

Monitoring resistance to Bt maize in field populations of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) from smallholder farms in the Eastern Cape Province of South Africa

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Post-release monitoring of transgenic Bt maize fields for resistant pest populations is an important activity that will contribute to early identification and mitigation of resistance evolution by target pests. An effective Bt maize pest resistance monitoring programme relies on well-established baseline susceptibility data. The target pest of Bt maize in South Africa, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), has evolved resistance to Bt maize expressing Cry1Ab proteins, with numerous reports of resistance from the highveld region of the country. Although Bt maize has been cultivated in the Eastern Cape province since 2001, no data exist on the resistance status of field populations of *B. fusca* to Bt maize in this region. In view of this, *B. fusca* larvae were collected from fields in two Bt maize cultivating areas and a non-Bt maize cultivating area of the Eastern Cape for laboratory assays to determine the level of susceptibility of *B. fusca* to Bt maize. Rearing colonies of each population were established and neonate larvae from each population were used to infest non-Bt maize plants, and Bt maize of events MON810 and MON89034. All larvae maintained on MON89034 died within seven days of infestation. Survival of all *B. fusca* populations maintained on MON810 declined rapidly during the first seven days and was significantly ($P < 0.001$) lower than larval survival on non-Bt maize. Similarly, mass of surviving larvae of all populations on MON810 from the first two weeks to the 21st day was significantly ($P < 0.001$) lower than the mass of larvae on non-Bt maize. These results indicate that field-collected populations screened in this study are still susceptible to Bt maize.

Key words: *Busseola fusca*, insect resistance management, refuge planting, resistance evolution, survival.

INTRODUCTION

The African maize stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and the spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) are the most important stem borer pests of maize in South Africa (Kfir 1998). These two pest species may occur in single or mixed populations (Van den Berg *et al.* 1991). Although *C. partellus* is a highly competitive coloniser, *B. fusca* is considered to be the most destructive lepidopteran pest of maize (Kfir *et al.* 2002). *Busseola fusca* infestation may lead to a yield reduction of up to 10 % or in severe infestations, total yield loss (Van Rensburg & Bate 1987). The availability of maize genetically modified (GM) to express *Bacillus thuringiensis* (Bt) proteins constitute an important *B. fusca* management tool (Van den Berg *et al.* 2015)

since it provides convenient and cost-effective options for mitigating yield losses (Hellmich *et al.* 2008; Brookes & Barfoot 2014) caused by *B. fusca* in South Africa.

Following the introduction of Bt maize to South Africa during 1998, the pest status of *B. fusca* in the country has diminished (Gouse *et al.* 2005; Kruger *et al.* 2012a). Yield advantage of Bt maize hybrids over conventional iso-hybrids of up to 32 % has been reported from smallholder Bt maize farms in the country (Gouse *et al.* 2006) and successful deployment of Bt maize against *B. fusca* resulted in a high rate of adoption of this technology in the country (Van den Berg *et al.* 2013). Currently an increasing number of smallholder farmers in many parts of the country, including the Eastern Cape, have been introduced to Bt maize through a

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number of Government development initiatives (Fischer *et al.* 2015; Kotey *et al.* 2016). The widespread planting of Bt maize may, however, place intense selective pressure on Bt maize target pest populations to evolve resistance (Tabashnik 1994; Gassman *et al.* 2014). Insect populations have a demonstrated ability to evolve resistance to insecticides and Cry proteins through selection on novel mutations (Orr & Betancourt 2001; Tabashnik *et al.* 2013) and become resistant to previously used highly effective and widely used pesticides, including Bt sprays (Tabashnik 1994). This is particularly so in environments where the adoption of Bt maize is not coupled with the implementation of effective insect resistance management (IRM) strategies, as exemplified by resistance evolution of *B. fusca* to Bt maize on commercial farms in South Africa (Van Rensburg 2007; Kruger *et al.* 2011). The most commonly used IRM strategy involves planting of refuges of non-Bt maize adjacent to the main Bt maize crop (Tabashnik *et al.* 2003). The main assumption of the refuge strategy is that the inheritance of resistance is recessive, that the plants express a high dose of the toxin and that refuges of non-Bt plants are present (Tabashnik *et al.* 2013). Refuges of non-Bt crops are expected to sustain populations of Bt-susceptible target pests which may mate with resistant individuals that survive on the Bt crop (Gould 1998; Siegfried & Hellmich 2012). Campagne *et al.* (2013) have recently reported the dominance of at least one type of resistance of *B. fusca* to Cry1Ab protein. The refuge strategy, however, remains the principal strategy for delaying resistance evolution. In South Africa, resistance development by *B. fusca* has been largely ascribed to non-compliance to the requirement for the planting of refuges (Kruger *et al.* 2009).

Smallholder maize farming systems in South Africa are characterised by numerous small contiguous fields (Aheto *et al.* 2013; Van den Berg & Campagne 2014) and limited access to extension support (Assefa & Van den Berg 2009; Jacobson & Myhr 2012; Kotey *et al.* 2016). All these factors may compromise the management of resistance evolution of lepidopteran stem borers that infest maize (Van den Berg & Campagne 2014) and possibly facilitate the evolution of resistance of *B. fusca* to Bt maize in smallholder maize systems. In view of this, the adoption of post-release resistance monitoring programmes is vital for sustaining the efficacy of Bt maize. Monitoring and reporting of

resistance development is a key tenet of resistance management (Van den Berg *et al.* 2013). An effective monitoring programme, however, requires well-established baseline susceptibility data (Glaser & Matten 2003). Currently, resistant populations of *B. fusca* are being reported at new locations in the highveld region of South Africa on a regular basis (Van den Berg *et al.* 2013). Despite reports of the prevalence of many of the factors implicated in resistance evolution in the Eastern Cape (Assefa & Van den Berg 2009; Jacobson & Myrh 2012; Kotey *et al.* 2016), there has been no study to determine the level of resistance of *B. fusca* larvae from the province to Bt maize. The objective of this study was therefore to evaluate the status of resistance of different populations of *B. fusca* from different maize cultivating areas of the Eastern Cape to Bt maize.

MATERIAL AND METHODS

Field surveys of Bt and non-Bt maize fields

Localities were identified where Bt maize had been cultivated continuously for at least two years. In line with this, Bt maize fields in 14 localities (three fields per locality) (Table 1) were visited and inspected for the presence of stem borers during the 2014/15 maize cropping season, prior to collecting stem borer larvae for evaluation of their resistance status in 2016. Information regarding the history of Bt maize cultivation and Bt maize variety cultivated in the area were obtained and recorded (Table 1).

Collection of Busseola fusca larvae

Glaser & Matten (2003) recommended that sampling locations for Bt resistance monitoring should focus on areas where Bt crops are intensively planted since these are the areas where selection pressure is expected to be high. Thus, on the basis of history and area under Bt maize cultivation, two Bt maize cultivating areas designated as ECBt001 (30.87372°S 29.62144°E) and ECBt002 (31.08722°S 29.53661°E) were selected for *B. fusca* larvae collection surveys (Table 1). A third locality, designated as ECRef001 (31.08271°S 29.32504°E) which is a rural area in the Alfred Nzo District Municipality where only open pollinated varieties (OPV) of maize are cultivated (Table 1), was also selected for the collection of a reference population of *B. fusca* larvae. Since no stem borer larvae could be found in fields of Bt maize, maize plants

Table 1. Bt maize cultivating localities in the Eastern Cape, cultivars planted and stem borer species recorded. (*B.f* = *Busseola fusca*, *C.p* = *Chilo partellus*).

Geographic coordinate of localities visited	Estimated Bt maize area (ha)	No. of fields visited	GM maize variety in field visited	Borer spp. in nearby non-Bt fields
S30.87372°E29.62144°	325	9	PAN 5Q-749BR ¹	<i>B.f</i> , <i>C.p</i>
S31.08722°E29.53661°	619	7 5	PAN 5Q-749BR PAN 6Q-745BR	<i>B.f</i>
S31.49170°E29.49802°	30	6	PAN 5Q-749BR	<i>B.f</i> , <i>C.p</i>
S31.80815°E28.75360°	17	3	BG 3792BR	<i>B.f</i>
S30.40422°E28.51627°	219	6	PAN 4P-716BR	<i>B.f</i>
S31.49633°E27.36287°	15	3	PAN 6Q-708BR	<i>B.f</i>
S31.37500°E28.00712°	10	3	Phb 33H52B ²	<i>B.f</i>
Total	1235	42		

¹ BR indicates that variety has 'stacked' traits (Bt insect resistance + herbicide tolerance) GM maize.

² B indicates that variety is a single-gene Bt maize event.

from inside 38 home gardens (19 from ECBt001 and 19 from ECBt002) adjacent to farms where Bt maize has been cultivated continuously for at least two cropping seasons were sampled in January 2016. In the non-Bt maize cultivating area, maize plants (OPVs) were sampled from inside 10 home gardens.

Each home garden visited in each area was demarcated into three zones and between 20 and 100 maize plants (depending on the size of the garden) from within each demarcated zone were randomly selected and closely inspected for signs of borer damage, including scarified or dry leaves and shoots (dead hearts), frass, or holes bored into stems (Moolman *et al.* 2014). The number of infested plants in each home garden was recorded, after which five of the most severely damaged plants in each garden were selected and dissected to collect *B. fusca* larvae. Collected larvae were identified *in situ* and individually placed in perforated, labelled vials containing pieces of tissue from the plant part from which they were collected. The GPS coordinates, number of infested plants and the number of larvae collected from each area were recorded. A total of 145 (ECRef001), 173 (ECBt001) and 210 (ECBt002) third to fourth instar larvae were collected at the different sites.

Establishment of Busseola fusca populations for laboratory screening

Collected larvae were pooled together according to the area from which larvae were collected, after

which they were transported to the Entomology Laboratory of the Grain Crops Institute (GCI) of the Agricultural Research Council (ARC), Potchefstroom, and used to initiate three *B. fusca* populations. For each population, groups of five larvae from each area were placed in a 100 ml plastic cup containing a 4 cm piece of non-Bt maize stem and reared until pupation. Larvae were provided with a fresh maize stem piece every five days until pupation. Pupae were removed from containers, sexed and placed in oviposition cages with 30 cm long pieces of maize stems as oviposition substrate and with cut maize whorl tissue as stimulus for oviposition. Cages were maintained at room temperature (23–24 °C) and 12L:12D hour photoperiod and 50 % relative humidity (RH). Maize stems were checked daily for the presence of eggs. Egg batches were removed from the stem with the aid of a scalpel blade and placed in sterile 100 ml plastic containers with stainless steel mesh-lined lids. Eggs from each population were incubated at 60 % RH, 25–27 °C and a 14L:10D hour photoperiod until eggs hatched.

The effect of Bt and non-Bt maize on Busseola fusca larval survival and mass

The experiment to determine *B. fusca* larval survival and mass on Bt and non-Bt maize consisted of nine treatments (three *B. fusca* populations on each of three maize hybrids) each replicated four times. The experiment was laid out in a completely randomised design. Maize plants

of two Bt maize events (MON810 and MON89034) and a non-Bt maize variety (iso-hybrid of the two Bt hybrids) were used. Maize plants of Event MON810 express Cry1Ab protein while those of Event MON89034 express Cry2Ab2 + Cry1A.105. These varieties were: DKC8010 (non-Bt iso-hybrid), DKC8012B (MON810) and DKC8012BGEN (MON89034). The presence of Bt proteins inside Bt maize plants and absence in non-Bt plants was confirmed using Bt test strips (Quickstix Bt test kit, EnviroLogix, Portland, U.S.A.).

The bottom of the 100 ml plastic cups were lined with five layers of square (4 cm × 4 cm) filter paper to absorb moisture. Four-week-old maize plants of each of the three maize types were harvested from the field by cutting at the base of the stem. All leaf sheaths were removed from the stems of cut plants by cutting at the base of the leaf with a pair of scissors. Two stem pieces (4 cm long) were cut from each plant and placed on the paper lining of each cup. Representative samples of neonate larvae from each population were weighed using an Ohaus Pioneer scale. Five neonate larvae were then randomly picked by means of a camel hair brush and inoculated onto maize whorls in each cup. Each cup was tightly sealed with stainless steel mesh-lined lids and placed in a climate controlled room at 27 °C, 50 % RH and 14L:10D hour photoperiod. The number and mass (mg) of the surviving larvae per cup were determined 7, 10, 14, 17 and 21 days after inoculation by carefully inspecting the whorl tissue in each cup. Whorls were replaced with fresh material from the same maize type after each assessment or as and when necessary. Dead larvae were removed during each assessment. The experiment was terminated 21 days after inoculation. Larval survival per cup was recorded and expressed as a percentage of the total number of larvae used per cup. The mean percentage larval survival was then calculated per treatment.

Data analysis

Data on field incidence, larval survival and mass of *B. fusca* were subjected to analysis of variance (ANOVA) using SPSS (version 24) statistics software (IBM Corporation, U.S.A.). Pearson's chi-square (χ^2) test (SPSS) was used to analyse the sex ratio of *B. fusca* pupae from the different areas.

RESULTS

Results of field surveys indicated that Bt maize was cultivated in seven sub-districts in the Eastern Cape during the 2014/15 cropping season. Five out of the six varieties cultivated were stacked trait varieties, a combination of insect resistance and herbicide tolerance traits in one variety (Table 1). The total estimated area under Bt maize cultivation was 1235 ha. Individual Bt maize field sizes ranged from 0.5 to 2.5 ha. The usual practice was to consolidate these small units into large units of between 10 and 150 ha to facilitate mechanisation operations. Structured refuge areas were not included in any of the fields visited (data not shown). With the exception of one Bt field in which neonate *B. fusca* larvae were recorded in the central whorl leaves of two maize plants, all 42 Bt maize fields inspected during the 2014/2015 cropping season were free of *B. fusca* infestation.

Mean incidence of *B. fusca* larvae and the number of larvae recovered per non-Bt maize plant in the non-Bt area (ECRef001) was higher than that in the Bt maize cultivating areas. These differences between infestation levels were, however, not significant ($P > 0.05$), ranging between 39 % and 56 % (Table 2). There were more male than female pupae in populations ECBt001 and ECRef001 as compared to population ECBt002 (Table 2).

Larval survival on Bt and non-Bt maize

One-hundred per cent larval mortality was observed in all three *B. fusca* populations on

Table 2. Incidence of stem borer-infested plants on non-Bt maize in home gardens and sex ratios of populations of *Busseola fusca* collected in the Eastern Cape.

Area	Mean (\pm SEM) percentage of infested plants/home garden	Mean (\pm SEM) number of <i>B. fusca</i> larvae/plant	Sex ratio (males:females)
ECBt001	39.00 (\pm 4.46)	1.73 (\pm 0.26)	1.1:1
ECBt002	42.11 (\pm 4.57)	2.21 (\pm 0.25)	0.81:1
ECRef001	56.36 (\pm 6.01)	2.64 (\pm 0.32)	1.14:1
<i>P</i> -value	0.073	0.090	$\chi^2 = 1.77$
<i>F</i> -value	2.77	2.53	

MON89034 plant tissue within seven days (Table 3). Survival on MON810 by larvae from population ECRef001 on the seventh day was significantly higher than that of populations ECBt001 and ECBt002. From the 10th to 21st days, there were no significant ($P > 0.05$) differences in survival between the different populations on MON810 and between populations on MON810 and MON89034. Survival on MON810 on day 21 ranged between 1.0 % (ECBt001 and ECBt002) and 1.50 % (ECRef001). Compared to non-Bt maize, larval survival on MON810 maize from the seventh to the 21st day was significantly ($P < 0.001$) lower in all populations (Table 3). Significantly more larvae from population ECBtRef001, compared to populations ECBt001 and ECBt002 survived on non-Bt maize for the first seven days. Survival on non-Bt maize at the end of the experiment (day 21) ranged between 22.0 % (ECBt002) and 53.0 % (ECBt001) (Table 3).

Larval mass on Bt and non-Bt maize

Larvae of population ECRef001 maintained on non-Bt maize had significantly ($P < 0.001$) higher mean mass during the first two weeks than larvae from populations ECBt001 and ECBt002 maintained on non-Bt maize. There were, however, no significant differences in mean larval mass between the three different populations on non-Bt maize between day 17 to day 21. Mean mass of larvae of

all *B. fusca* populations maintained on non-Bt maize was, however, significantly ($P < 0.001$) higher on all days as compared to the mean mass of larval populations on MON810 (Table 4). Mean larval mass of populations on non-Bt maize ranged from 66.76 mg (ECBt002) to 73.86 mg (ECBt001) whilst that on MON810 on day 21 ranged from 2.80 mg (ECBt001) to 7.48 mg (ECRef001). There were no significant differences in mean larval mass between the three different populations on MON810 (Table 4).

DISCUSSION

The total land cultivated to maize in South Africa in 2014 was estimated at 2.5 million ha (James 2014). About 69 % (1.73 million ha) of this area was cultivated with Bt maize (single and stacked Bt traits) and BR (insect resistance + herbicide tolerance trait) (James 2014). In the Eastern Cape, the total area planted with maize under the cropping programme in 2014 was 18 069 ha (DRDAR 2015). Estimates from information obtained during interviews with key stakeholders from the Department of Rural Development and Agrarian Reform (DRDAR) and smallholder maize projects in the Eastern Cape suggest that approximately 1240 ha of this area was cultivated with Bt maize. Approximately 99 % of the area under Bt maize was cultivated to stacked trait BR maize. This indicates that

Table 3. Larval survival (%) of different *Busseola fusca* populations maintained on Bt and non-Bt maize.

Treatments	Mean (\pm SEM) larval survival (%)				
	Day 7	Day 10	Day 14	Day 17	Day 21
ECBt001Control*	88.5 (\pm 2.49) a	83.0 (\pm 2.21) a	75.5 (\pm 2.84) a	70.0 (\pm 2.99) a	53.0 (\pm 3.16) a
ECBt001MON810	6.0 (\pm 2.49) d	3.5 (\pm 2.21) c	3.0 (\pm 2.84) c	2.0 (\pm 2.99) c	1.0 (\pm 3.16) c
ECBt001MON89034	0.0 (0.0) e	0.0 (0.0) c	0.0 (0.0) c	0.0 (0.0) c	0.0 (0.0) c
ECBt002Control	74.0 (\pm 2.49) b	64.0 (\pm 2.21) b	49.0 (\pm 2.84) b	42.0 (\pm 2.98) b	22.0 (\pm 3.16) b
ECBt002MON810	4.0 (\pm 2.49) de	1.5 (\pm 2.21) c	1.0 (\pm 2.84) c	1.0 (\pm 2.98) c	1.0 (\pm 3.16) c
ECBt002MON89034	0.0 (0.0) e	0.0 (0.0) c	0.0 (0.0) c	0.0 (0.0) c	0.0 (0.0) c
ECRef001Control	93.0 (\pm 2.49) a	86.0 (\pm 2.21) a	68.5 (\pm 2.84) a	54.5 (\pm 2.98) b	32.0 (\pm 3.16) b
ECRef001MON810	12.5 (\pm 2.49) c	2.5 (\pm 2.21) c	2.5 (\pm 2.84) c	2.0 (\pm 2.98) c	1.5 (\pm 3.16) c
ECRef001MON89034	0.0 (0.0) e	0.0 (0.0) c	0.0 (0.0) c	0.0 (0.0) c	0.0 (0.0) c
P-value	<0.001	<0.001	<0.001	<0.001	<0.001
F-value	409.59	457.57	195.72	134.04	55.85

Means within the same column followed by different letter(s) are significantly different at the 0.05 level.

Figures in brackets are standard error of means.

*ECBt001Control = Population ECBt001 fed with non-Bt maize, ECBt001MON810 = population ECBt001 fed with MON810 maize, ECBt001MON89034 = population ECBt001 fed with MON89034 maize, ECBt002Control = population ECBt002 fed with non-Bt maize, ECBt002MON810 = population ECBt002 fed with MON810 maize, ECBt002MON89034 = population ECBt002 fed with MON89034 maize, ECRef001Control = population ECRef001 fed with non-Bt maize, ECRef001MON810 = population ECRef001 fed with MON810 maize, ECRef001MON89034 = population ECRef001 fed with MON89034 maize.

Table 4. Mean larval mass of different *Busseola fusca* populations maintained on Bt and non-Bt maize.

Treatments	Mean (\pm SEM) larval mass (mg)				
	Day 7	Day 10	Day 14	Day 17	Day 21
ECBt001Control*	2.32 (\pm 0.20) b	9.81 (\pm 0.65) b	25.62 (\pm 1.29) a	65.99 (\pm 3.19) a	73.86 (\pm 4.18) a
ECBt001MON810	0.18 (\pm 0.85) b	0.28 (\pm 2.73) b	0.21 (\pm 5.37) c	0.64 (\pm 13.33) b	2.80 (\pm 17.47) b
ECBt001MON89034	–	–	–	–	–
ECBt002Control	1.84 (\pm 0.26) b	7.14 (\pm 0.84) b	22.06 (\pm 1.66) b	53.97 (\pm 4.11) a	66.76 (\pm 5.39) a
ECBt002MON810	0.51 (\pm 0.85) b	0.43 (\pm 2.73) b	0.72 (\pm 5.38) c	2.60 (\pm 13.33) b	4.27 (\pm 17.47) b
ECBt002MON89034	–	–	–	–	–
ECRef001Control	4.21 (\pm 0.24) a	13.86 (\pm 0.76) a	29.61 (\pm 1.49) a	66.09 (\pm 3.70) a	69.56 (\pm 4.84) a
ECRef001MON810	0.04 (\pm 0.65) b	0.66 (\pm 2.32) b	1.68 (\pm 4.39) c	6.02 (\pm 10.88) b	7.48 (\pm 14.26) b
ECRef001MON89034	–	–	–	–	–
P-value	<0.001	<0.001	<0.001	<0.001	<0.001
F-value	17.06	15.82	16.25	14.14	9.30

Means within the same column followed by different letter(s) are significantly different at the 0.05 level.

Figures in brackets are standard error of means.

*ECBt001Control = Population ECBt001 fed with non-Bt maize, ECBt001MON810 = population ECBt001 fed with MON810 maize, ECBt001MON89034 = population ECBt001 fed with MON89034 maize, ECBt002Control = population ECBt002 fed with non-Bt maize, ECBt002MON810 = population ECBt002 fed with MON810 maize, ECBt002MON89034 = population ECBt002 fed with MON89034 maize, ECRef001Control = population ECRef001 fed with non-Bt maize, ECRef001MON810 = population ECRef001 fed with MON810 maize, ECRef001MON89034 = population ECRef001 fed with MON89034 maize.

despite repeated introductions, the area under Bt maize on smallholder farms in the province still remains relatively small. Gouse *et al.* (2010) previously reported that many smallholder farmers in rural areas of South Africa, who were initially introduced to Bt maize, had a preference for herbicide-tolerant maize seed. In settings where labour availability is limited, the adoption of labour-saving technologies such as herbicide tolerant maize is also high (Manes 2013). Additionally, whilst stem borer pressure on maize is highly variable between cropping seasons (Van Rensburg *et al.* 1987), weeds are perennial problems on almost all agricultural fields in Africa (Gianessi & Williams 2011). The use of BR maize may therefore be an attempt to simultaneously benefit from the labour-saving trait and the buffer provided by the Bt trait against possible yield losses caused by target stem borer species (Fernandez-Cornejo & McBride 2002; Marra *et al.* 2003).

Incidence of *Busseola fusca* larvae on Bt and non-Bt maize

Results indicated *B. fusca* as the dominant stem borer pest of maize in smallholder farms in the province. *Chilo partellus* infestation on maize was observed only in areas close (about 50 km) to the coast or where maize was cultivated under irrigation (data not shown). Typical *B. fusca* damage was observed on non-Bt plants in all the areas surveyed.

However, on Bt maize plants only superficial feeding lesions caused by neonate *B. fusca* larvae were observed on two plants. Generally, the mean density of *B. fusca* larvae per maize field and plant was higher in the non-Bt maize area compared to Bt cultivating areas. Agronomic characteristics of the different varieties planted by farmers were not recorded during the survey, but it is known that there are differences in growing season length between these hybrids. Due to the general nature of stem borer infestation patterns and moth flight periods which extend over periods of several weeks, it is not expected that larval infestation levels would be differentially affected by differences in growing season length of the different varieties.

Although *B. fusca* infestation levels may be affected by several factors (Calatayud *et al.* 2014), the general reduction of the pest status of *B. fusca* in South Africa has been associated with the introduction of Bt maize (Van den Berg *et al.* 2015). Hutchison *et al.* (2010) have also associated reductions in estimated mean densities of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) in parts of the United States corn belt with the introduction of Bt maize. Similarly, Storer *et al.* (2008) associated reductions in the mean density of this pest on non-Bt maize in other parts of the U.S.A. to the adoption of Bt maize. The observed variation in the incidence of *B. fusca* in the Bt and non-Bt

areas of the Eastern Cape may therefore be associated with the cultivation of Bt maize in these areas.

Larval survival and mass gain on Bt and non-Bt maize

High numbers of *B. fusca* larvae from all populations survived on non-Bt maize. On MON89034, 100 % mortality was observed within seven days after introduction of larvae. The high level of mortality of neonate larvae of *B. fusca* on MON89034 is consistent with the findings of Erasmus *et al.* (2016) who reported no survival of this pest on this event. MON89034 is a stacked trait Bt event that was introduced in South Africa in 2011, purposely to counteract *B. fusca* resistance to the single transgene, Cry1Ab (Van den Berg *et al.* 2013). MON 89034 combines the transgene Cry2Ab2 with Cry1A.105, a chimeric protein incorporating domains I and II from Cry1Ac and domain III from Cry1Fa (USEPA, 2012). Each of the pyramided transgenes (Cry1A.105 and Cry2Ab2) have a different mode of action and binding characteristic to the mid-gut of target insects, and they are therefore highly effective against key lepidopteran pests (Storer *et al.* 2012). Larval survival on MON810 from day 10 onwards was similar to that on MON89034 across all populations. Survival of all three populations of *B. fusca* on MON810 was, however, significantly lower than on non-Bt maize. Growth of an insect on susceptible or resistant plants is commonly determined by measuring the weight gain of the larvae, and the development of larvae into pupae (Khan 1997). Although the experiment was terminated before the estimated duration of the larval period of 31 to 50 days (Onyango & Ochieng'-Odero 1994; Ratnadass *et al.* 2001; Kruger *et al.* 2012b) the very low mass of the few surviving larvae makes it likely that none of the individuals would have survived until pupation. Since similar levels of larval survival have been observed between laboratory and field trials conducted with *B. fusca* (Erasmus *et al.* 2016), it is expected that results observed in the laboratory trials during this study, would be similar under field conditions in the Eastern Cape region.

Bt maize is genetically engineered to express a high dose of Bt toxin (Caprio *et al.* 2000; Siegfried & Hellmich 2012) against target pests. It is assumed that for the high dose requirement to be satisfied, the protein concentration in tissues fed on by homozygous susceptible insects should be suffi-

ciently high that nearly all (>99.9 %) larvae feeding as neonates fail to complete development, and insects heterozygous for resistance alleles are expected to suffer at least 95 % mortality (USEPA 1998). It is worthy to note that pre-commercialisation field data indicate that Cry1Ab proteins (MON810) did not kill 99 % of larvae (Van Rensburg 1999). Given these facts coupled with the fact that the mortality observed in this study falls within the expected range (95–99.9 %) it can be concluded that *B. fusca* populations from Bt cultivating areas in the Eastern Cape are still highly susceptible to Bt toxin.

Continuous cultivation of transgenic Bt maize, however, increases selection pressure and consequently increases the risk that insect species directly exposed to Bt toxin may evolve resistance to Bt proteins (Ferré & Van Rie 2002). The Eastern Cape was amongst the provinces to which Bt maize was first introduced to smallholder farmers during 2001 (Gouse 2012). Since then, cultivation has been limited to farmers participating in various Government development initiatives such as the Massive Food Production Programme (2003–2009) and DRDAR cropping programme (2012 onwards). Adoption of Bt maize outside of Government development initiatives have been very limited. Consequently, Bt maize cultivation in the province has not been continuous and hence, larvae may not be subject to intense selection pressure derived from continuous exposure to Bt toxin. Rice & Pilcher (1998) observed that farmers' perception of transgenic Bt maize technology is an important determinant of its adoption. Previous studies of Bt maize introduction to smallholder farmers in the Eastern Cape indicated limited awareness of the fact that Bt maize provides resistance to stem borers (Assefa & Van den Berg 2009; Jacobson & Myrh 2012; Kotey *et al.* 2016). It is therefore possible that as awareness about the efficacy of the Bt trait against stem borer increases, the area under cultivation may increase. One possible threat posed by this is an increase in the selection of resistant insects to Bt plants, a possibility that could limit the use of Bt technology if increased use is not accompanied by good stewardship (Gould 1998).

CONCLUSION AND RECOMMENDATIONS

Results suggest that *B. fusca* populations in the Eastern Cape remain susceptible to Bt maize. However, as past experience with Bt maize else-

where in South Africa has shown, adoption of Bt maize without adherence to recommended stewardship requirements, particularly IRM, compromises the long term sustainability of the technology. Continuous monitoring of resistance levels and/or prediction of resistance evolution through the development of diagnostic tools and monitoring of fields for early identification of possible transgenic crop product failure, will be required. Recent studies indicating the dominance of at least one type of resistance of *B. fusca* to Bt maize showed the inherent ability of this species to evolve resistance to Cry proteins. This highlights the need to promote Bt maize not as a stand-alone pest control option but as part of a broader integrated pest management strategy.

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