Effects of Bt Maize on *Agrotis segetum* (Lepidoptera: Noctuidae): A Pest of Maize Seedlings

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ABSTRACT The lepidopteran stemborers *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae), and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) are effectively controlled by Bt maize that expresses the *Cry1Ab* insecticidal protein. Another noctuid species, the cutworm *Agrotis segetum* (Denis and Schiffermüller) (Lepidoptera: Noctuidae), which is the most common and injurious pest of maize seedlings in South Africa, is exposed to Bt toxin for a part of its life cycle. The effect of this exposure to Bt maize has not been studied yet. The aims of this study were to determine the effects of Bt maize (events MON810 and Bt11) on larval mass, development time, survival, and fecundity of *A. segetum*. Laboratory studies were conducted with first- and fourth-instar larvae and moths. Results showed that the effect of *Cry1Ab* toxin on the biology of *A. segetum* larvae and moths were largely insignificant. The effects of the two Bt maize events on the different parameters measured in this study was not similar between the Bt events and their respective iso-hybrids. Compared with larvae that fed on conventional (non-Bt) maize, Bt maize did not affect survival of first-instar larvae. However, mean mass of larvae that fed on Bt maize (Bt11) was significantly lower. Feeding on Bt maize did not have a significant effect on development and survival of fourth-instar larvae or moth longevity. It did, however, delay the development period to pupa formation. Fewer eggs were laid by moths fed as larvae on maize event Bt11 compared with MON810. This study indicates that Bt maize will most likely not have any significant effect on the control of *A. segetum* under field conditions.

KEY WORDS Bt maize, *Cry1Ab*, cutworm, *Agrotis segetum*

The target pests of Bt maize in South Africa are the lepidopterous stem borers *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae), and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). These pests are effectively controlled by *Cry1Ab* toxin of events MON810 and Bt11 (Van Wyk et al. 2009). Various cutworm species (Lepidoptera: Noctuidae) occur in South Africa namely the black cutworm (*Agrotis ipsilon*), gray cutworm (*A. subalba*), brown cutworm (*A. longidentifera*), spiny cutworm (*A. spinifera*), and the common cutworm (*A. segetum*). The latter is the only economically important species and recognized as the most common and injurious to maize seedlings (Du Plessis 2000). Damage caused by *A. segetum* larvae can be severe. During the day, larvae occur beneath the soil surface from where they emerge to be active nocturnally. At night, larvae move from one seedling to another, cutting stems near ground level that cause seedlings to die (Annecke and Moran 1982). Larvae consume part of the seedling stem and one larva can destroy several seedlings in one night (Drinkwater 1980). Larvae attacking crop seedlings are usually in the fourth and later instars of development (Blair 1975). Because moths lay their eggs on weeds and larvae are active throughout the winter it is generally accepted that an abundance of winter weeds may enhance cutworm infestations (Drinkwater 1980).

There are currently no transgenic maize hybrids in South Africa targeting *A. segetum* and the effect of *Cry1Ab* toxin on this species has not been reported. Lambert et al. (1996) did, however, report that *Cry9Ca* was toxic to *A. ipsilon* and *A. segetum* in Belgium. The *Cry1Ab* toxin is very selective for Lepidoptera (Pons et al. 2005, Eizaguirre et al. 2006) and the Monsanto user guide for the production of YieldGard (MON810) maize states that MON810 has no effect on cutworm (Monsanto 2007). In a field evaluation on Bt maize in Spain conducted by Eizaguirre et al. (2006), no effects by *Cry1Ab* (Event 176) on the percentage of plants killed by *A. segetum* were observed. Van Wyk et al. (2008) reported that the incidence of seedling damage caused by *A. segetum* in South Africa was significantly higher on a non-Bt than a Bt field. In the latter study, however, again only the incidence of cutworm damaged plants was recorded and the possible effect that exposure to Bt toxin could have had on larvae were not studied.

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An assessment of the ecological effects of Bt maize on components of the maize biocenosis other than stem borers is essential (Pons et al. 2005). Crawley (1999) emphasized the need to study the effects of genetically modified crops on the demography of nontarget species over their entire life cycle and several generations in the field. In selection of nontarget Lepidoptera species for ecological risk assessment of Bt maize in South Africa, Van Wyk et al. (2007) identified species that can be regarded as priority species for testing. Five nontarget Lepidoptera species including *A. segetum* were recommended for inclusion in postrelease monitoring of Bt maize in South Africa. Based on their distribution and the fact that the bionomics of *A. segetum* is well known, it could also be recommended for inclusion in prerelease testing (Van Wyk et al. 2007).

The objective of this study was to determine the effect of Bt maize expressing *Cry1Ab* toxin (events MON810 and Bt11) on *A. segetum* larval survival and mass gain as well as fecundity and fertility of moths.

Materials and Methods

Larval Survival Studies

Two studies, one with neonate larvae and the other with fourth instars, were conducted to determine the effect of Bt maize on larval growth and survival. These studies involved laboratory bioassays in which maize seedlings were fed to larvae. The "whole plant method" suggested by Birch et al. (2004) was used to evaluate the effect of transgenic maize (not only the transgenic product), which in the case of cutworm is only the maize seedling.

The Bt maize events MON810 and Bt11 are the only registered maize events with insecticidal properties in South Africa and both were evaluated in this study. The following four varieties were used: DKC 7815B (transgenic, MON810), CRN 3505 (non-Bt iso-hybrid for DKC 7815B), NK Mayor B (transgenic, Bt11), and Brasco (non-Bt iso-hybrid for NK Mayor B).

Experiment 1: Neonate Larvae. Larvae for the use in this experiment originated from larvae that were reared on artificial medium for one generation after collecting larvae from maize fields. First-instar survival and mass increase were evaluated under laboratory conditions. The experimental layout was a completely randomized design. Seven to 10-d-old seedlings were placed in test tubes (15 by 1.5 cm). One first-instar larva was placed per test tube that was plugged with cotton wool. Test tubes were kept in an incubator at 25°C and 65% RH. Each maize hybrid was replicated 50 times. Seedlings were replaced with new seedlings and larvae were weighed at 3- to 4-d intervals. Larvae were weighed until the pupal stage was reached. Percentage pupation over time was also determined. Autoclaved soil was placed in the test tubes when larvae reached the second instar to prevent accumulation of unnecessary moisture.

Experiment 2: Fourth Instars. This experiment was conducted using fourth instars, using the same methods described above. To obtain larger larvae of uniform age, first-instar larvae were fed spinach until they reached the fourth instar before they were used in the experiment. Because the weed species, *Amaranthus hybridus* (Amaranthaceae), was reported to support high levels of survival of *A. segetum* larvae (Mabuda 2001), larvae used in this study were reared on spinach [*Spinacia oleracea* (Amaranthaceae)], which belongs to the same plant family as *A. hybridus.* Each treatment was replicated 70 times. Larvae were starved for one day before the onset of the experiment. Larval survival and mean larval mass were recorded every 3–4 d until the onset of pupation. The duration of larval development from the onset of the experiment to pupation was recorded.

Oviposition Experiment

The fecundity, fertility, and longevity of moths derived from larvae fed on Bt- and non-Bt maize from the fourth instar until pupation was determined. Two larval colonies were maintained on Bt and non-Bt maize seedlings. One male and one female moth were kept in a round plastic container (9 by 12 cm) and were replicated 20 times. The container's opening was covered with gauze to serve as oviposition site. A zig-zag folded white paper (5 by 8 cm) was placed inside each container to allow for a daytime hiding place for moths, as well as extra oviposition sites. A non-Bt maize seedling leaf was placed inside each container to provide possible oviposition stimuli to moths. Drinking water was provided by means of a sponge (1.5 by 1.5 cm) saturated with sugar water. Mortality of male and female moths was recorded at 24-h intervals, and eggs were collected until moths died. The number of eggs laid each night as well as the number of eggs that hatched per moth was recorded and expressed as a percentage.

Data Analysis

Repeated-measures analysis of variance (ANOVA) were used to analyze larval mass, larval survival, and percentage pupation over time (STATISTICA 8.0). Data on larval mass were $\log(x + 1)$ transformed before analyses. Untransformed data are, however, provided in the figures. Longevity, fertility, and fecundity data were analyzed using one-way ANOVA (STATISTICA 8.0).

Results and Discussion

Larval Survival Studies

Experiment 1: Neonate Larvae. A differential response of larvae to the different Bt maize events were observed with the mass of larvae feeding on Bt maize (NK Mayor B) being significantly lower than those that fed on the non-Bt iso-hybrid (Brasco) ($F_{(1,98)} = 18.179$; $P = 0.00005$; Fig. 1A). However, mean larval mass was not significantly different between larvae feeding on the other pair of hybrids (CRN 3505 and DKC 78–15B; $F_{(1,98)} = 2.4038$; $P = 0.1242$; Fig. 1A). Mass of larvae that fed on Brasco (non-Bt) seedlings increased more rapidly than larvae that fed on NK Mayor B (Bt). There was a

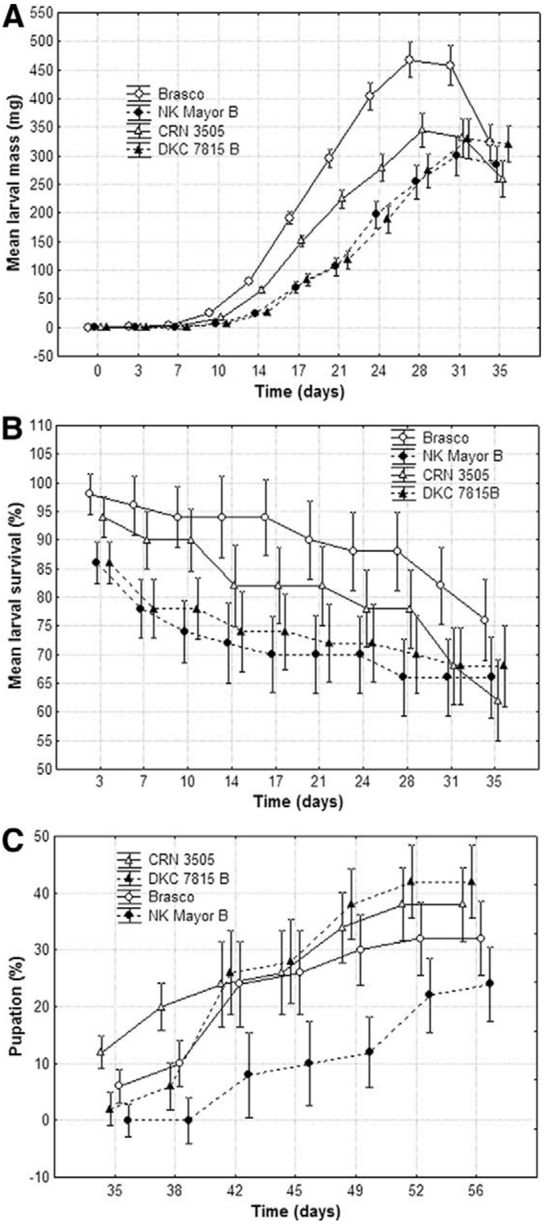

Fig. 1. (A) Mass, (B) survival, and (C) pupation of *A. segetum* larvae feeding on maize seedlings from the first instar onward. Event MON810 (DKC 78–15B) and its non-*Bt* iso-hybrid (CRN 3505) and event Bt11 (NK Mayor B) and its non-*Bt* iso-hybrid (Brasco). Bars indicate SE.

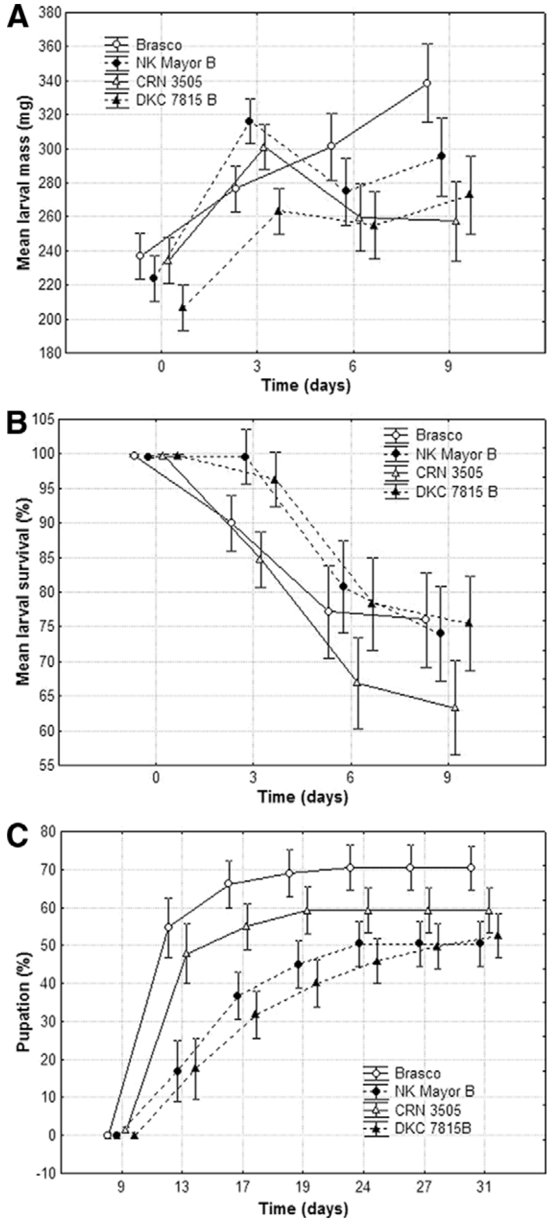

Fig. 2. (A) Mass, (B) survival, and (C) pupation of *A. segetum* larvae feeding on maize commencing seedlings as fourth-instar larvae. Event MON810 (DKC 78–15B and non-Bt iso-hybrid CRN 3505) and event Bt11 (NK Mayor B and non-Bt iso-hybrid Brasco). Bars indicate SE.

significant difference in larval mass between the two non-Bt hybrids ($F_{(1,98)} = 4.419$; $P = 0.038$) but not between the two Bt hybrids ($F_{(1,98)} = 0.144$; $P = 0.705$).

Larval survival decreased slowly over time but did not differ significantly between Bt and non-Bt maize seedlings for treatments CRN 3505 (non-Bt) and DKC 78–15B (Bt) ($F_{(1,8)} = 1.7925$; $P = 0.217413$) or Brasco (non-Bt) and NK Mayor (Bt; $F_{(1,8)} = 4.886$; $P = 0.058$; Fig. 1B).

The percentage pupation of larvae that studied feeding on maize as first instars did not differ significantly between CRN 3505 (non-Bt) and DKC 78–15B (Bt; $F_{(1,8)} = 0.021$; $P = 0.887$) or Brasco (non-Bt) and NK Mayor (Bt; $F_{(1,8)} = 3.741$; $P = 0.089$; Fig. 1C).

This study showed that feeding on Bt maize did not have a significant effect on survival of first-instar *A. segetum* larvae compared with feeding on conventional maize. Some effects were, however, observed with regard to mean larval mass. When first-instar larvae fed on maize event Bt11 seedlings for their entire larval period,

Table 1. Fecundity, fertility, and female and male longevity of *A. segetum* moths originating from larvae fed on *Bt* and conventional maize seedlings from the fourth instar onward

	Event: Bt11 with iso-hybrid		Event: MON810 with iso-hybrid	
	Brasco	NK Mayor B	CRN 3505	DKC 7815 B
Mean no. of eggs laid (±SE)	292.5 (±40.67)	134.4 (±35.06)	209.1 (±36.76)	201.0 (±30.51)
F-value	$F_{(1,36)} = 0.42$		$F_{(1,49)} = 0.02$	
P value	0.0077		0.8653	
Percent hatched (±SE)	64 (±7.82)	70 (±11.79)	40 (±7.26)	81 (±6.97)
F-value	$F_{(1,34)} = 0.18$		$F_{(1,48)} = 16.35$	
P value	0.6726		0.2462	
Mean female moth longevity (d) (±SE)	8.7 (±0.517)	8.1 (±0.811)	9.3 (±0.812)	7.7 (±0.550)
F-value	$F_{(1,36)} = 0.42$		$F_{(1,41)} = 3.05$	
P value	0.5206		0.0878	
Mean male moth longevity (d) (±SE)	7.0 (±0.716)	7.5 (±0.609)	5.4 (±0.658)	6.5 (±0.614)
F-value	$F_{(1,36)} = 0.16$		$F_{(1,41)} = 1.38$	
P value	0.6871		0.2462	

Event MON810 (DKC 78–15B and non-*Bt* iso-hybrid CRN 3505) and event Bt11 (NK Mayor B and non-*Bt* iso-hybrid Brasco).

larvae were smaller compared with larvae feeding on non-Bt seedlings. Larvae feeding on non-Bt seedlings reached the maximum mass sooner than larvae feeding on Bt seedlings, indicating that the former will reach physiological maturity faster. Larvae feeding on the non-Bt hybrid, Brasco, were significantly heavier than on any other hybrid indicating that this hybrid was more suitable as host for larval development.

Experiment 2: Fourth Instars. There where no significant differences between the mass of fourth instars feeding on either CRN 3505 (non-Bt) and DKC 78–15B (Bt; $F_{(1,142)} = 0.703$; $P = 0.403$), or Brasco (non-Bt) and NK Mayor (Bt; $F_{(1,142)} = 0.086$; $P = 0.769$; Fig. 2A). Similarly, no significant differences were observed in larval survival between Bt and non-Bt cultivars [Fig. 2B; $F_{(1,12)} = 1.630$; $P = 0.226$ for CRN 3505 (non-Bt) and DKC 78–15B (Bt; $F_{(1,12)} = 0.412$; $P = 0.533$) for Brasco (non-Bt) and NK Mayor].

The pupal stage started on day 9 on the non-Bt hybrid CRN 3505 and day 13 for the other hybrids (Fig. 2C). The incidence of pupation of larvae over time was significantly higher on non-Bt maize (Brasco) than on Bt maize (NK Mayor B; $F_{(1,12)} = 29.045$; $P = 0.00016$). However, no significant differences were observed between treatments CRN 3505 (non-Bt) and DKC 78–15B (Bt; $F_{(1,12)} = 2.605$; $P = 0.1325$).

Oviposition Experiment

Data on the effects of the consumption of Bt maize on fertility and fecundity of moths are provided in Table 1. In one of the treatments combinations, moths originating from larvae feeding on the non-Bt hybrid (Brasco) produced significantly more eggs than those from the Bt hybrid. Also, only in one of the treatments combinations was fertility significantly higher in moths originating from larvae feeding on Bt maize (DKC 7815B; Table 1).

The mean longevity of female or male moths did not differ significantly between any of the treatments (Table 1).

Results showed that the effect of *Cry1Ab* toxin on the biology of *A. segetum* larvae and moths were largely insignificant. Because small cutworm larvae do not feed on maize seedlings under field conditions, it is not realistic to extrapolate these results on first instars to field situations. This study was, however, conducted to determine the effects of Bt maize on cutworm larvae at the highest levels of exposure possible.

Cutworm moths lay their eggs on weeds (Drinkwater and Van Rensburg 1992) where larvae start feeding until the fourth instar. Only these larger larvae feed on maize seedlings. Blair (1975) reported that larvae attacking crop seedlings are usually fourth instars, which is why it was scientifically more realistic to rear larvae on another host plant until the fourth instar before using them in experiments.

Although there were no significant differences between survival and mass of fourth-instar larvae in the different treatments, significant differences were observed in the percentage pupation over time. Larvae feeding on non-Bt seedlings of hybrid Brasco reached a higher percentage pupation over a shorter period of time compared with larvae feeding on event Bt11. Under field conditions, this can possibly influence the number of seedlings that larvae may feed on before pupation. A delay in the onset of pupation may therefore result in more seedlings being damaged in a Bt maize field. Dutton et al. (2002) also showed that another noctuid species, *Spodoptera littoralis* (Boisduval), a polyphagous lepidopteran species, was partly affected by *Cry1Ab.* The survival rate and the time required to reach the second instar were affected significantly when larvae were reared on Bt maize compared with larvae reared on non-Bt maize.

No detrimental effects were observed with regard to moth longevity when fourth instars were fed Bt seedlings. Because pupal mass differed in one case, being lower in moths derived from Bt maize, it can be expected that these moths will lay fewer eggs, in accordance with observations by Moawad (1983) on *A. ipsilon.* Moawad (1983) reported a positive relationship between the numbers of eggs laid and the weight of the female pupa of *A. ipsilon.* It can thus be concluded that if the mass of pupae are reduced on Bt maize, fewer eggs may be laid compared with pupae derived from non-Bt maize. This was, however, only the case for the Bt 11 and not for MON810.

Weeds in maize fields cause an increase in cutworm numbers (Norris and Kogan 2000). In South Africa, this leaves the farmers with a choice between early cultivation or the use of chemicals to control *A. segetum* (Du Plessis 2000). It seems that Bt maize events MON810 and Bt11 will have no effect as control method for cutworm. Although some adverse effects, which were dependent on the Bt maize event, were observed on larval mass and number of eggs laid, it seems that the effect on cutworm under field conditions will be negligent. Pilcher et al. (1997) and Koziel et al. (1993) reported that *A. ipsilon* was not affected by the *Bacillus thuringiensis* protein (*Cry1Ab*) even when high concentrations of Bt protein were present in the leaves. However, *A. ipsilon* showed susceptibility to a separate subspecies (Burges 1981), so there is potential for using a *B. thuringiensis* crystal protein to control this pest.

It can be concluded that, although significant effects of transgenic maize expressing *Cry1Ab* on *A. segetum* was observed in some instances under laboratory conditions, Bt maize events MON810 and *Bt* 11 will most likely not have any effect on this nontarget pest under field conditions.

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