Resistance to Bt Maize in *Busseola fusca* (Lepidoptera: Noctuidae) From Vaalharts, South Africa

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ABSTRACT The Þrst report of resistance of the maize stem borer [*Busseola fusca* (Fuller)] to Bt maize (MON810) was made in the Christiana area of South Africa during 2007. The objective of this study was to evaluate the status of resistance of other populations of *B. fusca* to Bt maize. One greenhouse and two laboratory studies were conducted. *B. fusca* populations were collected on Bt maize as well as the adjacent refugia (conventional maize and non-Bt maize) in the Vaalharts area, 50 km from the Christiana site. Control populations were collected from sites where Bt maize was not planted. In the greenhouse study 720 potted plants were each artiÞcially infested with 10 neonate larvae of the F1-generation after the Þeld collected populations were reared through to adults. Numbers of live larvae and larval mass per plant were determined at regular intervals over a 35-d period. Larvae of the Christiana conventional population (Bt-susceptible) on Bt maize (CHR08Con-Bt) and Bethal conventional population (Bt-susceptible) on Bt maize (BET08Con-Bt) did not survive on Bt maize for longer than 12 d. The populations collected from both Bt (VAA08Bt-Bt) maize and refuges (VAA08Ref-Bt) at Vaalharts were resistant and the subsequent generation of larvae completed their life cycle on Bt maize. Similar results were observed in the laboratory experiments. This study conÞrmed resistance of *B. fusca* to the Cry1Ab toxin (MON810). The geographical distribution of resistance was shown to include at least the Vaalharts area, in addition to the original report for the Christiana area. These observations that larvae collected from refugia at Vaalharts was resistant, show that the efÞcacy of the refuge strategy is compromised in this area because the contribution of refugia did not produce large enough numbers of susceptible individuals to mate with moths of which larvae survived inside Bt maize Þelds.

KEY WORDS resistance evolution, insect resistance management, refugia

Large-scale planting of Bt crops in the world began during 1996 with South Africa quickly adopting Bt maize and cotton for control of lepidopteran pests. Based on surface area planted to genetically modiÞed (GM) crops, South Africa is currently ranked the eighth in planting these crops in the world (James 2009).

Since the Þrst deployment of Bt crops there has been concern with regard to evolution of resistance of target pests (Gould 1998). The ability of Lepidoptera to evolve resistance to Bt toxins have been noted by Tabashnik (1994) who indicated that species in the Noctuidae, Pyralidae, and Plutellidae can develop resistance when exposed to Bt in selection experiments under laboratory conditions and in the Þeld. The large scale use of Bt crops have put considerable selection pressure on target species. To date Þeld evolution of resistance has been rare and only detected in *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in South Africa (Van Rensburg 2007); *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in the southeastern United States (Tabashnik 2008, Tabashnik et al. 2008a); and *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in Puerto Rico (Matten et al. 2008, Storer et al. 2010). Resistance to Bt cotton has also recently been reported in the pink bollworm [*Pectinophora gossypiella* (Saunders)] (Lepidoptera: Gelechiidae) in India (Monsanto 2010).

The importance of refugia in the delay of resistance evolution has been pointed out by several authors (Tabashnik et al. 2003, Bourguet 2004, Tabashnik 2008). Refuges are deÞned as habitats in which the target pest is not under selection pressure because of the toxin and it therefore provides a sustainable habitat for pest development. The principle underlying the high dose and refuge strategy is that any resistant insects emerging from the Bt crop are more likely to mate with one of the much larger number of susceptible pest insects emerging from refugia than with each other, thereby decreasing the selection of Bt resistance alleles (Bourguet 2004). Analysis of more than a decade of resistance monitoring data for six Lepidoptera species targeted by Bt maize and cotton suggests that the principles of the refuge strategy may apply in the Þeld (Tabashnik et al. 2008b).

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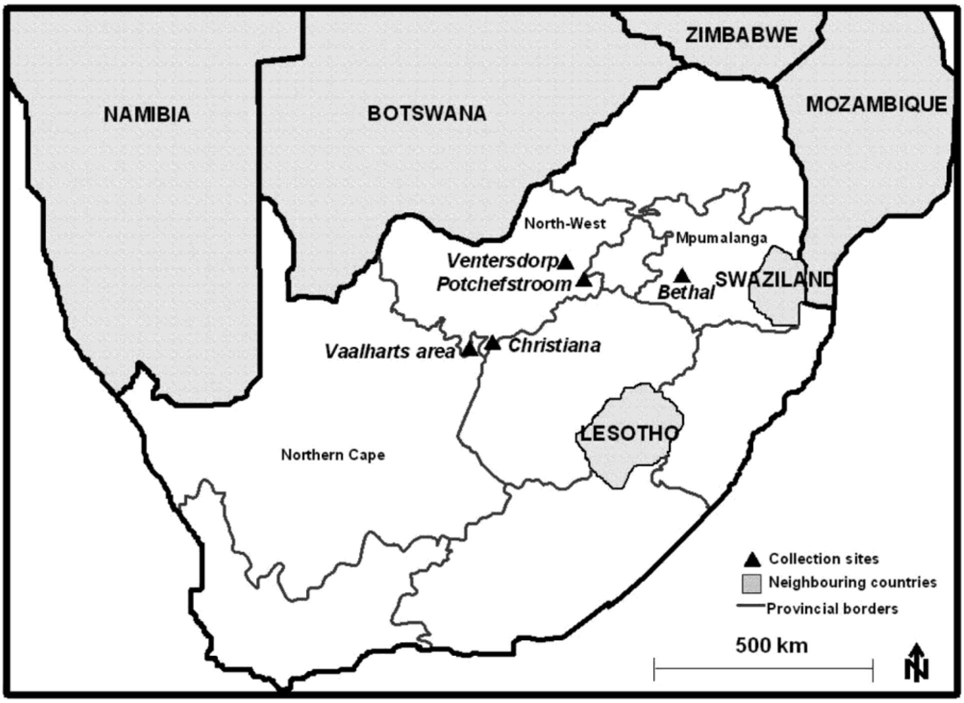

Fig. 1. Collection sites of different *B. fusca* populations in South Africa.

The Þrst evaluation of various Bt maize events for control of the maize stem borer, *B. fusca* in South Africa was conducted between the 1994 and 95 and 1996 and 1997 growing seasons (Van Rensburg 1999). This test was done by means of Þeld and greenhouse evaluations of Bt maize under artiÞcial infestation with the target pest. Results concluded that various events of the *Cry1Ab* gene were not equally effective for control of *B. fusca.* MON 810 and MON 802 were reported to be superior to other events, both in inbred material and in hybrid combinations. While Bt 11 has been released in South Africa the majority of hybrids contain event MON810. Bt maize was released in South Africa, during the 1998 and 1999 growing season when =50 000 ha were planted. The area planted to Bt maize increased to =1 million ha in 2007. At harvest of the 1999 growing season, crop damage to the lower stems caused by *B. fusca* was noticed on a considerable scale at a number of localities, involving various Bt maize hybrids (Van Rensburg 2001). Van Wyk et al. (2007) also reported increased incidence of *B. fusca* larvae on Bt maize during the postßowering period on the Highveld region of South Africa. Because no leaf feeding damage occurred during the vegetative growth stages of these plants, this damage seemed to indicate increased survival of larvae resulting from late oviposition, presumably during the period from tasseling to grain Þlling. No yield losses could be attributed to these infestations, but the observation caused concern because of the possibility that similar infestations may in future result in signiÞcant damage to ears, particularly in years when late spring rains necessitate the use of relatively late planting dates (Van Rensburg 2001). It also could contribute toward the evolution of Bt-tolerant stem borer populations. Because YieldGard technology (Monsanto, South Africa) claims control of only the Þrst two larval instars, it seems probable that increased survival of neonate and second instar larvae on Bt maize may result from feeding on some less toxic plant parts followed by stem tunneling at late plant growth stages (Van Rensburg 2001).

The Þrst report of Þeld resistance of *B. fusca* to Bt maize was subsequently made in the Christiana area by Van Rensburg (2007) who showed that the larvae on Bt maize at certain locations attained a level of resistance where some larvae were able to survive in the presence of the Bt toxin but not without some detrimental effect on larval growth rate.

Within one year of the Þrst report of resistance of *B. fusca* another reportedly resistant population was observed by farmers at the Vaalharts irrigation scheme, =50 km from the initial site.

The objective of this study was to evaluate the status of resistance of different populations of *B. fusca* to Bt maize and to evaluate the level of resistance of borer populations occurring on the refuge-plantings of maize at the locations where resistance was reported.

Materials and Methods

One greenhouse and two laboratory studies were conducted during 2008 and 2009, respectively.

Greenhouse Study (January–February 2008). *Collection Sites.* Four *B. fusca* populations were collected during December 2007 at three localities (Fig. 1) from infested maize plants that were between four and seven weeks old. Larvae were collected by dissecting between 100 and 200 plants inside the respective Bt and conventional and non-Bt Þelds described below. Approximately 250 fourth-instar larvae were collected from each of the Bt and conventional maize Þelds at each locality, reared through to adults on Bt and conventional maize respectively and the F1-generation used in the experiment.

After farmers reported another possible resistant population in the Vaalharts irrigation scheme, larvae were collected from Bt maize (designated VAA08Bt), as well as the refuge of conventional maize planted adjacently to the Bt maize Þeld (designated VAA08Ref). These Þelds were planted as prescribed in the user guide for the production of YieldGard maize (Monsanto 2007).

A susceptible population (designated CHR08Con) was collected on conventional maize planted on a farm near Christiana where Van Rensburg (2007) reported resistance, 50 km from the Vaalharts site (Fig. 1). Another susceptible population of stem borers outside of the Vaalharts region was collected from conventional maize in the Bethal area (Mpumalanga Province) ≈450 km away from the above mentioned sites (designated BET08Con). Planting of Bt maize is less common in the Mpumalanga Province where dry land maize production is the norm. The larvae were reared to adult stage and the F1 used in the larval survival study that was done under greenhouse conditions.

Larval Survival Study. The experiment comprised of eight treatments which consisted of four stem borer populations evaluated on two maize hybrids. These stem borer populations were designated the VAA08Bt, VAA08Ref, CHR08Con, and BET08Con populations, which were tested on each of a Bt- and conventional hybrid. The following two hybrids were used: DKC 78-15B (transgenic, MON810); and CRN 3505 (non-Bt iso-hybrid for DKC 78-15B).

The study was conducted in a greenhouse using potted maize plants. There were 90 potted plants for each treatment. Plants were infested by placing 10 neonate larvae into the whorl of each plant by means of a camel-hair brush, 20 d after plant emergence. Nine plants of each treatment were dissected 2, 4, 6, 8, 12, 16, 20, 25, 30, and 35 d after inoculation. Dissection of plants inoculated with larvae from the BET08Con population was terminated on day 25 because of a limited number of available F1 neonate larvae and infested plants.

The numbers and mass of live larvae per plant were determined at each sampling date. The experiment was terminated on day 35 when the Þrst pupae started to form. For this reason larval mass determined on day 30 was used in analyses to avoid the effect that the change to pupae could have on mass. Mean larval mass was calculated for each sampling date and survival was expressed as percentages.

The percentage and number of dead and live larvae of the different borer populations on Bt and conventional maize, 30 d after inoculation was also calculated. Lethal time (LT50), indicating the time (number of days) until 50% mortality was observed was calculated for each population.

Laboratory Study (February–March 2009). *Collection Sites.* In total, ≈1200 fourth-instar stem borer larvae (200 per farm) were collected from Bt maize on six farms at the Vaalharts irrigation scheme during December 2008, after reports of larvae surviving on Bt maize were received from farmers. Each population was reared on Bt maize for one generation to conÞrm resistance (reported below), after which populations were pooled (designated VAA09Bt). One population was also collected from the refugia (200 larvae) consisting of conventional maize on one of the six farms (designated VAA09Ref). A susceptible control population of 150 larvae was also collected from conventional maize at Viljoenskroon (designated Vil09Con) in the Free State province, 220 km away from the Vaalharts irrigation scheme. At this locality maize is planted under dry land conditions and Bt maize is less common. The F1-neonate larvae originating from populations collected from the Bt-, refuge-, and conventional Þelds were used in the laboratory experiment.

Larval Survival and Mass. The experiment consisted of six treatments viz. three stem borer populations (VAA09Bt, VAA09Ref, and Vil09Con) on each of a Bt- and conventional hybrid. The following two hybrids were used: DKC 78Ð15B (transgenic, MON810); and CRN 3505 (non-Bt iso-hybrid for DKC 78Ð15B).

Larvae were reared in glass test tubes (200 by 25 mm) containing a cut maize stem with the base of the whorl still intact. Stems were cut from 4- to 6-wk-old potted plants grown in a greenhouse. Ten neonate larvae per test tube were placed into the whorl of the cut stem. For the Þrst 15 d whorl tissue was provided because the stem was cut ≈10 cm below the whorl of the plant and 5 cm of the base of the whorl was left intact. From 18 d onwards, when larvae reached late third and fourth instars, only a 15-cm-long cut stem was provided as food. All tubes were kept at room temperature that ranged between 20 and 25 C0.

To ensure representative samples of each stem borer population, each test tube contained larvae originating from a different female moth. Because of the shortage of female moths, there were nine replicates for the Vil09Con, while the VAA09Ref and VAA09Bt consisted of 21 and 29 replications (offspring per female), respectively.

The number and mass of live larvae per plant were determined at 3-d intervals up to 35 d after inoculation. The experiment was terminated on day 35 when the Þrst prepupae were observed. Survival was recorded and expressed as percentages. Data on larval mass determined on day 30 was used in analysis to avoid the effect that the change to pupae could have on mass. The percentage and number of dead and live larvae 30 d after inoculation was calculated. Lethal time (LT50) was calculated for each population.

Statistical Analysis. Mean mass per larva was calculated for each of the different populations at day 30 after inoculation and compared between populations by means of analysis of variance (StatSoft 2009) after

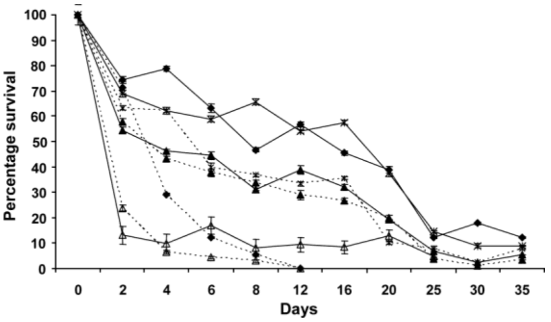

Fig. 2. Larval survival of different *B. fusca* populations on Bt and conventional maize under greenhouse conditions (Bars indicate Standard errors). Solid lines represent conventional maize and dotted lines represent Bt maize. Refuge and Bt populations were collected at Vaalharts, the Conventional population at Christiana and Bethal population at Bethal. VAA08Ref-Bt = Refuge population on Bt maize; VAA08Ref-NBt = Refuge population on conventional maize; VAA08Bt-Bt = Vaalharts Bt population on Bt maize; VAA08Bt-NBt = Vaalharts Bt population on conventional maize; CHR08Con-Bt = Christiana conventional population on Bt maize; CHR08Con-NBt = Christiana conventional population on conventional maize; BET08Con-Bt = Bethal population on Bt maize; BET08Con-NBt = Bethal population on conventional maize.

which means were separated by means of the Tukey test for signiÞcant differences. Lethal time (LT50), indicating the time (number of days) until 50% mortality was observed for each population was calculated using logistic regressions of larval survival over time (StatSoft 2009). Fiducial limits were calculated for each population.

Results

Greenhouse Study (January–February 2008; F1). *Larval Survival.* On Bt maize the lowest levels of survival were observed for the populations collected at Christiana (designated CHR08Con-Bt) and the Bethal population (BET08Con-Bt). Survival of these two populations decreased rapidly until 100% mortality was observed within 12 d (Fig. 2). The highest levels of survival were observed for the VAA08Ref-NBt and the CHR08Con-NBt populations on conventional maize. The F1-generation of the VAA08Bt-Bt population collected from Bt maize and the adjacent refuge planting (VAA08Ref-Bt) survived on Bt maize plants until the pupal stage was reached with 15.5 and 7.7% survival, respectively (Fig. 2). Survival of the VAA08Ref and VAA08Bt maize-collected populations was similar on Bt- and conventional maize, respectively. The VAA08Ref-Bt population had the lowest LT50 of 3.41 d and BET08Con-NBt population on conventional maize the highest LT50 of 4.8 d (Table 1). There were no signiÞcant differences observed between LT50-values of the VAA08Bt-Bt and VAA08Ref-Bt populations tested on Bt maize (Table 2).

Table 1. LT 50 (number of days) of different *B. fusca* populations on Bt and conventional maize in a greenhouse study

Population	LT50 (d)	95% Fiducial limits
BET08Con-Bt	2.22	1.82Ð2.60
CHR08Con-Bt	2.41	2.08Ð2.72
VAA08Bt-NBt	3.21	2.47Ð3.95
VAA08Bt-Bt	3.27	2.47Ð4.05
VAA08Ref-Bt	3.41	2.70Ð4.11
BET08Con-NBt	4.80	4.27Ð5.32
VAA08Ref-NBt	7.21	6.10Ð8.33
CHR08Con-NBt	8.71	7.49Ð9.95

VAA08Ref-Bt = Refuge population on Bt maize, VAA08Ref-NBt = Refuge population on conventional maize, VAA08Bt-Bt = Vaalharts Bt population on Bt maize, VAA08Bt-NBt = Vaalharts Bt population on conventional maize, CHR08Con-Bt = Christiana conventional population on Bt maize, CHR08Con-NBt = Christiana conventional population on conventional maize, BET08Con-Bt = Bethal population on Bt maize, BET08Con-NBt = Bethal population on conventional maize.

Larval Mass. Mean mass increased steadily and did not differ signiÞcantly between any of the treatments up to 25 d after inoculation (Fig. 3). Because of the shortage of inoculated plants for the BET08Con population only one dissection was done 25 d after inoculation. Larvae of this population had the greatest mass on day 25.

There were signiÞcant differences in mean larval mass between different populations collected from VAA08Ref, VAA08Bt, and CHR08Con maize on day 30 (Table 3) with larvae from the CHR08Con-NBt population on conventional maize being the largest. There were no signiÞcant differences in larval mass between the VAA08Bt-Grh populations collected from Bt and conventional maize. However, larvae from the CHR08Con population collected at Christiana were signiÞcantly heavier than any of the other populations from Vaalharts.

Laboratory Study (February–March 2009; F2). *Larval Survival.* 100% mortality was observed for larvae of the Vil09Con-Bt population, 6 d fter inoculation onto Bt maize. The highest levels of survival were observed for larvae of the VAA09Bt-Bt population on Bt maize and those collected from the VAA09Ref-Bt population feeding on Bt maize (Fig. 4). The decline in larval survival of the Vil09Con-NBt population from Viljoenskroon on conventional maize was similar to that of

Table 2. LT 50 (number of days) of different *B. fusca* populations on Bt and conventional maize in a laboratory experiment

Population	LT50 (d)	95% Fiducial limits
Vil09Con-Bt	3.02	Ð
VAA09Ref-Bt	6.84	5.77Ð7.83
Vil09Con-NBt	7.57	6.41Ð8.61
VAA09Bt-Bt	8.96	8.19Ð9.69
VAA09Ref-NBt	12.77	11.79Ð13.71
VAA09Bt-NBt	18.02	17.23Ð18.80

VAA09Ref-Bt = Refuge on Bt maize, VAA09Ref-NBt = Refuge population on conventional maize, VAA09Bt-Bt = Vaalharts Bt population on Bt maize, VAA09Bt-NBt = Vaalharts Bt population on conventional maize, Vil09Con-Bt = Viljoenskroon conventional population on Bt maize, Vil09Con-NBt = Viljoenskroon conventional population on conventional maize.

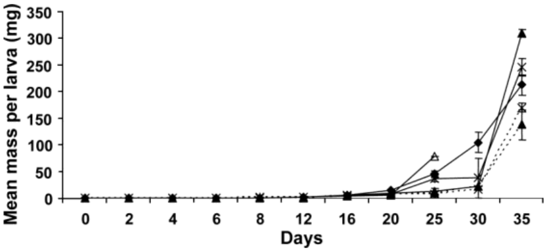

Fig. 3. Mean larval mass of different *B. fusca* populations on Bt and conventional maize under greenhouse conditions (Bars indicate Standard errors). Solid lines represent conventional maize and dotted lines represent Bt maize. Refuge and Bt populations were collected at Vaalharts, the Conventional population at Christiana and Bethal population at Bethal. VAA08Ref-Bt = Refuge population on Bt maize; VAA08Ref-NBt = Refuge population on conventional maize; VAA08Bt-Bt = Vaalharts Bt population on Bt maize; VAA08Bt-NBt = Vaalharts Bt population on conventional maize; CHR08Con-NBt = Christiana conventional population on conventional maize; BET08Con-NBt = Bethal population on conventional maize.

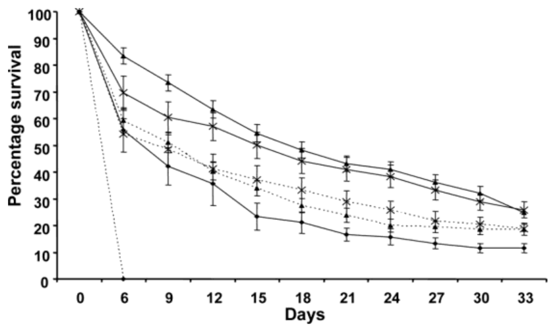

Fig. 4. Larval survival of different *B. fusca* populations on Bt and conventional maize under laboratory conditions (Bars indicate Standard errors). Solid lines represent conventional maize and dotted lines represent Bt maize. The Conventional population was collected at Viljoenskroon while Refuge and Bt populations were collected at Vaalharts. VAA09Ref-Bt = Refuge on Bt maize; VAA09Ref-NBt = Refuge population on conventional maize; VAA09Bt-Bt = Vaalharts Bt population on Bt maize; VAA09Bt-NBt = Vaalharts Bt population on conventional maize; Vil09Con-Bt = Viljoenskroon conventional population on Bt maize; Vil09Con-NBt = Viljoenskroon conventional population on conventional maize.

the VAA09Bt-Bt population on Bt maize (Fig. 4). In this study the VAA09Bt-NBt population on conventional maize had the highest LT50 (18.02 d) of the different *B. fusca* populations, while the Vil09Con-Bt population on Bt maize had the lowest LT50 and died within 3 d after inoculation (Table 2).

Larval Mass. The mean mass per larva increased steadily up to day 18 after which a rapid increase was observed for all treatments (Fig. 5). Mass of larvae of the VAA09Bt and VAA09Ref populations were significantly greater on conventional than on Bt maize compared with those in the greenhouse study. Mass of larvae of the Vil09Con-NBt population on conventional maize was greatest throughout the study and was signiÞcantly different from all the other populations 30 d after inoculation (Table 4).

Table 3. Mean larval mass and percentage survival of *B. fusca* populations feeding on Bt and conventional maize under greenhouse conditions

B. fusca pop	Mean mass (mg) on day 30 ± SE[a]	Percentage survival on day 30 ± SE[b]	Percentage survival on day 35 ± SE[c]
VAA08Ref-Bt	0.8 a ± 0.001	2.2 a ± 1.9	7.7 a ± 2.77
VAA08Bt-Bt	2.4 a ± 0.001	1.1 a ± 0.90	3.3 a ± 1.66
VAA08Bt-NBt	5.0 a ± 0.001	2.2 a ± 1.46	5.5 a ± 1.76
VAA08Ref-NBt	15.7 a ± 0.04	8.8 ab ± 5.04	8.8 a ± 3.80
CHR08Con-NBt	69.5 b ± 0.02	17.7 b ± 5.47	12.2 a ± 3.23

VAA08Ref-Bt = Refuge population on Bt maize, VAA08Ref-NBt = Refuge population on conventional maize, VAA08Bt-Bt = Vaalharts Bt population on Bt maize, VAA08Bt-NBt = Vaalharts Bt population on conventional maize, CHR08Con-NBt = Christiana conventional population on conventional maize.

[a] Means ± SE with columns followed by the same letter do not differ signiÞcantly according to Tukey test for highest signiÞcant differences ($F = 10.25$, df = 40, $P = 0.0008$).

[b] Means ± SE with columns followed by the same letter do not differ signiÞcantly according to Tukey test for highest signiÞcant differences ($F = 3.84$, df = 40, $P = 0.009$).

[c] Means ± SE with columns followed by the same letter do not differ signiÞcantly according to Tukey test for highest signiÞcant differences ($F = 1.68$, df = 40, $P = 0.171$).

Discussion

Evolution of resistance is deÞned as a heritable decrease in a populationÕs susceptibility to a toxin (Tabashnik 1994, Tabashnik et al. 2008b), Gassmann et al. 2009). Results from these studies conÞrmed resistance of *B. fusca* at Vaalharts to Bt maize because larvae that were collected from the Þeld were reared to the adult stage on Bt after which the F1-generation

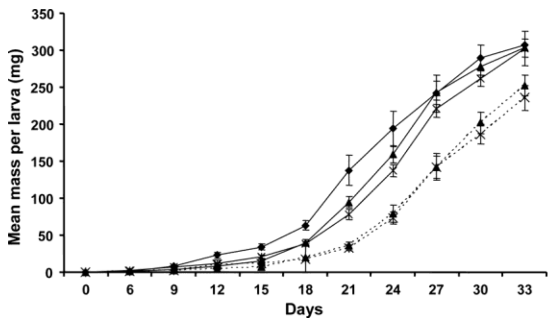

Fig. 5. Mean larval mass of different *B. fusca* populations on Bt and conventional maize under laboratory conditions (Bars indicate Standard errors). Solid lines represent conventional maize and dotted lines represent Bt maize. The Conventional population was collected at Viljoenskroon while the Refuge and Bt populations were collected at Vaalharts. VAA09Ref-Bt = Refuge on Bt maize; VAA09Ref-NBt = Refuge population on conventional maize; VAA09Bt-Bt = Vaalharts Bt population on Bt maize; VAA09Bt-NBt = Vaalharts Bt population on conventional maize; Vil09Con-NBt = Viljoenskroon conventional population on conventional maize.

Table 4. Mean larval mass and survival of *B. fusca* populations feeding on Bt and conventional maize under laboratory conditions

B. fusca pop	Mean mass (mg) on day 30 ± S[a]	Percentage survival on day 30 ± SE[b]	Percentage survival on day 33 ± SE[c]
VAA09Ref-Bt	186.5 a ± 0.26	12.0 a ± 2.45	10.3 ab ± 2.01
VAA09Bt-Bt	201.8 a ± 0.21	14.1 a ± 2.19	12.1 a ± 2.26
VAA09Ref-NBt	261.9 a ± 0.30	17.9 a ± 3.23	15.1 a ± 2.96
VAA09Bt-NBt	278.4 b ± 0.26	30.6 c ± 2.62	24.8 c ± 2.51
VIL09Con-NBt	289.9 b ± 0.16	2.4 b ± 0.94	2.4 b ± 0.94

VAA09Ref-Bt = Refuge on Bt maize, VAA09Ref-NBt = Refuge population on conventional maize, VAA09Bt-Bt = Vaalharts Bt population on Bt maize, VAA09Bt-NBt = Vaalharts Bt population on conventional maize, Vil09Con-NBt = Viljoenskroon conventional population on conventional maize.

[a] Means ± SE with columns followed by the same letter do not differ signiÞcantly according to the Tukey test for highest signiÞcant differences ($F = 10.39$, df = 140, $P = 0.0411$).

[b] Means ± SE with columns followed by the same letter do not differ signiÞcantly according to Tukey test for highest signiÞcant differences ($F = 18.14$, df = 140, $P = 0.0001$).

[c] Means ± SE with columns followed by the same letter do not differ signiÞcantly according to Tukey test for highest signiÞcant differences ($F = 13.00$, df = 140, $P = 0.0001$).

also survived on Bt maize. In the greenhouse study the observed tendencies on larval survival showed three groupings of lines (Fig. 2), indicating resistance levels that seemingly varied from susceptible to resistant. The borer population with expected resistance to Bt maize (VAA08Bt) as well as its VAA08Ref population successfully completed their life cycles on Bt maize, with no signiÞcant difference observed in either the level of survival (Table 3) or mean larval mass (Fig. 3) compared with controls on non-Bt maize. This result indicated that the larvae that occurred in the refuge were also resistant to Bt maize. The question arises whether the use of refugia could still serve its purpose in the Vaalharts irrigation scheme where resistance now seems to be wide-spread. If a large enough susceptible pest population is not maintained, alternative strategies will have to be investigated to manage the further spread of resistance.

Larvae of the Vaalharts population used in the greenhouse study were reared successfully for another three generations on Bt maize after completion of the experiment. Similarly, larvae from the laboratory experiment were successfully reared for another generation after completion of the experiment.

In the greenhouse study, larvae of both the Christiana- (CHR08Con population) and Bethal populations (BET08Con) died on Bt maize within 12 d, indicting that these populations were highly susceptible to Bt maize. The latter survival levels were similar to those observed for Bt-susceptible *B. fusca* populations during initial resistance evaluations by Van Rensburg (2001) and another noctuid species, *Sesamia calamistis* (Van den Berg and Van Wyk 2007, Van Wyk et al. 2009) on Bt maize. The survival of larvae collected from non-Bt maize at Christiana indicates that the speciÞc population that was collected was susceptible to Bt maize and that resistance was not wide spread in the area where the Þrst report of resistance to Bt maize was made by Van Rensburg (2007).

The decrease in survival over time observed in all treatments can also partly be ascribed to density dependent larval migration. A signiÞcant number of larvae disappeared because of emigration from plants and only the larvae present on plants were counted. The reduced numbers of larvae inside plants were ascribed to emigration and not mortality of larvae. Stem borer larvae have cryptic feeding habits in whorls and stems of plants, and the remains of dead or dying larvae are present and can easily be observed. Previous studies done by Van Rensburg and Van Rensburg (1987) on conventional maize under Þeld conditions reported a gradual decline in *B. fusca* survival over time until 16 d after infestation. A marked decrease occurs around 20 d after infestation and, under Þeld conditions, larvae leave the whorl and migrate to adjacent plants in a search of suitable feeding and pupating sites (Van Rensburg and Van Rensburg 1987).

An effective high dose and refuge strategy requires three main components. 1) The increase in Þtness conferred by resistance alleles must be recessive so that individuals heterozygous for a resistance allele are killed by the toxin produced by plant tissues, 2) resistance alleles must be rare so that few homozygotes survive on the Bt crop, and 3) one of the assumptions of the high dose and refuge strategy is that resistant insects selected on Bt crops mate randomly, or preferentially with susceptible insects preserved on non-Bt crops (Bourguet 2004). Results from this study indicates that the high dose and refuge strategy may be compromised in effectiveness in this geographical area because larvae that occur on non-Bt maize plants inside refugia were also shown to be resistant to Bt maize.

Although the evolution of resistance of *B. fusca* can probably be ascribed to several factors, the low initial levels of compliance to refuge requirements most likely played an important role. Although the planting of refugia is compulsory to limit evolution of resistance (Monsanto 2007), the level of compliance between 1998 and 2006 was shown to be low in the region where resistance was reported in South Africa (Kruger et al. 2009). Furthermore, Van Rensburg and Van Rensburg (1987) indicated that rainfall and humidity are important environmental factors affecting the abundance of *B. fusca* moths. Moths possibly prefer irrigated maize, which could have contributed to increased selection pressure toward the evolution of resistance to the Bt toxin (Van Rensburg 2007). Van Wyk et al. (2008) also indicated that the strong linkage of stem borers to the maize ecosystem in irrigated areas and especially the planting of Bt maize in these systems results in strong selection pressure for evolution of resistance.

This study conÞrmed resistance by *B. fusca* to the *Cry*1Ab toxin, indicating the geographical distribution of resistant populations to include at least the Vaalharts area, in addition to the original report by Van Rensburg (2007) for the Christiana area. Further research is needed on possible Þtness costs associated with resistance evolution as well as insect resistance management and the high and dose refuge strategy to limit the evolution and spread of Bt-resistant populations to other maize production regions in the country.

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