Effect of Bt-Maize Expressing Cry1Ab Toxin on Non-Target Coleoptera and Lepidoptera Pests of maize in South Africa

Author(s): A. Erasmus & J. Van den Berg
Source: African Entomology, 22(1):167-179. 2014.
Published By: Entomological Society of Southern Africa
DOI: http://dx.doi.org/10.4001/003.022.0110
URL: http://www.bioone.org/doi/full/10.4001/003.022.0110

Effect of Bt-maize expressing Cry1Ab toxin on non-target Coleoptera and Lepidoptera pests of maize in South Africa

A. Erasmus[1,2]* & J. Van den Berg[2]

[1]*ARC-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520 South Africa*
[2]*School of Biological Sciences, North-West University, Potchefstroom, 2520 South Africa*

While many studies have been conducted on possible effects of Bt-maize on beneficial arthropods, information on its effects on non-target pests of maize is scarce. Bt-maize has been planted in South Africa since 1998 for control of the maize stem borers, *Busseola fusca* (Lepidoptera: Noctuidae) and *Chilo partellus* (Lepidoptera: Pyralidae). No information is available on the possible effects that feeding on Bt-maize may have on other pests exposed to Cry1Ab protein when they attack seedlings or ears of Bt-maize. Important pests attacking maize seedlings are *Heteronychus arator* (Coleoptera: Scarabaeidae) and *Somaticus angulatus* (Coleoptera: Tenebrionidae), while *Helicoverpa armigera* (Lepidoptera: Noctuidae) is a sporadic pest of maize ears. The objectives of this study were to determine the effects of Bt-maize, expressing Cry1Ab protein, on these non-target pests. Laboratory studies in which larvae or adults of these species were fed with Bt and non-Bt-maize leaves or ears were conducted and mortality, growth, fecundity and fertility determined. Feeding on Bt-maize did not have a significant effect on any of these life-history parameters of *H. arator* or *S. angulatus*. In greenhouse and laboratory studies with *H. armigera* no larvae survived to the pupal stage on Bt-maize. This study showed that Cry1Ab-producing maize may protect the crop against *H. armigera* feeding damage. However, the likelihood of *H. armigera* becoming resistant to Cry1Ab protein over time, and it becoming an important secondary pest, is high.

Key words: *Busseola fusca, Helicoverpa armigera, Heteronychus arator,* non-target pests, secondary pests, *Somaticus angulatus.*

INTRODUCTION

The most important pests of maize in South Africa are the stem borers *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), and the soil pests cutworm, *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae), black maize beetle, *Heteronychus arator* Fabricius (Coleoptera: Scarabaeidae) and false wire worm, *Somaticus terricola* Fåhraeus (Coleoptera: Tenebrionidae) (Annecke & Moran 1982). While stem borers attack stems and ears of plants, soil pests attack seed and seedlings.

Except for studies on *A. segetum* (Erasmus *et al.* 2010), no evaluation of the effect of Cry1Ab-expressing Bt-maize on other non-target primary consumers of Bt-maize has been done in South Africa. Although the value of determining the effect of Lepidoptera-targeting Bt-maize on other pests may at first glance be questioned, this is important in both the risk assessment process as well as in the bigger picture of integrated pest management (IPM) and insect resistance management (IRM). Non-target pest species that are screened for their sensitivity to insecticidal proteins can serve as surrogates for non-target arthropods (Romeis *et al.* 2008) and information on the effect on pests that are closely related to target species provide baseline data and may shed light on possible secondary pest development. For example, the effect of Bt-maize on *Eldana saccharina* Walker (Lepidoptera: Pyralidae), which does not attack maize in South Africa but in other parts of Africa, was studied by Keeping *et al.* (2007) who reported that this pest was highly susceptible to the Cry1Ab protein.

The effect of exposure of these non-target primary consumers to Cry1Ab protein would not necessarily result in mortality but could be in the form of individual reduced fitness (Van Wyk *et al.* 2007) which could lead to suppressed pest status in the field.

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae), which attacks maize leaves and ears, is common on maize, but regarded as a minor or sporadic pest (Van Wyk *et al.* 2008). The effects of

*Author for correspondence. E-mail: erasmusa@arc.agric.za

Bt-maize on *H. armigera* as a pest of minor importance has not been reported previously but could be relevant in IPM programmes in maize. Bt-maize in South Africa was designed for specific target pests and are not intended for control of secondary pests such as *H. armigera*. These secondary pests may, however, show some degree of susceptibility to Cry1Ab proteins and their numbers may be suppressed on Bt-maize. Resistance can thus develop in this pest of which populations are under selection pressure, giving rise to increased importance and affording them secondary pest status. For example, damage caused by *H. armigera*, which is not the target pest of Cry1Ab Bt-maize in South Africa, is significantly suppressed by this toxin under field conditions (Van Wyk *et al.* 2008). Farmers have experienced a decline in *H. armigera* pest status during the past decade (Van Wyk *et al.* 2008; Erasmus and Van den Berg, pers. observations), but increased bollworm infestation levels are anticipated if this pest becomes resistant to this Bt toxin.

The first transgenic crop commercialized in South Africa for the control of bollworm was Bt-cotton encoding for the Cry1Ac protein between 1997 and 2011, after which Bt-cotton expressing both Cry1Ac and Cry2Ab2 proteins. *Helicoverpa armigera* is susceptible to both Cry1Ab (Bt-maize), Cry1Ac and Cry2Ab2 (Bt-cotton) proteins, though considerably less so than other heliothine species such as *Heliothis virescens* Fabricius which is the primary target for Bt-cotton in the U.S.A. (Fitt *et al.* 2004). Although *H. armigera* is considered a non-target species for Cry1Ab-expressing Bt-maize, it is regarded as one of the main non-target pests of concern in risk assessments for release of maize expressing proteins with insecticidal properties (Van Wyk *et al.* 2007).

Although outbreaks of *H. arator* are sporadic, it is the most important coleopteran pest of maize in South Africa. Larvae feed on organic material in the soil and do not cause damage to maize. Adults, however, cause damage to subterranean stems of seedlings by eating frayed holes into them. *Somaticus angulatus* on the other hand, is the only economically important tenebrionid species of the many that occur in maize. Its outbreaks are highly sporadic and it is only important in the western maize-production regions of South Africa (Drinkwater 1990). Larvae damage seedlings by chewing holes into the subterranean stems and one individual may damage several seedlings during its life cycle. Adults of *S. angulatus* do not cause any damage to crops (Drinkwater 1990).

The objectives of this study were to determine the effects of Bt-maize expressing Cry1Ab insecticidal protein on life history parameters of important non-target pests of maize, *i.e. H. armigera, H. arator* and *S. angulatus*.

MATERIAL AND METHODS

In this study the 'whole plant method' approach was used to expose the insect to the actual plant parts that pests would consume under natural conditions, as suggested by Birch *et al.* (2004). The life history parameters referred to in this study are commonly used to evaluate the fitness of arthropods exposed to Bt crops and have been indicated to provide accurate measurements of fitness (Kruger *et al.* 2012; Kruger *et al.* 2013).

Helicoverpa armigera

Laboratory and greenhouse experiments were conducted with F_1-larvae originating from field-collected larvae. Larvae were collected from ears of non-Bt-maize in the Potchefstroom area (46°43'S 27°06'E) of the North West Province, South Africa. Larvae were reared on artificial medium (chickpea-based agar diet, developed for *Chilo partellus*) until pupation. Moths derived from these pupae laid eggs on nylon gauze and the first-instars that hatched were used in various bioassays.

Larval survival and mass gain on Bt- and non-Bt-maize leaves and ears were evaluated in a laboratory bioassay and a greenhouse trial. In the laboratory, first-instar larvae were allowed to feed on whorl leaves whereas ears were used under greenhouse conditions. Both experimental layouts were completely randomized designs.

Larval survival on maize whorl leaves

One first-instar larva was placed in a glass test tube with a 15 cm long piece of maize leaf cut from the central whorl leaves of 3–4-week-old maize plants of the various hybrids. The hybrids were DKC 78-15B (expressing Cry1Ab protein) with non-Bt iso-hybrid CRN 3505 and NK Mayor B (event Bt11) with its iso-hybrid Brasco. In this experiment each treatment was replicated 50 times. Test tubes were kept under natural day/night conditions in a laboratory where temperatures ranged between 20 and 25 °C. Larval mass and survival were determined every fourth day and leaf material was replaced with each assessment.

Larval survival on maize ears

The non-Bt hybrid CRN 3505 and Bt hybrid DKC 78-15B were used in this study. Ears in the soft dough stage of development were infested with 10 first instars per ear without removing ears from the plants. Larvae were placed on the tips of ears between the silks by means of a camel-hair brush. Seventy ears of each hybrid were infested. Each infested ear was then covered with a white fine organza bag. Ten ears were removed from randomly selected maize plants of each hybrid 12 days after infestation and dissected to collect surviving larvae and to determine larval mass. Seven further samplings were done at 3-day intervals until larvae reached the pre-pupal stage.

Heteronychus arator

Two laboratory experiments in which different rearing methods were used were conducted to compare beetle mass, mortality, and fertility when feeding on 2–4-week-old stems of Bt and non-Bt-maize seedlings. Overwintering sexually immature beetles that are active from late January until late April were collected from maize fields in the eastern region of the maize-production area (Balfour district, Mpumalanga) in South Africa. Beetles were collected by using light traps at the end of February. The experimental layout was a completely randomized design. The Bt and non-Bt iso-hybrids used were DKC 78-15B and CRN 3505, respectively.

Experiment 1

The effect of Bt-maize on beetle survival and mass gain was studied in this experiment. Beetle mass and mortality was recorded at weekly intervals from commencement of the experiment until day 47 and then at fortnightly intervals until day 119, when the study was terminated. Thirty males (replicates) and 30 females were evaluated per hybrid (60 replicates). Beetles were kept separately in 10 cm-long glass vials, filled with 20 ml washed sand at the bottom and provided with 7 cm-long pieces of seedling stems at weekly intervals.

Experiment 2

The effect of Bt-maize on beetle survival, fecundity and fertility was studied in this experiment, with the difference from the experiment above being that beetles were kept in groups in containers. Beetles were fed with Bt or non-Bt-maize and the experiment was replicated three times. Each replicate consisted of a group of 30 female and 20 male beetles, placed in plastic containers (40 × 40 × 22 cm). The containers were filled with a 10 cm layer of washed sand. Drinking water was provided using water-filled test-tubes (7 × 1 cm) topped with cotton wool plugs. Beetles in each container were provided with five 20 cm-long pieces of four-week-old maize stems at weekly intervals. The experiment commenced in early March 2010 and continued for 319 days. The number of live beetles in each container was initially determined at weekly intervals but during winter months this was done fortnightly. The sand was sifted once a week and later in the season every second week to collect eggs. The total number of eggs laid per 30 females was determined. Eggs were kept in glass vials on a mixture of moist peat and sandy soil to determine the number of viable eggs.

Somaticus angulatus

Four experiments were conducted to study the effect of Bt-maize on various life-history parameters of *S. angulatus* larvae, exposed to Bt-maize for different periods during their life cycle. Stems of Bt and non-Bt-maize seedlings were fed to larvae in these experiments. The hybrids used were DKC 78-15B (expressing Cry1Ab protein) with non-Bt iso-hybrid CRN 3505 and NK Mayor B (expressing Cry1Ab protein) with its iso-hybrid Brasco.

A rearing colony was established by collecting adults from maize fields in the Hoopstad area (Free State Province, South Africa) (27°51′33.68″S 26°06′25.89″E) during April and May. Beetles were kept in plastic containers as described above, with the bottoms covered with a 5 cm layer of sifted, washed sand. Beetles were fed apples and green maize leaves which also provided shelter. Drinking water was provided as described above. Eggs were left inside plastic containers and first instars collected once eggs started to hatch. First instars were kept at 25 °C and 65 % humidity since larvae do not feed until the second instar (Drinkwater 1987).

Second-instar larvae

Two experiments, which differed in terms of maize hybrids used as well as food supplements, were conducted under laboratory conditions. Survival of second-instar larvae on maize seedlings of different hybrids was evaluated in test tubes. Each hybrid was replicated 50 times. One second-instar larva was placed per test tube (75 mm long, 10 mm diameter) and cuttings (1.5 cm long) of seedling stems provided as food. Test tubes were filled with autoclaved soil in a sufficient quantity to cover stem cuttings completely and kept in an incubator at 25 °C and 65 % humidity. Seedling stems were replaced every 3–4 days when larval mass was also determined.

During this experiment in which hybrids CRN 3505 and DKC 78-15B were used, it was observed that larvae were reluctant to commence feeding. In a follow-up experiment, using hybrids Brasco and NK Mayor B, dry cereal bran (ProNutro™) was added to the soil to serve as a feeding stimulus. Adding cereal to the soil was stopped at day 49, when stem cuttings started to exhibit damage caused by larval feeding. Larval mass and survival was determined until the pre-pupal stage was reached.

Fourth-instar larvae

An experiment similar to the one above was also conducted using larger larvae. To obtain larger larvae of uniform age, second-instar larvae collected from the rearing colony were reared as described above, on ProNutro™, until they reached the fourth-instar before they were used in the experiment. One larva and a 1.5 cm seedling stem cutting were placed per test tube (125 mm long, 13.5 mm diameter) and covered with the ProNutro™ cereal/soil mix. The mix was used for the first week after which only soil was used. Survival and mass were recorded every 3–4 days until pre-pupae started to form.

Evaluation of fertility and fecundity

Field-collected beetles were used in this experiment to determine the effect of Bt-maize on beetle fecundity and fertility. Ten pairs of males and females were placed in each of 22 plastic containers (17 × 12 × 8 cm), using 11 containers (replicates) for each of the two maize hybrids (DKC 78-15B and CRN 3505). The containers were filled with a 3 cm deep layer of sand. Green maize leaves of each hybrid were provided as food and replaced daily. Sand was sifted every third day to collect eggs. The number of eggs laid per container was determined. Eggs were kept in an incubator at 25 °C and 65 % humidity until hatching after which the numbers of larvae were determined.

Data analysis

Repeated measures ANOVA was used to analyse beetle mortality, beetle mass, larval survival, larval mass, fertility and fecundity over time (STATISTICA 8.0).

RESULTS

Helicoverpa armigera

Larval survival on maize whorl leaves

Mean larval mass was significantly lower when larvae fed on Bt-maize whorl leaves compared to non-Bt-maize (Fig. 1a). These differences were significant between CRN 3505 and DKC 78-15 B ($F_{(1,98)} = 28.16$, $P < 0.00001$) as well as Brasco and NK Mayor B ($F_{(1,98)} = 79.78$, $P < 0.00001$) (Fig. 1b). Larval mass was also significantly lower on the two Bt hybrids compared to the non-Bt hybrids ($F_{(1,98)} = 9.00$, $P = 0.003$). The mass of larvae feeding on Brasco increased rapidly over the first 12 days followed by a rapid decrease until day 17.

Larval survival on leaves in general was low and decreased rapidly on both Bt hybrids over the first four days compared to the non-Bt hybrids (Fig. 1b). Larval survival was significantly lower on DKC 78-15 B compared to CRN 3505 ($F_{(1,98)} = 69.74$, $P = 0.00003$) as well as on NK Mayor B compared to Brasco ($F_{(1,8)} = 200.30$, $P < 0.00001$).

Larval survival on maize ears

Mass of larvae that fed on Bt-maize ears was significantly lower than those that fed on the non-Bt ears ($F_{(6,60)} = 20.28$, $P < 0.00001$) (Fig. 2a). No larval growth was observed and the few that did survive remained small and had a shrivelled appearance. A decrease in mass of larvae feeding on non-Bt ears on day 30 was due to the onset of the pre-pupal stage. Similar tendencies of decreased mass during pre-pupal stages were reported for the maize stem borer, *B. fusca* (Kruger *et al.* 2012, 2014).

Larval survival decreased slowly over time (Fig. 2b) and differed significantly between Bt and non-Bt ears ($F_{(6,60)} = 11.02$, $P < 0.00001$).

Heteronychus arator

Experiment 1

The percentage mortality of beetles increased over time but did not differ significantly between the Bt and non-Bt iso-hybrid over the 119-day

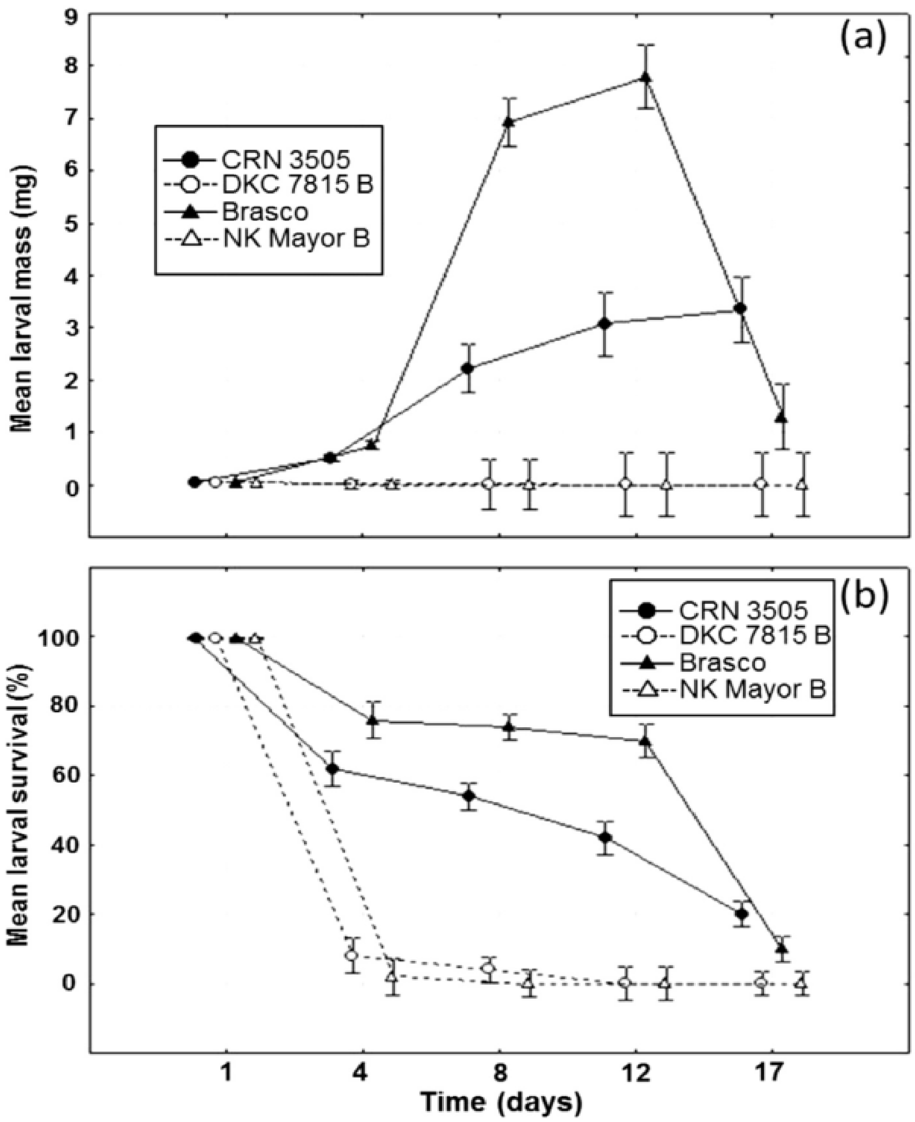

Fig. 1. Mean mass (**a**) and survival (**b**) of *Helicoverpa armigera* larvae feeding on maize whorl leaves from the first-instar onwards (Event MON810 hybrid, DKC 78-15 B with non-Bt iso-hybrid, CRN 3505 and event Bt11 hybrid, NK Mayor B with non-Bt iso-hybrid, Brasco) (bars indicate S.E.).

period (Fig. 3a) ($F_{(1,8)} = 1.06$, $P = 0.419$). No differences were observed between mass of male beetles ($F_{(1,4)} = 0.062$, $P = 0.816$) or female beetles ($F_{(1,4)} = 4.252$; $P = 0.108$) feeding on Bt and non-Bt-maize (Fig. 3b). Beetle mass decreased slowly over time with no differences between the hybrids for male ($F_{(1,58)} = 0.288$, $P = 0.593$) and female beetles ($F_{(1,58)} = 1.472$, $P = 0.229$) (Fig. 3b).

Experiment 2

The mortality of male ($F_{(1,4)} = 0.919$, $P = 0.392$) and female ($F_{(1,4)} = 0.161$, $P = 0.705$) beetles feeding on Bt and non-Bt-maize did not differ over the 173-day period (Fig. 4a). Fecundity peaked between 182 and 265 days on both hybrids with no significant differences observed between the total number of eggs laid ($F_{(1,4)} = 0.002$, $P = 0.969$) (Fig. 4b). The number of eggs that hatched also did not differ significantly between hybrids ($F_{(1,4)} = 0.063$, $P = 0.814$) (Fig. 4c).

Somaticus angulatus

Survival of second-instar larvae

There were no significant differences in mean larval mass between groups that fed on Bt and

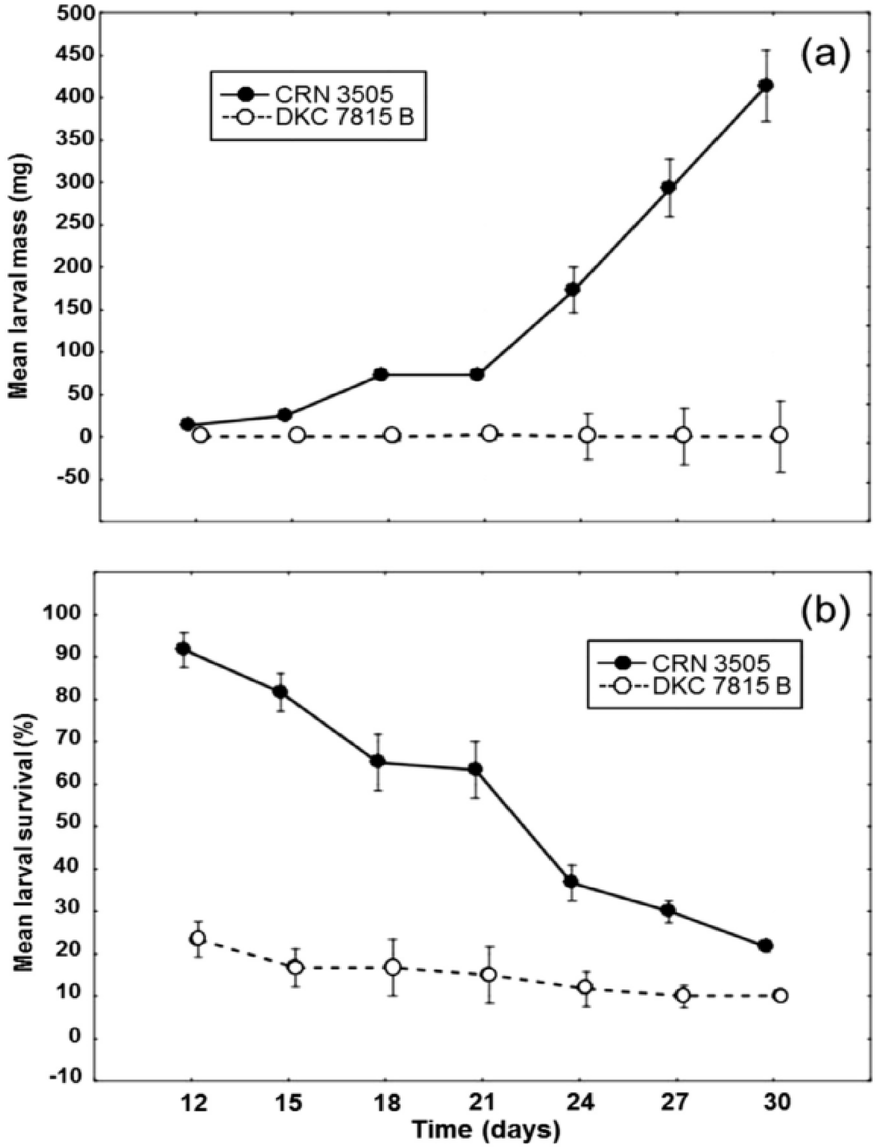

Fig. 2. Mean mass (**a**) and survival (**b**) of *Helicoverpa armigera* larvae feeding on maize ears from the first-instar onwards (DKC 78-15 B with non-Bt iso-hybrid, CRN 3505) (bars indicate S.E.).

non Bt-maize in any of the treatments combinations (DKC 78-15B with CRN 3503 ($F_{(1,198)} = 0.497$, $P = 0.481$) and Brasco with NK Mayor B ($F_{(1,198)} = 2.794$, $P = 0.096$) (Fig. 5a).

Larval survival in the treatments without the cereal bran was low (<20 %) and adding cereal bran resulted in an increase in survival to between 43 and 55 %. High levels of mortality occurred during the first three weeks of the experiment but no significant differences in survival were observed between the groups feeding on Bt (DKC 78-15B) and non-Bt-maize (CRN 3505) ($F_{(1,18)} = 0.169$, $P = 0.686$) (Fig. 5b). A slower rate of decrease and no significant differences in survival were observed for Brasco and NK Mayor B ($F_{(1,18)} = 1.195$, $P = 0.289$).

Survival of fourth-instar larvae

No significant differences were observed between mean larval mass on Bt and non-Bt-maize in either the experiment with CRN3505 and DKC 78-15B ($F_{(1,48)} = 0.888$, $P = 0.351$), or Brasco and NK Mayor B ($F_{(1,48)} = 0.244$, $P = 0.624$) (Fig. 6a).

Larval survival over the first 50 days was very high (>98 %). This was followed by a decrease in survival to between 32 and 72 % over the follow-

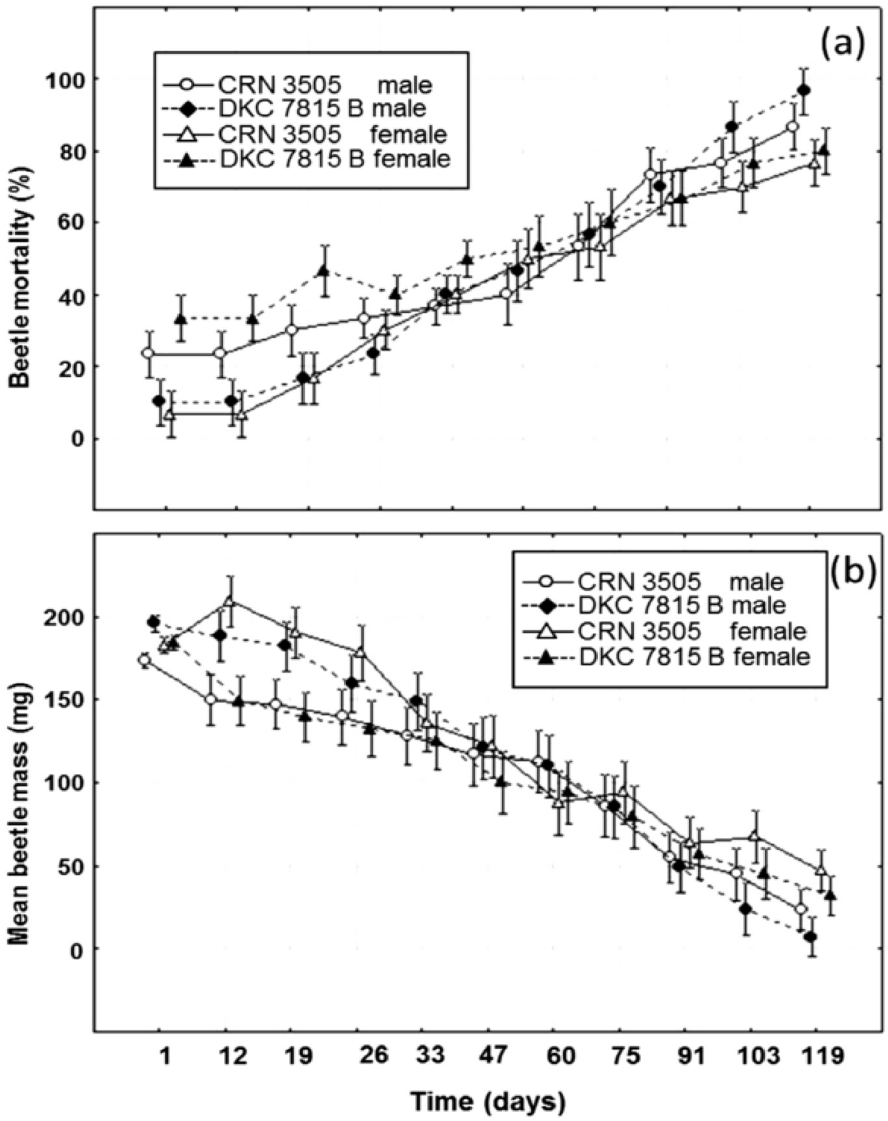

Fig. 3. Mean percentage mortality (**a**) and mean mass (**b**) of male and female *Heteronychus arator* beetles feeding on Bt (DKC 78-15B) and non-Bt-maize (CRN 3505) in glass vials (bars indicate S.E.).

ing 90 days. Larval survival did not differ significantly between the Bt and non-Bt feeding groups in the CRN3505 and DKC 78-15B ($F_{(1,8)} = 1.941$, $P = 0.201$) or Brasco and NK Mayor B comparison ($F_{(1,8)} = 2.149$, $P = 0.181$) (Fig. 6b).

Fecundity and fertility

There were no significant differences between the mean number of eggs laid per 10 female beetles after feeding for a 29-day period on Bt and non-Bt-maize ($F_{(1,20)} = 1.728$, $P = 0.204$). The number of eggs laid per 10 females were 31.2 (S.E. ± 3.64) for those that fed on non-Bt-maize while those that fed on Bt-maize laid 25.6 (S.E. ± 2.24) eggs per 10 females. Fertility did not differ between treatments ($F_{(1,20)} = 4.138$, $P = 0.055$) and the percentage eggs that hatched were 58.7 % (S.E. ± 2.13) and 49.8 % (S.E. ± 3.80) for the non-Bt and Bt-treatments, respectively.

DISCUSSION

Helicoverpa armigera

Although African bollworm larvae damage maize leaves under field conditions, the poor growth and survival on both Bt and non-Bt hybrids observed in this study shows that maize leaf tissue is not a suitable food source for first

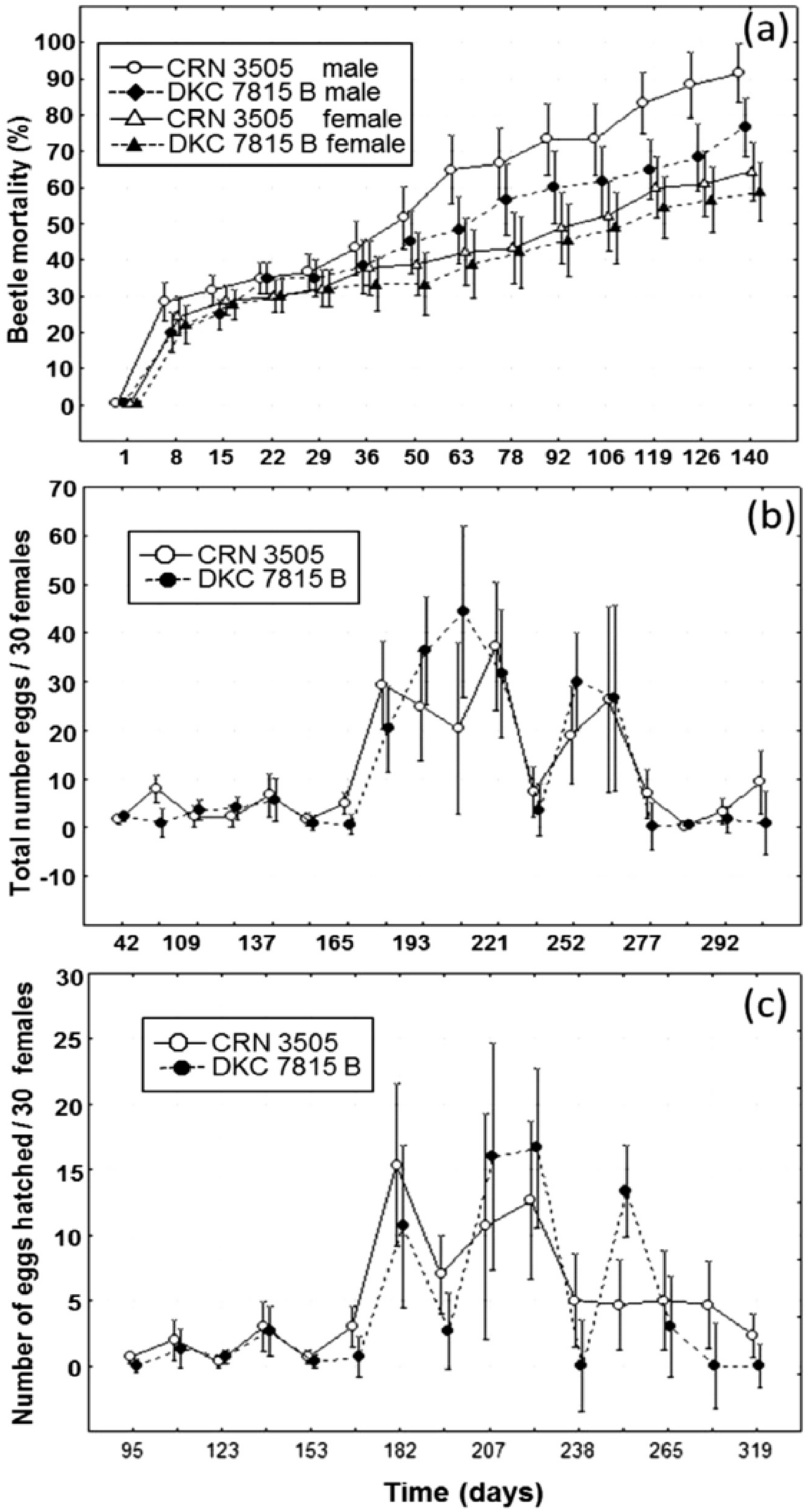

Fig. 4. Mean percentage mortality (**a**), fecundity (**b**) and fertility (**c**) of *Heteronychus arator* beetles feeding on Bt (DKC 78-15B) and non-Bt-maize (CRN 3505) in plastic containers (bars indicate S.E.).

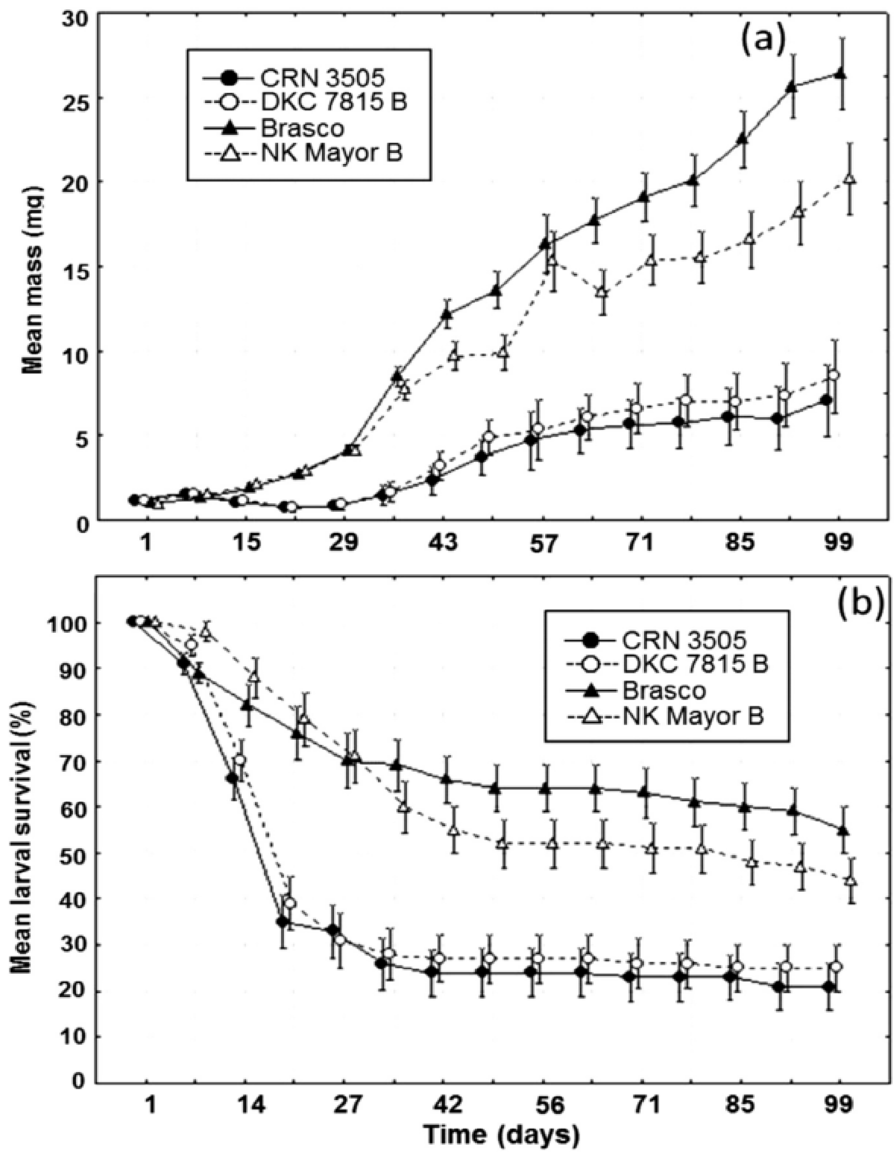

Fig. 5. Mean mass (**a**) and percentage survival (**b**) of *Somaticus angulatus* larvae commencing feeding on maize seedlings as second-instar larvae. [(DKC 78-15B) and its non-Bt iso-hybrid (CRN 3505) and event Bt11 (NK Mayor B) and its non-Bt iso-hybrid (Brasco)] (bars indicate S.E.).

instars. Similar observations were made by Wu *et al*. (2002) who reported that *H. armigera* first-instar larvae that fed on maize whorls did not perform well and that they preferred ears.

Larvae that fed on Bt-maize ears were always smaller than larvae feeding on non-Bt ears, which also contributed to a delay in development. Larval establishment occurred on a few ears of Bt-maize plants, but once established inside ears, larval development rate was significantly reduced. Because of this delay in development on Bt-maize ears, much less ear damage can be expected to occur compared to non-Bt plants. A study conducted by Van Wyk *et al.* (2008) indicated that *H. armigera* larvae survive on Bt-maize ears under field conditions but their numbers were always significantly lower in Bt-maize fields compared to non-Bt fields. The latter result was confirmed in this study conducted under greenhouse conditions. Buntin *et al*. (2001) also reported that Bt-maize expressing Cry1Ab protein suffered reduced whorl infestation and damage by both *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae)

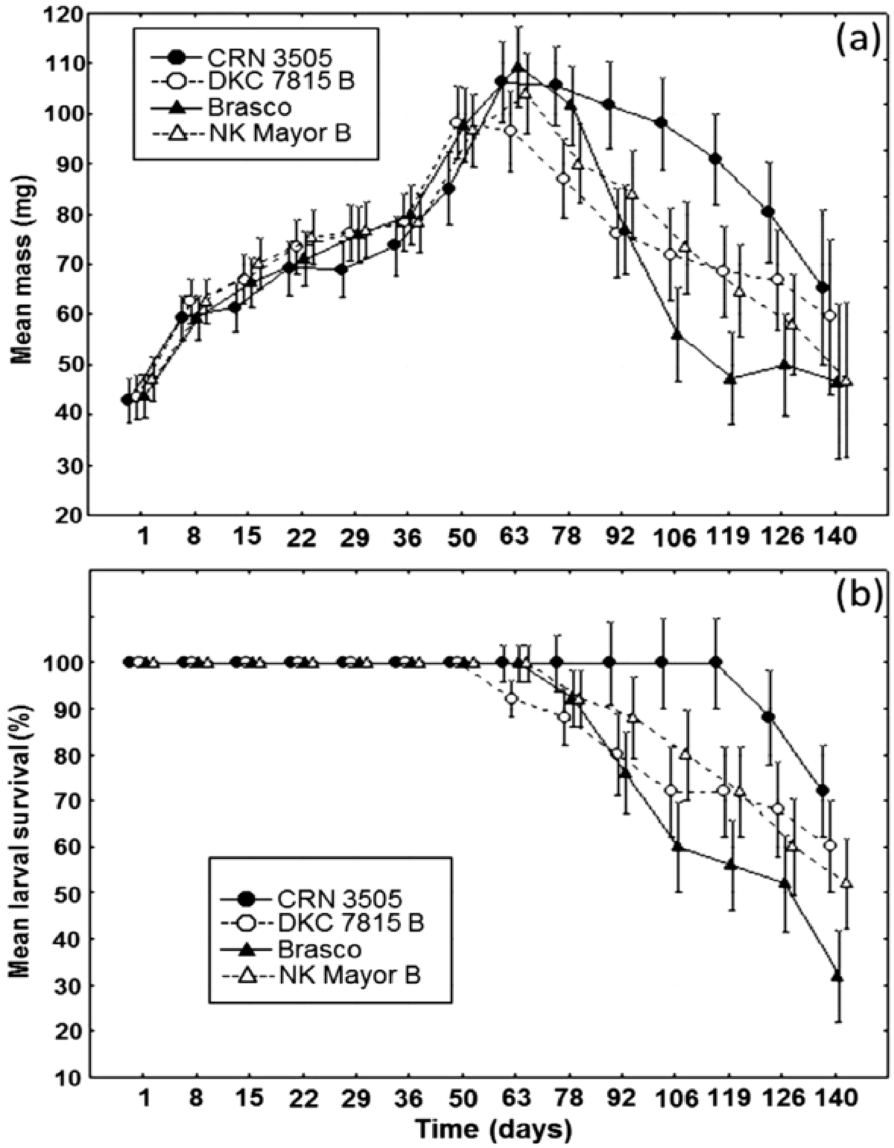

Fig. 6. Mean mass (**a**) and percentage survival (**b**) of *Somaticus angulatus* larvae commencing feeding on maize seedlings as fourth-instar larvae. [(DKC 78-15B) and its non-Bt iso-hybrid (CRN 3505) and event Bt11 (NK Mayor B) and its non-Bt iso-hybrid (Brasco)] (bars indicate S.E.).

in the U.S.A. Bt-maize that expressed Cry1Ab protein was found to cause mortality of *H. zea* but permitted 15–40 % survival to the prepupal stage compared with non-Bt-maize (Storer *et al*. 2001). A delay in development was also observed by Storer *et al*. (2001) who reported that larvae of *H. zea* that did survive grew more slowly on Bt than on non-Bt-maize, and that pupation and adult eclosion were delayed by 6–10 days when feeding on Bt-maize ears.

Pilcher *et al*. (1997) reported that *H. zea* larvae, which are secondary pests of maize in the U.S.A. where *O. nubilalis* is the main pest, also do not survive when feeding on Bt-maize leaf tissue, and that larvae do survive in much higher numbers in maize ears than the target pest, *O. nubilalis*. The differences in numbers of surviving larvae between different plant parts (leaf and ear) probably relate to the levels of Bt protein expressed in different tissues (Pilcher *et al*., 1997). Pilcher *et al*. (1997) suggested that a higher dose of Cry1Ab protein would be required to affect *H. zea* to the same extent as *O. nubilalis.* MON810 maize, which was used in this study, expresses the Cry-protein in silk tissue and kernels as well as leaves, tassels, and stalks; thus it is also biologically active against *H. zea* (Horner *et al.* 2003). Padidam (1992) showed that Cry1Ac expressed in Bt-cotton was about 12-times more toxic to *H. armigera* than Cry1Ab.

Although this study showed 100 % mortality of *H. armigera* under laboratory conditions, it is concluded that Bt-maize, under field conditions, will suppress larval numbers but may not provide complete protection against the pest. In a two-year study by Burkness *et al.* (2001), control of *H. zea* in Bt hybrids was reported to range between 85 and 88 % when compared with non-Bt hybrids, suggesting that Bt hybrids provide high but not complete levels of larval control. Archer *et al.* (2001) also studied ear damage caused by *H. zea* to four events of Bt-maize (Mon810, Bt11, Bt176 and CBH354) and reported that no Bt-maize hybrid provided complete control of *H. zea* larvae feeding on kernels. A study conducted by Dowd (2001) indicated that although *H. zea* feeding was reduced on Bt-maize expressing high levels of the protein in the kernels, incidence of infestation was often not affected, and larvae remained alive and eventually damaged an equivalent number of kernels.

Helicoverpa armigera has a history of demonstrated potential in developing resistance to virtually all the insecticide molecules used against it (Kranthi *et al.* 2005). With constitutive expression of Bt toxins throughout the plant and for the entire growing season, Bt crops exert a higher selection pressure for resistance development, compared to any insecticide deployed to date (Storer *et al.* 2003). Because the insecticidal activity of transgenic plants also declines significantly as the plants mature (Fitt & Wilson 2000), some *H. armigera* larvae are able to complete their development under field conditions later in the season (Van Wyk *et al.* 2008). This survival poses a serious risk to sustainability of the technology because it contributes to resistance development in this non-target pest.

Heteronychus arator and *Somaticus angulatus*

This study indicated that Bt-maize had no effect on *H. arator* mortality, mass, fertility or fecundity. Similar results were observed for *S. angulatus* with no effect on survival of second or fourth instars, larval mass, fecundity and fertility. These results show that no further testing of these beetle species on non-coleopteran active Cry proteins is necessary.

Similar results were reported for other non-target Coleoptera species in the U.S.A. Dowd (2000) reported that control of non-lepidopteran pests such as sap beetles (Coleoptera: Nitidulidae) by Bt-maize expressing Cry1Ab is expected to be low because of the lack of efficacy of the protein against Coleoptera. Numbers of Nitidulidae are, however, expected to be affected indirectly because lepidopteran damage, which attracts sap beetles, is reduced in Bt-maize.

Pons *et al.* (2005) also observed that Bt-maize did not affect the incidence of the wireworm, *Agriotes lineatus* (Linnaeus) (Coleoptera: Elateridae), which attacks maize seed and seedlings in the U.S.A. Eizaguirre *et al.* (2006) also reported following a six-year study in Spain, that Bt-maize did not have a negative impact on *A. lineatus*. Daly & Buntin (2005) found no consistent effect of Bt-maize event MON810 on phytophagous coleopterans.

The two coleopteran species used in this study can be considered to be polyphagous, especially the *Somaticus* beetles, which are adapted to live in semi-arid conditions where they feed on dead plant material. The level of feeding on Bt-maize that adults and larvae were subjected to in this study was therefore unrealistically high, yet no effects were observed.

CONCLUSIONS

This study shows that Bt-maize events expressing Cry1Ab toxin that targets certain lepidopteran pests, did not have an effect on the coleopteran species evaluated in this study and would most likely not have effects on other coleopteran species. To the advantage of the farmer, the Cry1Ab toxin has the potential to protect maize from *H. armigera* feeding damage. This effectiveness may, however, contribute to resistance development by *H. armigera* and it becoming an important secondary pest of maize.

ACKNOWLEDGEMENTS

This work forms part of the Environmental Biosafety Cooperation Project between the Republic of South Africa and the Kingdom of Norway coordinated by the Department of Environmental Affairs, Directorate of Nature Management and the South African National Biodiversity Institute. We accordingly give due acknowledgment.

REFERENCES

AL-DEE, M.A. & WILDE, G.E. 2003. Effect of Bt corn expressing the *Cry3Bb1* toxin for corn rootworm control on aboveground non-target arthropods. *Environmental Entomology* **32**: 1164–170.

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

ANNECKE, D.P. & MORAN, V.C. 1982. *Insects and Mites of Cultivated Plants in South Africa*. The Butterworth Group, Durban/Pretoria, South Africa.

ARCHER, T.L., PATRICK, C., SCHUSTER, G., CRONHOLM, G., BYNUM, E.D. Jr. & MORRISON, W.P. 2001. Ear and shank damage by corn borers and corn earworms to four events of *Bacillus thuringiensis* transgenic maize. *Crop Protection* **20**: 139–144.

BIRCH, A.N.E., WHEATLEY, R., ANYANGO, B., APRAIA, S., CAPALBO, D., GETU DEGAGA, E., FONTES, E., KALAMA, P., LELMEN, E., LOVEI, G., MELO, I.S., MUYEKHO, F., NGI-SONG, A., OCHIENO, D., OGWANG, J., PITELLI, R., SCHULER, T., SETAMOU, M., SITHANANTHAM, S., SMITH, J., VAN SON, N., SONGA, J., SUJII, E., TAN, T.Q., WAN, F.H. & HILBECK, A. 2004. Biodiversity and non-target impacts: a case study of Bt-maize in Kenya. In: Hillbeck, A. & Andow, D.A. (Eds) *Environmental Risk Assessment of Genetically Modified Organisms, Vol. 1: A Case Study of Bt Maize in Kenya*. 116–185. CAB International, Wallingford, U.K, pp. .

BUNTIN, G.D., LEE, R.D., WILSON, D.M. & McPHERSON, R.M. 2001. Evaluation of Yieldgard transgenic resistance for control of fall armyworm and corn earworm (Lepidoptera: Noctuidae) on corn. *Florida Entomologist* **84**: 37–42.

BURKNESS, E.C., HUTCHISON, W.D., BOLIN, P.C., BARTELS, D.W., WARNOCK, D.F. & DAVIS, D.W. 2001. Field efficacy of sweet corn hybrids expressing a *Bacillus thuringiensis* toxin for management of *Ostrinia nubilalis* (Lepidoptera: Crambidae) and *Helicoverpa zea* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* **94**: 197–203.

DALY, T. & BUNTIN, G.D. 2005. Effect of *Bacillus thuringiensis* transgenic corn for lepidopteran control on non-target arthropods. *Environmental Entomology* **34**: 1292–1301.

DOWD, P.F. 2000. Dusky sap beetles (Coleoptera: Nitidulidae) and other kernel damaging insects in Bt and non-Bt sweet corn in Illinois. *Journal of Economic Entomology* **93**: 1714–1720.

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

DRINKWATER, T.W. 1987. Die valsdraadwurms (Coleoptera: Tenebrionidae) wat 'n rol speel by mielieverbouing, met spesiale verwysing na die biologie van *Somaticus angulatus* (Fåhraeus). Ph.D. (Agric.) dissertation, University of Stellenbosch, South Africa.

DRINKWATER, T.W. 1990. The geographical distribution of false wireworms, *Somaticus* species (Coleoptera: Tenebrionidae) associated with maize in South Africa. *Journal of Entomological Society of Southern Africa* **53**: 127–136.

DU TOIT, H.A. 1998. *The Black Maize Beetle in South Africa*. Crop Protection series No. 9. ARC-Grain Crops Institute, Potchefstroom, South Africa.

EIZAGUIRRE, M., ALBAJES, R., LÓPEZ, C., ERAS, J., LUMBIERRES, B. & PONS. X. 2006. Six years after the commercial introduction of Bt-maize in Spain: field evaluation, impact and future prospects. *Transgenic Research* **15**: 1–12.

ERASMUS, A., VAN DEN BERG, J. & VAN RENSBURG, J.B.J. 2010. Effects of Bt-maize on the cutworm *Agrotis segetum* (Lepidoptera: Noctuidae), a pest of maize seedlings. *Environmental Entomology* **39**: 702–706.

FITT, G.P. & WILSON, L.J. 2000. Genetic engineering in IPM: a case study. In: Kennedy, G.G. & Sutton, T.B. (Eds) *Emerging Technologies in Integrated Pest Management: Concepts, Research and Implementation*. 108–125. APS Press, St. Paul, MN, U.S.A.

FITT, G.P., ANDOW, D.A., CARRIÈRE, Y., MOAR, W.J., SCHULER, T.H., OMOTO, C., KANY, A.J., OKECH, M.A., ARMA, P. & MANIANIA, N.K. 2004. Resistance risks and management associated with Bt-maize in Kenya. In: Hilbeck, A., Andow, D.A. (Eds) *Environmental Risk Assessment of genetically Modified Organisms: Vol. 1. A Case Study of Bt-maize in Kenya.* 209–250. CAB International, Wallingford, U.K.

HORNER, T.A., DIVELY, G.P. & HERBERT, D.A. 2003. Development, survival and fitness performance of *Helicoverpa zea* (Lepidoptera: Noctuidae) in MON810 Bt field corn. *Journal of Economic Entomology* **96**: 914–924.

KEEPING, M.G., RUTHERFORD, R.S. & CONLONG, D.E. 2007. Bt-maize as a potential trap crop for management of *Eldana saccharina* Walker (Lep., Pyralidae) in sugarcane. *Journal of Applied Entomology* **131**: 241–250.

KRANTHI, K.R., DHAWAD, C.S., NAIDAU, S., MATE, K., PATIL, E. & KRANTHI S. 2005. Bt-cotton seed as a source of *Bacillus thuringiensis* insecticidal Cry1Ac toxin for bioassays to detect and monitor bollworm resistance to Bt-cotton. *Current Science* **88**: 796–800.

KRUGER, M., VAN RENSBURG, J.B.J. & VAN DEN BERG, J. 2012. Reproductive biology of Bt-resistant and susceptible field-collected larvae of the maize stem borer, *Busseola fusca* (Lepidoptera: Noctuidae). *African Entomology* **20**: 35–43.

KRUGER, M., VAN RENSBURG, J.B.J. & VAN DEN BERG, J. 2014. No fitness costs associated with resistance of *Busseola fusca* (Lepidoptera: Noctuidae) to genetically modified Bt-maize. *Crop Protection* **55**: 1–6.

MATTHEE, J.J. 1974. Pest of graminaceous crops in South Africa. Entomology Memoir No. 40. *Department of Agricultural Technical Services. Pretoria.* South Africa.

PADIDAM, M. 1992. The insecticidal crystal protein Cry1A(c) from *Bacillus thuringiensis* is highly toxic for *Heliothis armigera*. *Journal of Invertebrate Pathology* **59**: 109–111.

PILCHER, C.D., RICE, M.E., OBRYCKI, J.J. & LEWIS, L.C. 1997. Field and laboratory evaluations of transgenic *Bacillus thuringiensis* corn on secondary lepidopteran pests (Lepidoptera: Noctuidae). *Journal of Economic Entomology* **90**: 669–678.

PONS, X., LUMBIERRE, S.B., LÓPEZ, C. & ALBAJES, R. 2005. Abundance of non-target pests in transgenic *Bt*-maize: a farm scale study. *European Journal of Entomology* **102**: 73–79.

ROMEIS, J., BARTSCH, D., BIGLER, F., CANDOLFI, M.P., GIELKENS, M.M.C., HARTLEY, S.E., HELLMICK, R.L., HUESING, J.E., JEPSON, P.C., LAYTON, R., QUEMADA, H., RAYBOULD, A., ROSE, R.I., SCHIEMANN, J., SEARS, M.K., SHELTON, A.M., SWEET, J., VAITUZIS, Z. & WOLT, J.D. 2008. Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. *Nature Biotechnology* **26**: 203–208.

STORER, N.P., PECK, S.L., GOULD, F., VAN DUYN, J.W. & KENNEDY, G.G. 2003. Spatial processes in the evolution of resistance in *Helicoverpa zea* (Lepidoptera: Noctuidae) to Bt transgenic corn and cotton in mixed agroecosystem: a biology-rich stochastic simulation model. *Journal of Economic Entomology* **96**: 156–172.

STORER, N.P., VAN DUYN, J.W. & KENNEDY, G.G. 2001. Life history traits of *Helicoverpa zea* (Lepidoptera: Noctuidae) on non-Bt and Bt transgenic corn hybrids in Eastern North Carolina. *Journal of Economic Entomology* **94**: 1268–1279.

VAN WYK, A., VAN DEN BERG, J. & VAN HAMBURG, H. 2007. Selection of non-target Lepidoptera species for ecological risk assessment of Bt-maize in South Africa. *African Entomology* **15**: 356–366.

VAN WYK, A., VAN DEN BERG, J. & VAN HAMBURG, H. 2008. Diversity and comparative phenology of Lepidoptera on Bt and non-Bt-maize in South Africa. *International Journal of Pest Management* **54**: 77–78.

WU, K., GUO, Y. & GAO, S. 2002. Evaluation of the natural refuge function for *Helicoverpa armigera* (Lepidoptera: Noctuidae) within *Bacillus thuringiensis* transgenic cotton growing areas in North China. *Journal of Economic Entomology* **95**: 832–837.

Accepted 12 November 2013