10D photoperiod. Larval survival and mass were determined seven days after inoculation, followed by assessments at 3 – 4 day intervals until the pupal stage was reached. Maize plant material and filter paper were replaced upon determining larval mass and survival.

3.3.4 Data analyses

Data on larval mass and survival were analysed by means of Statistica. Data on larval survival was done by means of repeated measures ANOVA. One-way ANOVA's were also calculated using survival collected on the last day of the experiment, and data on larval mass, on the day that pre-pupae were observed for the first time.

3.4. Results

3.4.1. *Chilo partellus*

Potchefstroom population

There was a significant treatment x time interaction for larval survival ($F_{(30,360)} = 180.06$; $P \leq 0.05$). Due to very low or no survival on any Bt event after 7 days, no further statistical analyses were done. A rapid decline in percentage survival was observed in all Bt events between the onset of the experiment and day 7 (Fig. 3.1). After a period of 7 days, larval survival on non-Bt maize was 88.0%, 0.0% (MON810), 0.4% (Bt11) and 0.8% (MON89034). Larval survival on MON89034 maize decreased between day 7 and day 10 to 0.4% and on day 14 no survival was observed. On day 17, larval survival on non-Bt maize was 50% and on day 38 it was 35.6%

A slight increase in larval mass was observed between the onset of the experiment and day 7 for Bt11 and MON89034. Between day 7 and 10 larval mass decreased on MON89034 (Fig 3.2). Mean larval mass on non-Bt maize showed a rapid increase between day 10 and 24, followed by a gradual decrease due to the onset of the pre-pupal stage. Since no survival occurred on MON810 and very little survival on Bt11 and MON89034 seven days after the commencement of the experiment, no statistical analysis was performed.

Vaalharts population

There was a significant treatment x time interaction for larval survival ($F_{(30,360)} = 92.40$; $P \leq 0.05$). No survival was recorded on any of the Bt events 7 days after inoculation (Fig 3.3). Due to very low or no survival on any Bt event after 7 days, no further statistical analyses were done. Percentage larval survival on non-Bt maize was 78.4% on day 7 and 32.8% when the experiment was concluded on day 38.

No larval mass was recorded on any of the Bt events, due to 100% mortality occurring before the 1st 7 day assessment (Fig. 3.4). A rapid increase in mean larval mass was observed on non-Bt maize between day 10 and 24. Since no survival occurred on any of the Bt events 11 days after the commencement of the experiment, no statistical analyses was performed on larval mass.

3.4.2. *Eldana saccharina*

Feeding on maize ears

There was a significant treatment x time interaction for larval survival ($F_{(30,120)} = 6.30$; $P \leq 0.0001$). Ten days after the commencement of the experiment, survival was 78.0% on non-Bt maize, 46.0% on MON810, 20.0% on Bt11 and 27.0% on MON89034 maize (Fig. 3.5). The mean percentage of surviving larvae on non-Bt, 38 days after inoculation was 36.0%, 8.0% (MON810), 3.0% (Bt11) and 6.0% (MON89034). Survival on Bt11 was the lowest throughout the experiment. Mean

larval survival differed significantly between treatments on the last day of the experiment (Table 3.1)

There was a significant difference in mean larval mass between treatments after 28 days ($F_{(3,11)} = 6.57$; $P \leq 0.05$). Larvae feeding on Bt events had lower mean mass than larvae feeding on non-Bt, especially Bt11 and MON89034 (Fig. 3.6). However, when the larvae started changing into pre-pupae on the non-Bt, a decrease in mass was observed and the mean mass of non-Bt and MON810 were almost equal at the end of the experiment. Mean larval mass differed significantly on day 28 of the experiment (Table 3.1).

Table 3.1. Mean larval mass and survival of *Eldana saccharina* on Bt and non-Bt maize ears.

Treatment	Mean larval mass (mg) (day 28)	Mean larval survival (%) (day 38)
Bt11	0.056 a	3.0 a
MON89034	0.069 a	6.0 a
MON810	0.070 ab	8.0 a
Non-Bt	0.121 b	36.0 b
	$p < 0.05$	$p < 0.0001$

Means within columns followed by the same letter do not differ significantly at the indicated level of significance.

Feeding on maize whorl tissue

There was a rapid decrease in larval survival on all Bt events within the first 10 days of the experiment. There was a significant treatment x time interaction for larval survival ($F_{(42,504)} = 129.37$; $P \leq 0.05$). Ten days after commencement of the experiment no surviving larvae were recorded on MON810 and Bt11 and the mean percentage survival of MON89034 was 2.8%, with the mean percentage of surviving larvae on non-Bt maize being 71.6% (Fig. 3.7). Mean larval survival differed significantly between treatments on the last day of the experiment (Table 3.2)

Larvae feeding on Bt events only had a slight increase in mean mass 7 days after inoculation. A decrease in mean mass was recorded for MON89034 between day 7 and 10 (Fig 3.8). Larval mass on non-Bt plants showed a rapid increase between day 17 and 28, followed by a gradual decrease from day 38 onwards due to the onset of the pre-pupal stage. There was a significant difference in mean larval mass on day 7 of the experiment ($F_{(3,30)} = 36.35$; $P \leq 0.0001$) (Table 3.2).

Table 3.2. Mean larval mass and survival of *Eldana saccharina* on Bt and non-Bt maize leaf whorls.

Treatment	Mean larval mass (mg) (day 7)	Mean larval survival (%) (day 7)
Bt11	0.100 a	3.2 a
MON810	0.171 a	4.8 a
MON89034	0.388 a	16.4 b
Non-Bt	1.412 b	81.9 c
	$p \leq 0.00001$	$p \leq 0.001$

Means within columns followed by the same letter do not differ significantly at the indicated level of significance.

3.4.3. *Sesamia calamistis*

Feeding on maize ears

No survival for *S. calamistis* feeding on the stacked Bt event MON89034 was recorded 21 days after inoculation. Mean percentage of surviving larvae on MON810 and Bt11 21 days after inoculation were 40.7% and 30.0%, respectively (Fig 3.9). There was a significant treatment x time interaction for larval survival ($F_{(33,220)} = 10.27$; $P \leq 0.0001$). Mean percentage of surviving larvae on the non-Bt event was 41.3%, 42 days after inoculation, whereas, MON810 had 18.7% survival and Bt 11 16.0% survival. Mean larval survival differed significantly between treatments on the last day of the experiment (Table 3.3). Since no survival occurred on MON89034 on day 42 of the experiment, no statistical analyses was performed.

There was a significant difference in mean larval mass between treatments ($F_{(2,15)} = 2.16$; $P = 0.15$) (Table 3.3). Larval mass on MON89034 decreased between 7 and 18 days after inoculation until there were no surviving larvae. Larval mass for the non-Bt event as well as MON810 and Bt11 events had a rapid increase from day 14 to day 28 (Fig 3.10). A decrease in larval mass was observed for the non-Bt event and Bt11 between day 28 and day 42. Larvae feeding on MON810 had a mass decrease from 39 to 42 days after inoculation. This decrease in mass was a result of larvae changing into pre-pupae on the non-Bt and Bt event. MON89034 was not included in the statistical analyses, due to no survival on day 25 of the experiment.

Table 3.3. Mean larval mass and survival of *Sesamia calamistis* on Bt and non-Bt maize ears.

	Mean larval mass (mg) (day 25)	Mean larval survival (%) (day 42)
Bt11	0.113 a	16.0 a
MON810	0.117 a	18.67 a
Non-Bt	0.138 a	41.33 b
MON89034	-	-
	$p = 0.15$	$p < 0.001$

Means within columns followed by the same letter do not differ significantly at the indicated level of significance.

Feeding on maize whorl tissue

A rapid decrease in larval survival was observed on MON810, Bt11 and MON89034 (Fig 3.11). There was a significant treatment x time interaction for larval survival ($F_{(24,96)} = 149.53$; $P \leq 0.0001$). No larval survival occurred on any of the Bt events 7 days after inoculation. Due to very low or no survival on any Bt event after 7 days, no further statistical analyses were done. Mean percentage of surviving larvae on non-Bt was 90% after 7 days and 58% after 32 days.

At the first interval where mass and survival was determined (day 7), there was no survival on any of the Bt events, thus no mass was recorded (Fig 3.12). A rapid increase in larval mass was recorded between day 11 and 21 on the non-Bt maize tissue followed by a decrease in larval mass from day 21 to 32 when larvae started forming pre-pupae. Since no survival occurred on any of the Bt events 7 days after the commencement of the experiment, no statistical analyses was performed on larval mass.

3.5. Discussion

3.5.1. *Chilo partellus*

Bt maize was initially developed for control of two North American lepidopteran stem borers. These stem borers are *Diatraea grandiosella* (Dyar) (Lepidoptera: Crambidae) (Archer *et al.*, 2001) and *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) (Ostlie *et al.*, 1997). In South Africa, the target pests of Bt maize are the lepidopteran stem borers *Busseola fusca*, *Chilo partellus*, and *Sesamia calamistis* (Van Rensburg, 1999; Van Wyk *et al.*, 2008; Erasmus *et al.*, 2010).

Van Rensburg (1999), conducted a study on *B. fusca* (Fuller) (Lepidoptera: Noctuidae) and *C. partellus* to evaluate the efficacy of Bt maize event MON810 against these pests. Results showed that *C. partellus* was more susceptible than *B. fusca* to Bt maize. Andow *et al.* (2004) also observed that *C. partellus* and *Chilo orichalcociliellus* (Strand) (Lepidoptera: Crambidae) were highly susceptible to event 176 (Cry1Ab) and event 1835 (Cry1B).

Two studies were conducted by Singh *et al.* (2005) to determine if *C. partellus* is tolerant to Cry1Ab expressed in event MON810. The insecticidal activity of Cry1Ab expressed in MON810, against first instar larvae of *C. partellus*, was assayed using the diet-incorporation method. Solubilised Cry1Ab was serially diluted to 7 concentrations in a general lepidopteran diet. This bioassay was done to determine the lethal concentrations (LC₅₀ and LC₉₀) and second instar moult inhibitory concentrations (MIC₅₀ and MIC₉₀). Cry1Ab had significant activity against the first

instar larvae of *C. partellus*. The mean LC₅₀ and LC₉₀ were determined to be 0.116 and 2.770 parts per million (ppm) respectively, and the mean MIC₅₀ and MIC₉₀ were 0.058 and 0.804 ppm. The second study was a greenhouse evaluation of MON810 maize with non-Bt maize as control. Plants were infested with *C. partellus*, and when the plants were harvested 20 days later, no larvae were obtained from the Bt maize plants.

The results obtained from this experiment showed that first instar larvae of *C. partellus* is highly susceptible to the Bt maize events tested. *Chilo partellus* from the Potchefstroom population had an almost insignificant percentage of larval survival on all the Bt events and a significant difference was observed between the mean mass of larvae feeding on non-Bt and Bt events. *Chilo partellus* from the Vaalharts population, feeding on Bt events, had no survival 7 days after the commencement of the experiment.

The Vaalharts population was chosen for the experiment to see whether or not there is resistance development of *C. partellus* to the selected Bt maize events, because it is in relative close proximity to where *B. fusca* first developed resistance to Cry1Ab expressed by event MON810 (Van Rensburg, 2007).

3.5.2. *Eldana saccharina*

A study was conducted in Kenya on the efficacy of Bt maize against *E. saccharina* using event 176 (Cry1Ab), event 1835 (Cry1B), event 5207 (Cry1Ac), Event 5601 (Cry1B) and event 7 (Cry1Ab). Results from this study showed that the susceptibility levels were very low and the 1st instar larvae were not significantly affected by any of the events. These events were therefore not investigated or developed further in Kenya after 2001 (Andow *et al.* 2004).

Keeping *et al.* (2007) conducted a study on *E. saccharina* using the 'whole plant' methodology (Birch *et al.*, 2004). Bt maize expressing Cry1Ab was used as a potential trap crop for this pest. Significant differences were recorded between Bt and non-Bt for all variables relating to plant damage, percentage larval survival and

mean larval mass. At the trial harvest, a total of 100 larvae were recovered from non-Bt and only three were recovered from Bt plants.

The results obtained in this study on larvae feeding on maize whorls, showed that *E. saccharina* was highly susceptible to the Bt maize events. No survival occurred on any of the Bt events 14 days after inoculation and only slight increases in mean larval mass was observed for larvae feeding on Bt maize during the experiment.

Larval feeding on maize silks resulted in significant differences in percentage larval survival between non-Bt and the Bt events. Surprisingly, the stacked Bt event performed poorer than Bt11 for *E. saccharina*. Bt11 was also significantly more effective than MON810. This cannot be explained but indicates that Bt11 maize may be of use in areas such as western Africa where this pest attacks maize. No significant differences were observed in mean larval mass between non-Bt and MON810 (Cry1Ab) and MON89034 (Cry2Ab2 + Cry1A.105), however, between non-Bt and Bt11 (Cry1Ab) there was a significant difference in mean larval mass.

According to Dutton *et al.* (2003), there are numerous commercial transgenic maize hybrids and different promoters have been used within these different maize hybrids. It has been shown that concentrations of the toxin may differ in other plant tissues.

3.5.3. *Sesamia calamistis*

According to Dutton *et al.* (2003), “Expression of Bt toxins in maize is often cited in literature to be constitutive, meaning that expression occurs in all tissues at all times”, and that is misleading, because there are various commercial transgenic maize hybrids and different promoters have been used in these hybrids. Within these different hybrids it has been shown that toxin concentrations in different plant tissues may vary (Dutton *et al.*, 2003). An example of the abovementioned is MON810 and Bt11 (Cry1Ab) that contain and is regulated by the cauliflower mosaic virus (CaMV) 35s promoter, also known as the enhanced 35s promoter (e35s) (EPA, 2000; Andow *et al.*, 2004), whereas there are two promoters present in MON89034. The Cry2Ab2

expression is regulated by the figwort mosaic virus (FMV) and the Cry1A.105 expression is regulated by the enhanced 35s (e35s) promoter (EPA, 2010).

Van den Berg and Van Wyk (2007) reported that *S. calamistis* is highly susceptible to MON810 (Cry1Ab). Furthermore, a study was conducted by Van Wyk *et al.* (2008), that compared the susceptibility of *S. calamistis* to MON810 (Cry1Ab) and Bt11 (Cry1Ab). Results obtained indicated that the larvae of *S. calamistis* was just as highly susceptible to Bt11 (Cry1Ab) as to MON810 (Cry1Ab), with no larval survival 7 days after the commencement of the experiment.

Results from this study indicate that *S. calamistis* feeding on maize whorls, was highly susceptible to the stacked Bt event, MON89034 as well as MON810 and Bt11, with no larval survival occurring 7 days after commencement of the experiment. Survival of *S. calamistis* feeding on maize silks was also significantly higher on non-Bt than Bt events. However, low survival occurred on MON810 and Bt11 at day 42, which was the end of the experiment. Survival was observed on MON89034 until 21 days after commencement of the experiment. A significant difference in mean larval mass was observed between non-Bt and the two Bt events expressing Cry1Ab (MON810 and Bt11), until larvae feeding on non-Bt started forming pre-pupae.

Sesamia calamistis was highly susceptible to the Bt events. MON810 is also highly effective against certain other stem borers, such as *E. saccharina* and *C. partellus* (Van Rensburg, 2001; Singh *et al.*, 2005; Keeping *et al.*, 2007). According to Van Rensburg (2001), larval survival of *B. fusca* was affected by the plant part they feed on. Protein expression during vegetative stages of plant development was sufficient when larvae fed only on leaf and stems of maize plants, however, larvae of *B. fusca* survived when they fed on maize silks. Other noctuid species that are not highly susceptible to Bt maize when feeding on maize silks or maize ears is *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Archer *et al.*, 2001; Andow and Hillbeck, 2004).

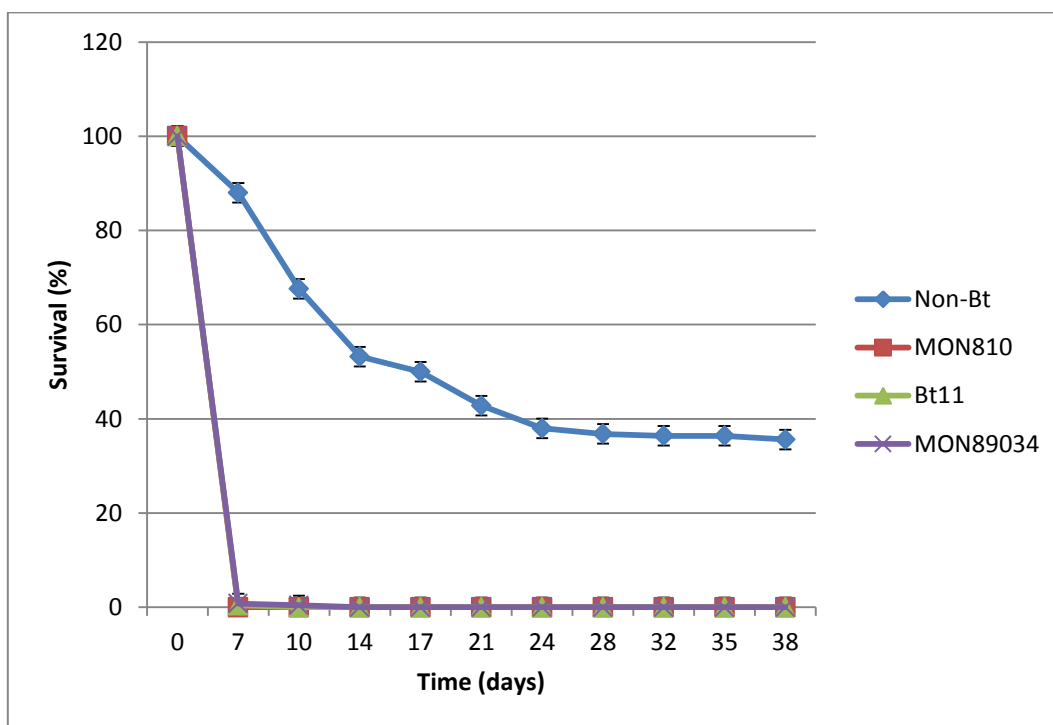


Figure 3.1. Mean percentage survival of *Chilo partellus* (Potchefstroom population) larvae feeding on maize whorls from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

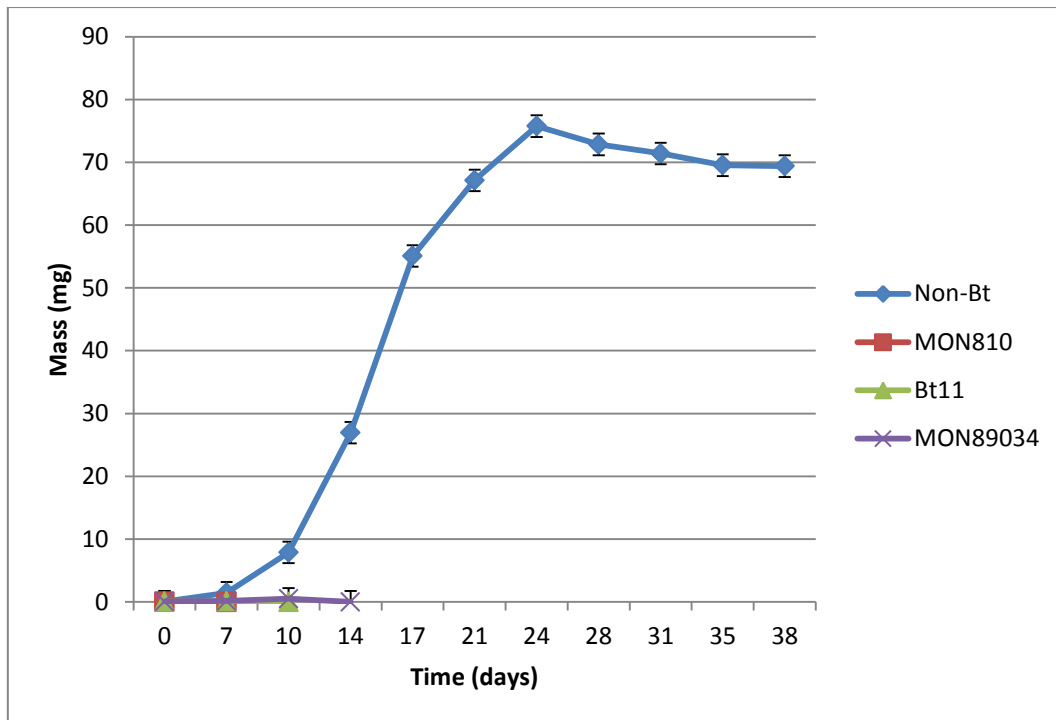


Figure 3.2. Mean larval mass of *Chilo partellus* (Potchefstroom population) larvae feeding on maize whorls from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

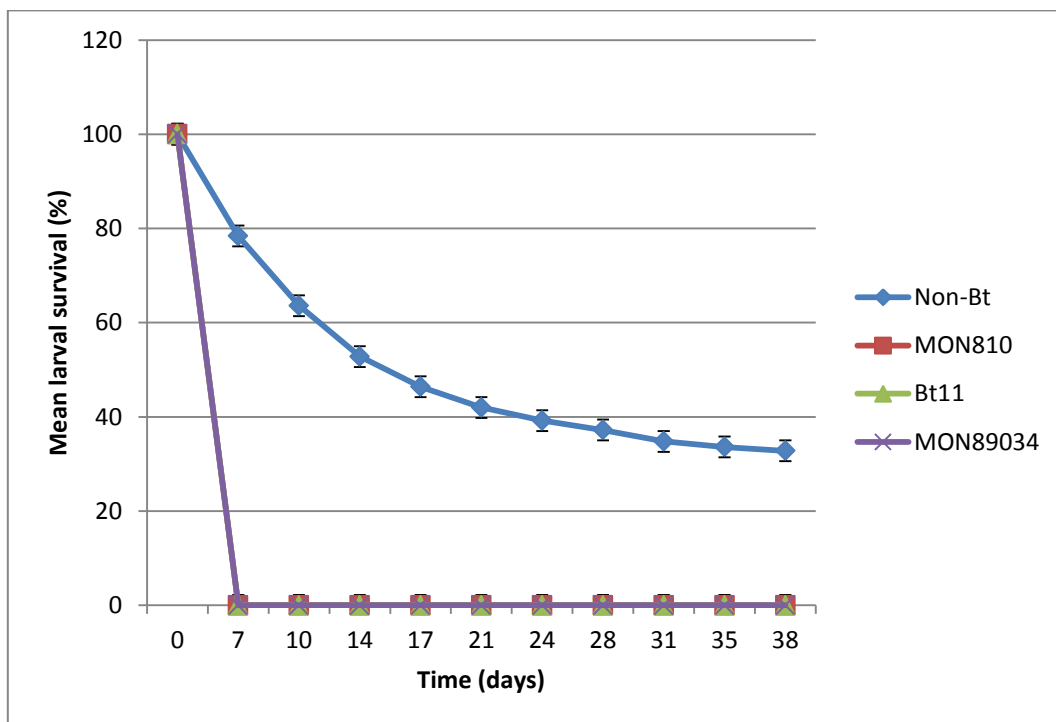


Figure 3.3. Mean percentage survival of *Chilo partellus* (Vaalharts population) larvae feeding on maize whorls from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

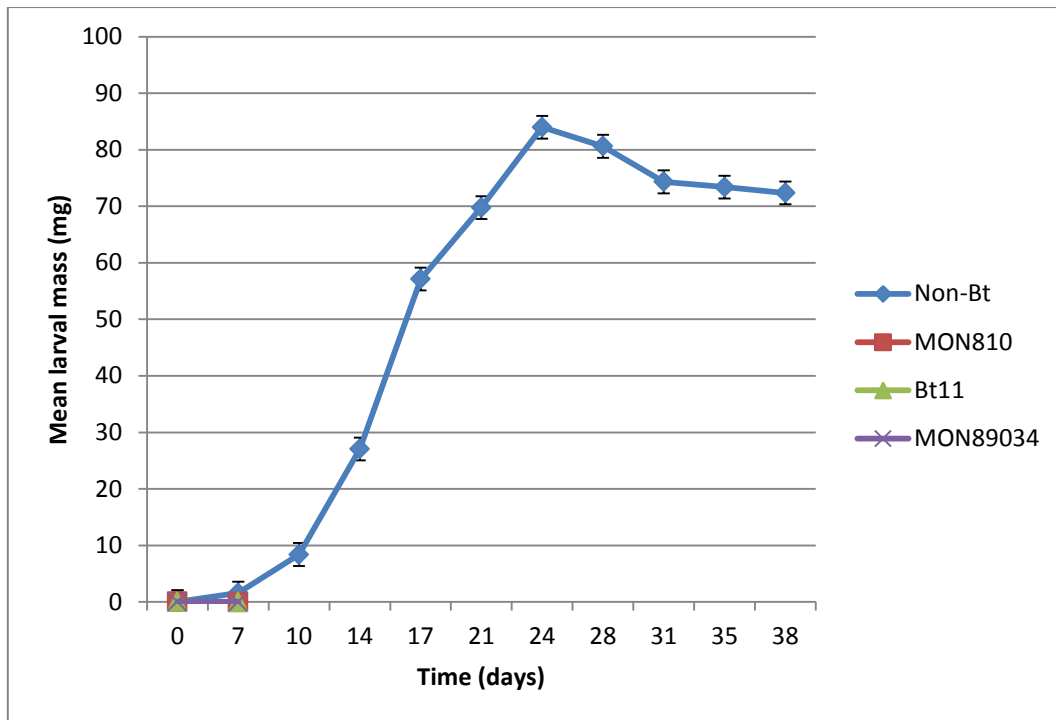


Figure 3.4. Mean larval mass of *Chilo partellus* (Vaalharts population) larvae feeding on maize whorls from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

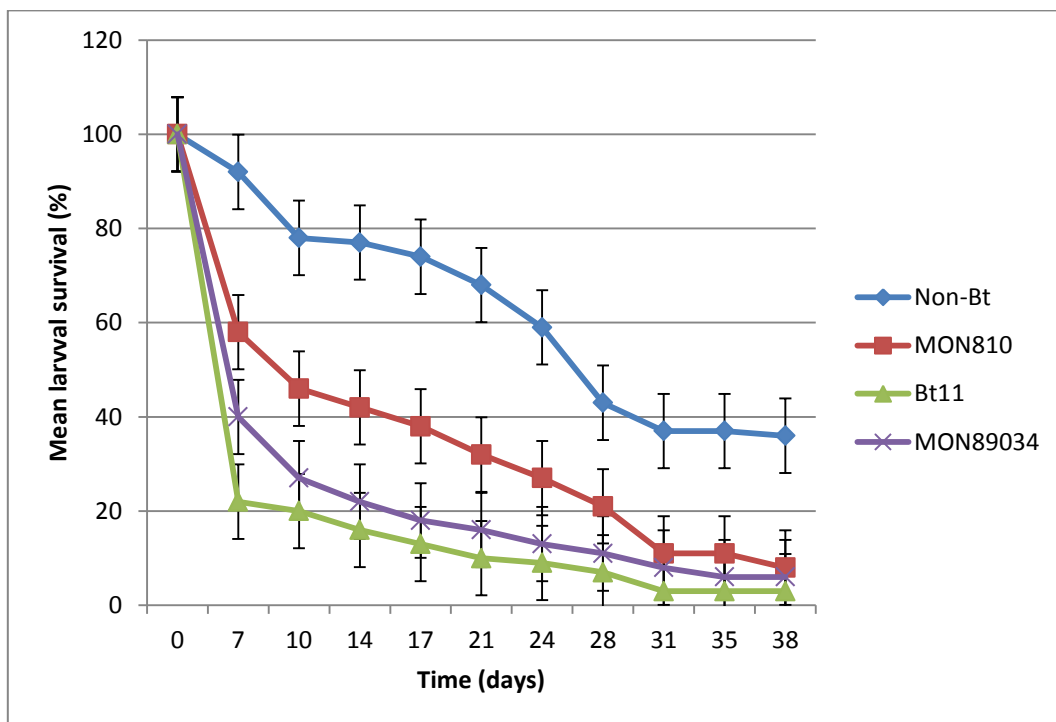


Figure 3.5. Mean percentage survival of *Eldana saccharina* larvae feeding on maize ears and silks from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

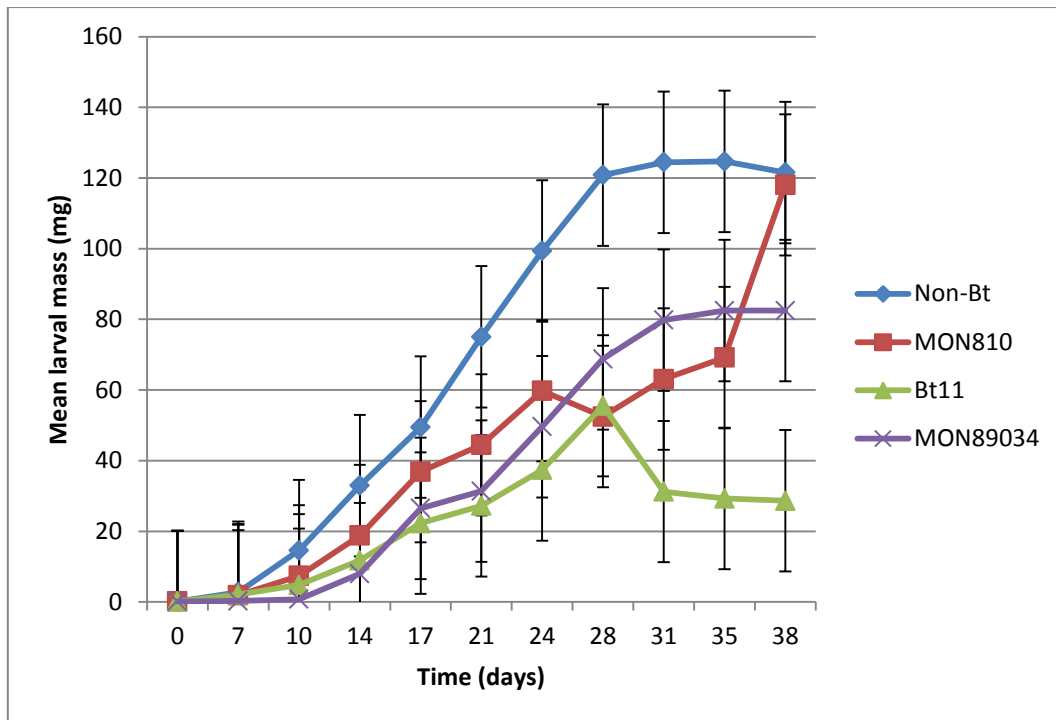


Figure 3.6. Mean larval mass of *Eldana saccharina* larvae feeding on maize ears and silks from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

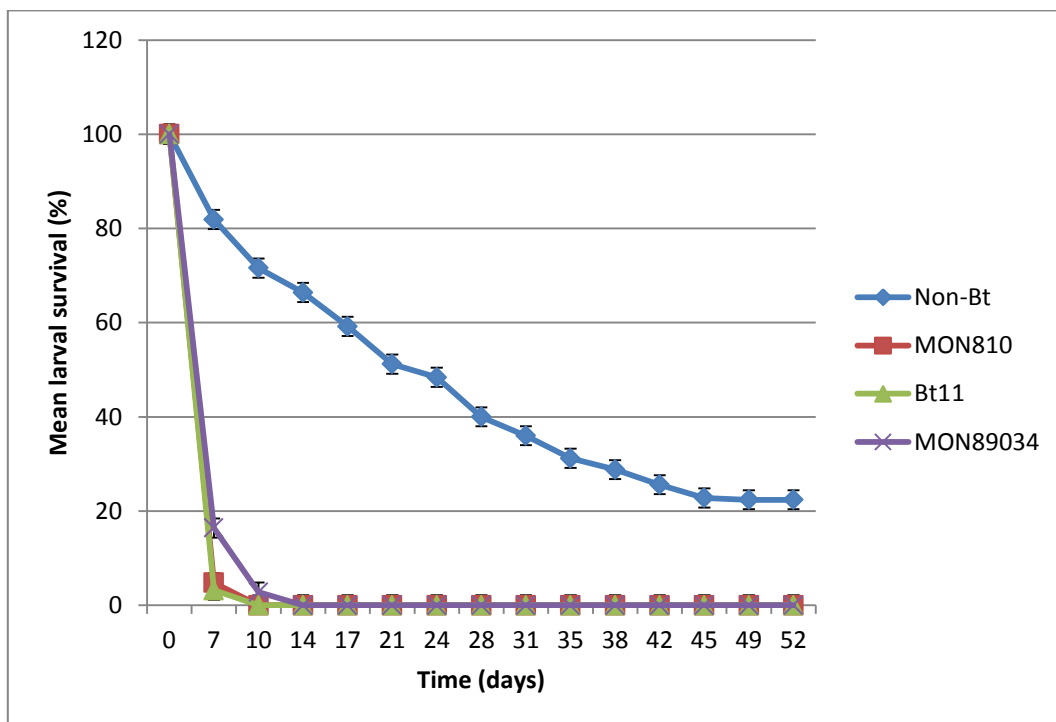


Figure 3.7. Mean percentage survival of *Eldana saccharina* larvae feeding on maize whorls from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

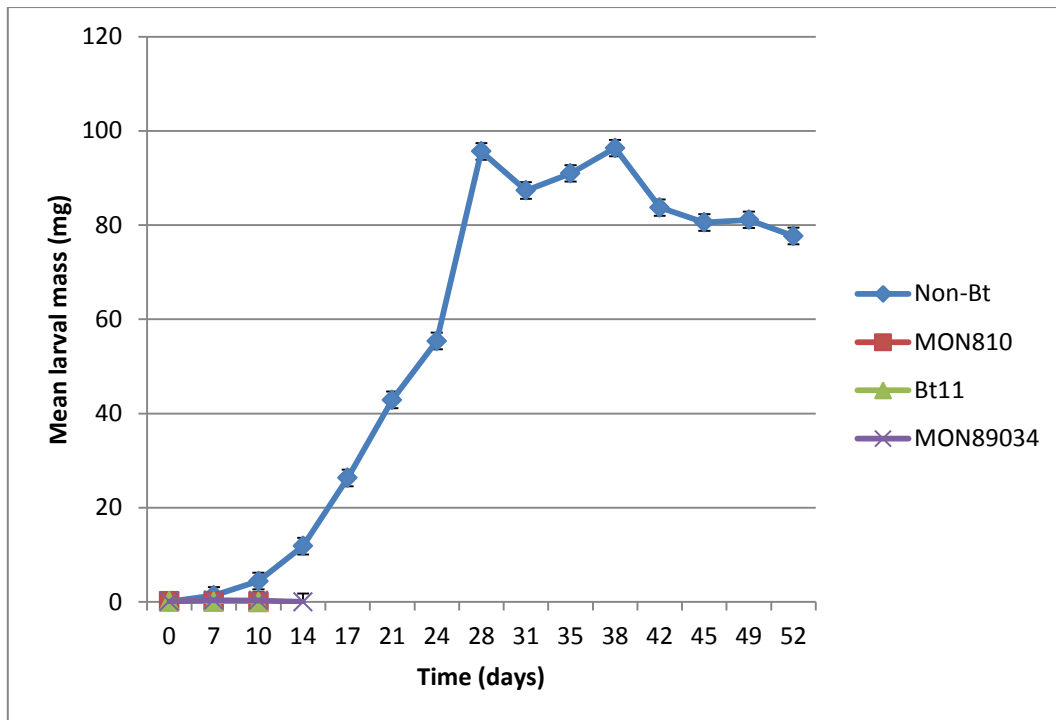


Figure 3.8. Mean larval mass of *Eldana saccharina* larvae feeding on maize whorls from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

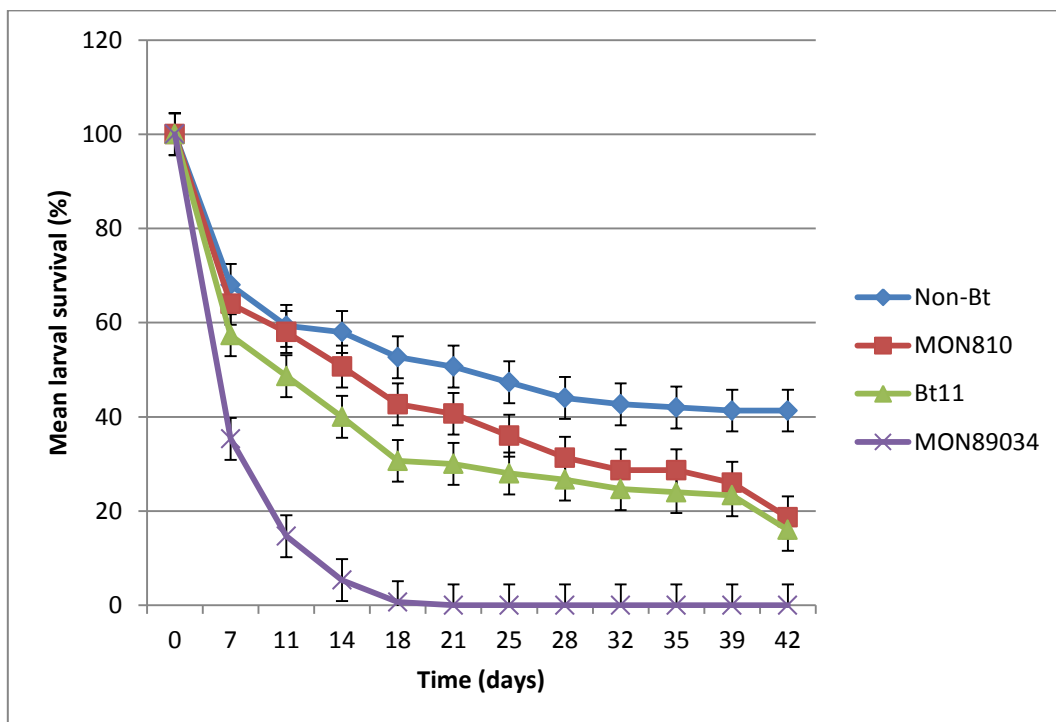


Figure 3.9. Mean percentage survival of *Sesamia calamistis* larvae feeding on maize ears and silks from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

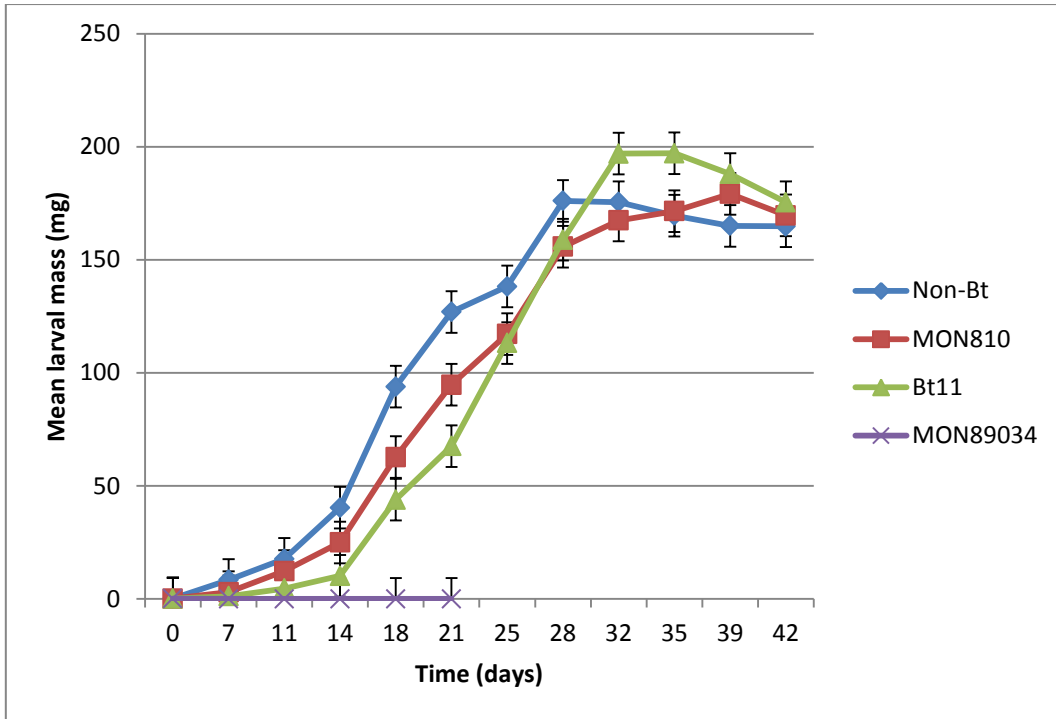


Figure 3.10. Mean larval mass of *Sesamia calamistis* larvae feeding on maize ears and silks from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

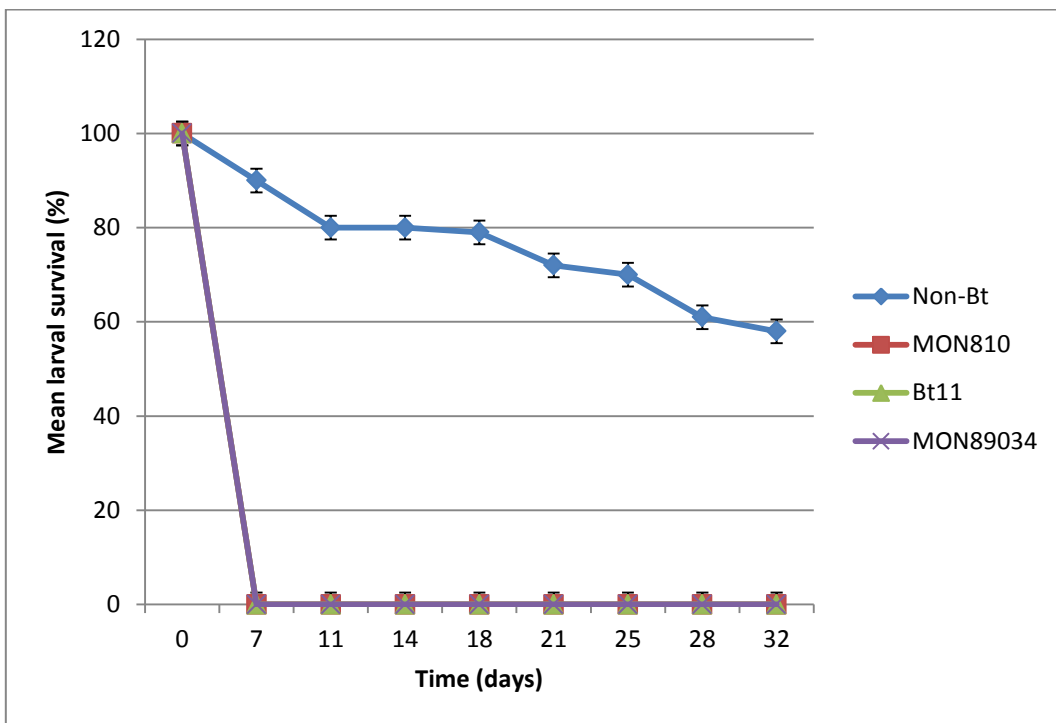


Figure 3.11. Mean percentage survival of *Sesamia calamistis* larvae feeding on maize whorls from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

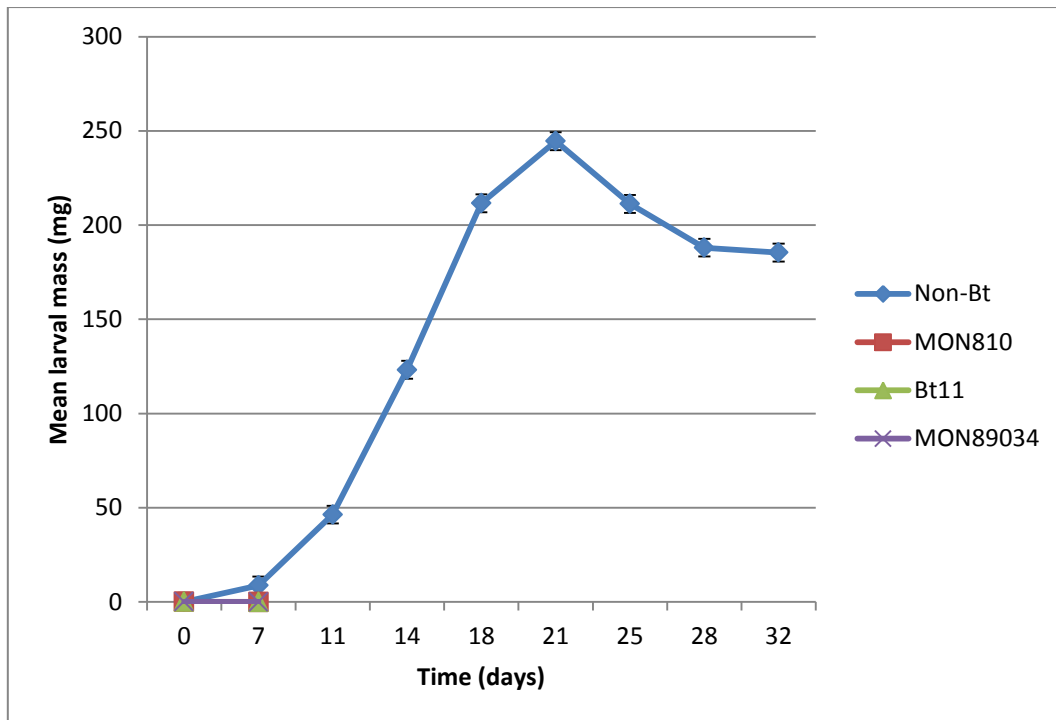


Figure 3.12. Mean larval mass of *Sesamia calamistis* larvae feeding on maize whorls from 1st instar onwards (non-Bt, event MON810, event Bt11 and event MON89034) (Bars indicate SE).

CHAPTER 4: Conclusion

Before the 2012/13 growing season, all commercialised Bt maize events in South Africa expressed the Cry1Ab crystal protein, until the stacked Bt event, MON89034 was cultivated on a large scale in South Africa. This stacked Bt event expresses two different Cry proteins, Cry1A.105 and Cry2Ab2.

A study conducted by Van Rensburg (1999) between 1994 and 1997 to evaluate the efficacy of numerous Bt maize events against *B. fusca* showed that the efficacy of event MON810 was sufficient to protect maize from damage caused by stem borers. Since then it has been shown that Bt maize hybrids provide effective control against *C. partellus* and partial to good control against *S. calamistis* and *B. fusca* (van Rensburg, 1999; Van den Berg and Van Wyk, 2007; Tende *et al.*, 2010).

The first commercial plantings of Bt maize in South Africa occurred in the growing season of 1998/99. The first official report of resistance development to Bt maize in field conditions was made in 2006, when Van Rensburg (2007) showed that significant numbers of *B. fusca* F1 generation originating from diapause larvae survived on Bt maize. Larvae were collected from the Christiana area (27° 57 ` S, 25° 05 ` E) in the Northern Cape Province and studies were conducted. Other cases of resistance were reported and confirmed throughout South Africa (Kruger *et al.*, 2011, 2012 2014).

Helicoverpa armigera feed primarily on maize silks and ears. A study conducted by Van Rensburg (2001) reported that larvae of *B. fusca* feeding on maize silks had greater survival than larvae feeding on leaf and stem tissue when larvae fed on a Bt event expressing Cry1Ab. Van Wyk *et al.* (2007) showed that *H. armigera* can complete its life cycle on Bt maize expressing Cry1Ab under field conditions, prolonging the exposure to Bt toxin. No experiments were previously conducted with Bt maize expressing Cry1A.105 + Cry2Ab2. There are uncertainties regarding the expression levels of Bt toxin in different plant tissues, therefore it is unknown what exposure to the toxin may have on species that feed on different plant tissues (Andow and Hillbeck, 2004). Dowd (2001) reported infestations of up to 50% of *H.*

zea on Bt maize with high expression levels of toxin in the ears and silks in the U.S.A.

Erasmus *et al.* (2010) reported that larvae of *A. segetum* were insensitive when feeding on Bt maize events expressing Cry1Ab, however, the mass of the larvae feeding on Bt maize was significantly lower than larvae feeding on non-Bt maize hybrids. Lambert *et al.* (1996) reported that Cry9Ca1 to be the first insecticidal crystal protein with activity against cutworms

Spodoptera exempta cannot be as effectively controlled by Cry1A proteins as other *Spodoptera* spp., however, the threat of resistance development is insignificant (Fitt *et al.*, 1985). In Puerto Rico *S. frugiperda* has reduced sensitivity towards Cry1F expressed in TC1507, however, in mainland U.S.A. Cry1F effectively controls *S. frugiperda* (Storer *et al.*, 2010, 2012).

Cultivating genetically modified crops in conjunction with implementing integrated pest management (IPM) can contribute towards reducing maize yield losses. Kogan (1998) defined IPM as "a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impact on producers, society, and the environment." IPM consists of four pillars: cultural control, chemical control, biological control and host plant resistance (Van Emden, 1983). These strategies can be used singly or harmoniously to control pest populations (Kogan, 1998).

Stem borers are of economic importance in the agricultural context, because they can seriously limit the potential attainable yield from the seedling stage to maturity of the plant. In South Africa, the target pests of Bt maize are the lepidopteran stem borers *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) (Van Wyk *et al.*, 2009; Erasmus *et al.*, 2010). *Eldana saccharina* is also an important pest of maize, however, it is not considered a target pest of Bt maize in South Africa. *Busseola fusca* and *S. calamistis* occur almost throughout sub-Saharan Africa. Typically *C. partellus* occurs in the eastern and southern parts of

Africa (Le Ru *et al.*, 2006a; Ong'amo *et al.*, 2013). *Eldana saccharina* occurs almost throughout sub-Saharan Africa where suitable host plants are found (Girling, 1978).

All stem borers that attack Bt maize can be considered to be target species, however, these target species differ between regions. *Busseola fusca* and *C. partellus* are the main target species in eastern and southern Africa. In Egypt the target pests are *Sesamia cretica* (Lederer) (Lepidoptera: Noctuidae) and *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae) (El-Shazly *et al.*, 2013). In West Africa the major stem borers are *B. fusca*, *S. calamistis* and *E. saccharina* (Bosque-Perez and Schulthess, 1998).

All the non-target pest species selected for this study occur in the rest of Africa. *Agrotis segetum* has a cosmopolitan distribution in temperate and sub-tropical regions and occurs throughout Africa (Svensson *et al.*, 1998). *Helicoverpa armigera* can be found in southern Europe, Africa, Asia and Australasia (Riley *et al.*, 1992). *Spodoptera exempta* occurs in eastern sub-Saharan Africa, South West Arabia and South East Asia (Haggis, 1986; Brown and Dewhurst, 1975).

South Africa and Egypt are the only countries in Africa currently cultivating Bt maize. South Africa started cultivating Bt maize in 1997 (Gouse *et al.*, 2005) and Egypt started in 2008 (El-Shazly *et al.*, 2013). Bt maize may have an important role in all of Africa, especially in controlling the stem borer species, *B. fusca*, *C. partellus*, *S. calamistis* (target pests of Bt maize in South Africa) and *E. saccharina*. Gouse *et al.* (2005) reported that adopters of genetically modified (GM) maize had increased income when compared to adopters of non-GM maize through savings on reduced pesticide application.

This study showed that larvae of *C. partellus* feeding on Bt maize had less than 1% survival on all the maize Bt hybrids 7 days after commencement of feeding. The results were the same for the Potchefstroom and Vaalharts populations. *Eldana saccharina* feeding on maize ears was partially controlled by the Bt maize hybrids and larval survival was reduced to below 40% within the first 17 days of the experiment on all the Bt maize events. No larval survival occurred on Bt maize hybrids 14 days after commencement of the experiment when *E. saccharina* fed on

maize whorl tissues. Larval survival of *S. calamistis* feeding on Bt11 and MON810 maize ears were 28.0% and 36.0%, respectively, on day 25. No larval survival occurred on MON89034 at day 21. *Sesamia calamistis* feeding on Bt maize whorls had no larval survival 7 days after commencement of the experiment.

Survival of *A. segetum* from the Polokwane population did not differ significantly between larvae feeding on non-Bt maize and larvae feeding on Bt maize. However, significant differences were observed between larval survival on non-Bt and MON89034 maize for the Potchefstroom population of *A. segetum*. *Helicoverpa armigera* feeding on MON89034 had no survival 10 days after the commencement of the experiment, at the same stage larvae feeding on MON810 and Bt11 had 26% and 22% survival, respectively. Larval survival of 1st instar *S. exempta* feeding on Bt maize events had no survival 7 days after the onset of the experiment. *Spodoptera exempta* 3rd instar larvae feeding on MON89034 had no survival on day 11 of the experiment and MON810 had 6% survival at the same stage.

Survival of *A. segetum* occurred on the single-gene and stacked Bt events, however, these Bt events contain no Cry proteins that has insecticidal activities against *A. segetum*. Therefore, the cultivation of a Bt maize event expressing Cry9Ca1 is recommended in areas where *A. segetum* is a major pest of maize.

No survival of *H. armigera* occurred on the stacked Bt event 10 days after the commencement of the experiment, however, survival occurred on both the single-gene Bt events. Thus, the cultivation of the stacked maize Bt event will be more effective in controlling this pest.

Larvae of *S. exempta* feeding on maize from the 3rd instar onwards had survival until pupation when feeding on the single gene Bt event and no survival after 11 days when feeding on the stacked Bt event. It is recommended that the stacked Bt event be cultivated in areas where *S. exempta* is a major pest of maize, since this pest only attacks maize from the 3rd instar onwards.

Eldana saccharina feeding on maize ears survived on all the Bt maize hybrids until pupation. The stacked Bt event performed poorer than Bt11, and this may indicate

the increased likelihood of resistance development to the stacked Bt event in areas where this pest attacks maize. Bt11 maize may be more effective in controlling this stem borer pest species in those particular areas where this pest attacks maize.

Sesamia calamistis feeding on maize ears had survival on the two single-gene Bt events until pupation. No larval survival occurred on the stacked Bt event 21 days after commencement of the experiment. Resistance developing to MON810 and Bt11 is a possibility if survival of this stem borer pest species also occurs on these Bt events under field conditions.

When larvae of *C. partellus* fed on maize leaf whorls, no survival occurred after 7 days on any of the Bt events. The above mentioned stem borer species also had no to very low survival when feeding on maize leaf whorl Bt events, however, survival occurred when larvae fed on maize ears of different Bt events. It is recommended that an experiment be done with larvae of *C. partellus* feeding on maize ears of these three maize Bt events.

These data are relevant for Africa due to the poorer performance of the single gene Bt maize events, such as MON810 and Bt11. It is therefore recommended that a stacked or pyramided variety (MON89034) is released, since the stacked Bt event had better performance against the majority of species.

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