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Full Length Research Paper

Control of *Busseola fusca* and *Chilo partellus* stem borers by *Bacillus thuringiensis* (Bt)-δ-endotoxins from Cry1Ab gene Event MON810 in greenhouse containment trials

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Previous testing of several public *Bacillus thuringiensis* (Bt)-maize events did not show control of the African stem borer (*Busseola fusca* Fuller), an important stem borer species, without which stewardship would be compromised by the possibility of rapid development of resistance to Bt delta-endotoxins. This study was carried out to test Bt-maize Event MON810 as an option to control all major stem borer species in Kenya. Two Bt-maize hybrids, DKC8073YG and DKC8053YG, both containing Bt Event MON810 of *Cry1Ab* gene were imported to carry out greenhouse containment trials. The hybrids together with the controls were grown in 10 replications upto the V6 and V8 stages. Infestations on whole plants were carried out at two stages of growth using 5 neonates of the spotted stem borer (*Chilo partellus* Swinhoe) and *B. fusca*. Bt-maize Event MON810 hybrids showed resistance to both stem borer species with low leaf damage scores and few surviving larvae recovered from the whole plant. The public Bt-maize Event 223 did not control *B. fusca*. Deploying Bt-maize Event MON810 may, therefore, be used to control the two species of stem borers. However, the efficacy of Bt-maize Event MON810 will, need to be evaluated under field environments.

Key words: *Bacillus thuringiensis* (Bt) maize, cry1A (b) proteins, stem borers, transgenic.

INTRODUCTION

The rising food prices are a reflection of food scarcity that could affect Kenya and other net food importers. There is continuing need to increase food production in Kenya through adoption of technologies that increase productivity and minimize losses. One practical means of achieving greater yields is to minimize insect pest losses through the use of stem borer resistant maize that could reduce annual grain losses by more than 10%, (De Groote, 2002).

Food security awareness and concerns have become household issues globally, due to the sharply rising food prices currently estimated at over 40% (FAO, 2008). This exceptional hike in food prices resulted in severe economic, social and political consequences to African agriculture (FAO, 2008). Unfavorable government policies and low investments have contributed to Africa becoming the worst hit by food insecurity issues. Other factors that have contributed to food insecurity include

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Abbreviations: Bt, *Bacillus thuringiensis*; **HPR,** host plant resistance; **ITK,** indigenous technical knowledge; **CFT,** confined field trials; **IRM,** Insect Resistance Management.

drought, high costs of farm inputs such as fertilizers and improved seeds, pests and diseases, and insufficient quantities of relief food.

Insect pests, including stem borers, cause direct losses and reduced food production worldwide. The impact of climate change and new methods of farming, including conservation agriculture on insect pests, can only be speculated or tested using considerable time and resources. However, it is known that insect pests respond to changes in moisture and temperature.

Kenya loses an estimated 13.5% of its maize production, the major staple food, to pests annually (De Groote, 2002). These makes Kenya a net annual maize importer of an average 400,000 metric tons (Mugo et al., 2001).

Past efforts to reduce stem borer losses include use of indigenous technical knowledge (ITK), cultural methods, chemical pesticides, biological control, and host plant resistance (HPR) using conventional breeding (Mugo et al., 2005; Mugo et al., 2007). The applications of biotechnology tools using HPR include molecular markers and genetic engineering. Most of the insect resistant transgenic plants have been developed by using modified genes from *Bacillus thuringiensis* (Bt) which when ingested by target pests, release δ-endotoxins. Several public Bt maize events were tested in the biosafety level 2 laboratory, and in a confined field trials (CFT) site (Mugo et al., 2011). Complete control was found for the three major stem borer species (spotted stem borer (*Chilo partellus Swinhoe),* the sugarcane borer (*Eldana saccharina* Walker) and the pink stem borer (*Sesamia calamistis* Hampson), but not for *Busseola fusca*, the pest causing the highest level of maize damage in farmers fields (Mugo et al., 2005). Due to considerations of stewardship and insect resistance management (IRM), these events could not be deployed, and a search for additional effective events was initiated. Bt-maize event MON810 that carries Bt gene *Cry1Ab* was used as a candidate.

The *cryIA(b)* gene is 3468 nucleotides in length and encodes a full-length *B.t.k.* HD-1 [CryIA(b)] protein of 1156 amino acids (Fischoff et al., 1987), which when subjected to trypsin, yields an active trypsin-resistant protein product of approximately 600 amino acids *in planta* and *in vitro* (Lee et al., 1995). The *cryIA(b)* gene sequence was modified to increase the levels of expression in maize (Perlak et al., 1991). The *cryIA(b)* gene encodes the natural identical CryIA(b) protein (Fischoff et al., 1987).

Maize line, MON810, was developed by particle acceleration technology with a DNA solution containing two plasmids: PV-ZMBK07 [which contains the c*ryIA(b)* gene] and PV-ZMGT10 [which contains the CP4 EPSPS and *gox* genes] (Kania et al., 1995). This maize line contained one integrated DNA on an approximately 5.5Kb fragment with a single copy of the E35S promoter, the *hsp*70 intron, and the *cryIA(b)* gene.

This paper reports the evaluation of Bt-maize Event MON810 against the two major stem borer species in Kenya, the spotted stem borer (*C. partellus*) and the African stem borer (*B. fusca*).

MATERIALS AND METHODS

Application for introduction of Bt-maize Event MON810

An application was made to Kenya Agricultural Research Institute (KARI) Insitutional Biosafety Committee (IBC) in November 2006, then to National Biosafety Committee (NBC) in April 2007, to introduce maize Hybrids DKC8073YG and DKC8053YG. The application was revised from an earlier one approved for research at KARI Kibos. The revised application was based on a bilateral agreement between KARI and Monsanto to jointly test Bt-maize Event MON810. It reflected the new testing location, and research objectives that would include comparison of efficacy with the IRMA Bt-maize public events.

Experimental design and treatments

The experimental design was a 4x4 alpha-lattice with 10 replications, each plant being a replicate. The maize seeds were treated with Murtano®, which contains a fungicide and insecticide before sowing. The planting media was first treated with Lamdex® 50EC (a.i. lambda cyhalothrin 50 g/L) a broad-spectrum contact and stomach acting pesticide at the rate of 0.5ml per 1.25 liters of clean water to control soil-borne pests.

Three seeds were grown in large pots (30 cm diameter by 36 cm high) but later thinned down to one plant per pot. The pots were spaced at 0.75 m apart to ensure that larvae could not migrate across treatment pots via leaf contact. A heater was placed to regulate temperatures to 18°C night and 22°C day to maximize growth. Soil moisture was maintained at field capacity. Fertilizers were applied to adequate levels with a starter water soluble fertilizer, followed by an application of nitrogenous and phosphate fertilizers as required (Murenga et al., 2004).

Test maize germplasm and plant materials

The test materials were two Bt-maize Event MON810 hybrids, DKC8073YG hybrid (MON810) and DKC8053YG hybrid (MON810), and their non-transgenic equivalent DKC9355YG (Non-Bt) hybrid. The public Bt-maize, Bt Cry1Ab/CML202 BC2F1 (Event 223) was also used for comparison.

Stem borer species and infestation

Five neonates of each of the two stem borer species, *B. fusca* and *C. partellus,* were used for artificially infesting the plants. The infestation was done when the plants had complete collar formed around the central stem by the fully expanded leaf with six to eight visible leaves, but only six of them had visible leaf ligules or 'collars' around the stem, usually referred to as V6 and V8 stages of growth.

Data collection

Leaf damage scores for each of the 10 plants were taken 10 days

Table 1. Means of traits after infestation of whole plant with *Busseola fusca* larvae at the V6 stage.

Entry	Pedigree	Leaf damage score	Live larvae	Larvae weight	Larvae instar	Plant height	Leaf stage
		(1-9)	no.	mg	no.	cm	no.
1	DKC8073YG hybrid (MON 810)	1.1±0.1	0.1±0.1	0.35±0.27	1.3±0.22	70±2.40	10.5±0.17
5	DKC8053YG hybrid (MON 810)	1.3±0.14	0.14±0.11	0.44±0.16	1.1±0.16	81.1±3.83	10.8±0.25
9	DKC8053YG hybrid (Non-Bt)	3.4±0.4	1.7±2.33	7.64±2.33	1.6±0.10	70.6±3.30	10.8±0.25
13	Event 223 Bt Cry1Ab/CML202 BC_2F_1	3.2±0.13	1.5±7.72	10.65±7.72	1.6±0.12	56.7±1.87	10.2±0.22
	Mean	2.3	0.9	4.9	1.6	70.2	10.6
	CV(%)	29	93	266	27	12	6
	LSD	0.6**	0.8**	-	-	7.8**	-

after infestation, using a scale of 1–9 scale, where 1 refers to least damage and 9 is when all leaves have extensive borer damage. The leaf damage scores and number of recovered larvae were determined for the four borer species. Destructive sampling was done on the plants 10 days after infestation by carefully splitting the stems to expose the larvae. The larvae were counted, mortality assessed, the instar stage of the developing larvae, and the weight of larval mass.

Data analysis

Analysis of Variance (ANOVA) on data from insect resistance, agronomic traits such as plant stand, 50% anthesis, ear height, and leaf bioassays from the confined field trials was done using PROC General Linear Model (GLM) of Statistical Analysis Software (SAS, 2003), program and least significant difference (LSD) used to separate means.

RESULTS

Responses due to *B. fusca* infestation

There were significant differences observed (p=0.001) for leaf damage score, total number of recovered larvae, and number of live larvae recovered in plants infested with *B. fusca*. Hybrids DKC8073YG (MON810) and DKC8053YG (MON810) showed very little leaf damage at score of 1.1 and 1.3, respectively. Extensive leaf damage of 3.4 to and 3.2 scores was observed in the non-Bt check, DKC8053YG, and the public Bt Event 223, respectively (Table1). Similar results were observed in the number of live larvae recovered with a mean of 0.10 and 0.14 larvae, from MON810 hybrids, compared to a mean of 1.70 and 1.50 larvae for the non-Bt and public Bt Event 223, respectively. The number of larvae recovered from the non-Bt check and Event 223 was higher than in the Bt-maize Event MON810 hybrids. Event 223 and the non-Bt hybrids also showed significantly higher leaf damage than the two Bt-maize Event MON810 hybrids. No differences were observed in plant height and leaf stage between Bt-maize Event MON810 hybrids and non-Bt hybrid, but Event 223 had shorter plants (Table 1).

Significant differences were observed for leaf damage score (p=0.001), number of dead larvae (p=0.005), number of live larvae (p=0.005), larvae weight (p=0.005), and plant height (p=0.001) in the v8 stage plants infested with *B. fusca* (Table 2). Bt maize Event MON810 hybrids and the non-Bt check showed less damage of scores 1.3-1.5 compared to 2.00 of the public Bt Event 223. Similar differences were found in the number of live larvae with the hybrid DKC8073YG showing no live larvae recovered, and very few live larvae from hybrid DK8053YG (0.00 and 0.22 respectively). The non-Bt check and public Bt Event 223 had higher live larvae recovered (0.40 and 0.60 respectively). Plant height for Event 223 was significantly shorter than all other plants (Table 2).

Responses due to *C. partellus* infestation

The Event MON810 hybrids showed significant differences in leaf damage score after infestation using *C. partellus* at the V6 leaf stage (1.0) whereas the non-Bt hybrid and Event 223 showed higher damage scores (2.99 and 2.70, respectively) (Table 3). There were no live larvae recovered from the two MON810 hybrids. A relatively high number of live larvae (2.14) was recovered from non-Bt hybrid check compared to Event 223 (1.70). Larvae recovered from the MON810 hybrids had lower weight compared to those recovered from non-Bt hybrid and Event 223.

Table 2. Means of traits after infestation of whole plant with *Busseola fusca* larvae at the V8 stage.

Entry	Pedigree	Leaf damage score	Live larvae	Larvae weight	Larvae instar	Plant height	Leaf stage
		(1-9)	no.	mg	no. (1-3)	cm	no.
1	DKC8073YG hybrid (MON 810)	1.4±0.16	0±0	0.01±0.01	1.3±0	170.5±4.81	16.2±0.25
5	DKC8053YG hybrid (MON 810)	1.3±0.15	0.2±0.14	1.5±0.84	1.3±0.13	182.3±6.37	16.5±0.17
9	DKC8053YG hybrid (Non-Bt)	1.5±0.17	0.4±0.10	0.2±0.10	1.6±0.18	176.9±3.06	15.8±0.29
13	Event 223 Bt Cry1Ab/CML202 BC_2F_1	2±0.21	0.6±0.02	0.1±0.02	1.1±0.15	143.1±5.96	16.1±0.23
	Mean	1.6	0.3	0.3	1.3	168.2	16.2
	CV(%)	36.4	236.7	354.9	28.8	9.2	4.6
	LSD	0.5**	0.7*	2.1*	0.8	14.2***	0.7*

Table 3. Means of traits after infestation of whole plant with *Chilo partellus* larvae at the V6 stage.

Entry	Pedigree	Leaf damage score	Live larvae	Larvae weight	Larvae instar	Plant height	Leaf stage
		(1-9)	no.	mg	no. (1-3)	cm	no.
3	DKC8073YG hybrid (MON 810)	1±0	0±0	0.14±0.14	0.9±0	86.3±3.89	10.9±0.27
7	DKC8053YG hybrid (MON 810)	1±0	0±0	0.03±0.03	0.9±0	86±2.22	11.0±0.25
11	DKC8053YG hybrid (Non-Bt)	2.99±0.31	2.1±1.12	5.1±1.12	1.7±0.11	78.9±2.75	10.7±0.30
15	Event 223 Bt Cry1Ab/CML202 BC_2F_1	2.7±0.36	1.7±1.55	3.39±1.55	1.8±0.08	67.2±8.77	10.4±0.22
	Mean	2.1	0.9	0.3	1.6	74.9	10.7
	CV(%)	36.73	118	271	23.20	17.58	6.89
	LSD	0.96**	1.59**	-	-	14.34**	-

Similar trend was observed from larvae in the first to third instar developmental stages with fewer larvae observed in the Bt-maize hybrids (0.9), compared to non-Bt check and Event 223 (1.7 and 1.8 respectively). The lowest plant height was observed in Event 223 plants with mean of 56.7cm at V6 infested with *B. fusca* (Table 3).

A similar trend of lower leaf damage, fewer live larvae recovered, lower larvae weight, fewer live larvae in each of the first three developmental stages, was observed from MON810 hybrids as compared to the non-Bt-check and Event 223. The lowest plant height was recorded from Event 223 with a mean of 138.80 cm (Table 4). There were no significant differences in the number of leaves on all plants of MON810 hybrids, non-Bt hybrids, Event 223 at V6 and V8 stages infested by *B. fusca* or *C. partellus*.

DISCUSSION

The leaf damage scores were significantly lower among the two MON810 hybrids than for the non-Bt hybrid and the public Bt-Event 223 for *C. partellus* and *B. fusca* at the V6 and V8 growth stages (Figure 1). This observation has also been reported by the significantly lower number of live *B. fusca* and *C. partellus* larvae (Mugo et al., 2005) supported by the Bt MON810 hybrids compared to the non-Bt hybrid and the public Bt Event 223. The susceptibility of Event 223 to *B. fusca* has previously been reported (Mugo et al., 2011), and these results are consistent with those findings (Mugo et al., 2005), however the levels of susceptibility recorded from this study are higher

Table 4. Means of traits after infestation of whole plant with *Chilo partellus* larvae at the V8 stage.

Entry	Pedigree	Leaf damage score (1-9)	Live larvae no.	Larvae weight mg	Larvae instar no. (1-3)	Plant height cm	Leaf stage no.
3	DKC8073YG hybrid (MON 810)	1.2±0.14	0.3±0.15	0.1±0.04	1.0±0	165.4±6.55	15.8±0.25
7	DKC8053YG hybrid (MON 810)	1.4±0.17	0.04±0	1.3±0.02	0.9±0	181.1±3.34	15.8±0.11
11	DKC8053YG hybrid (Non-Bt)	2.9±0.35	3.3±2.33	9.5±2.33	1.6±0.12	160.1±5.46	15.8±0.13
15	Event 223 Bt Cry1Ab/CML202 BC_2F_1	3.1±0.1	3.5±0.92	2.4±0.92	1.7±0.11	138.8±5.22	16.1±0.31
	Mean	2.2	1.8	2.6	1.3	161.3	15.9
	CV(%)	31.4	78.7	87.6	23.2	9.6	4
	LSD	0.7***	1.3***	4.3***	0.6**	14.4***	0.6

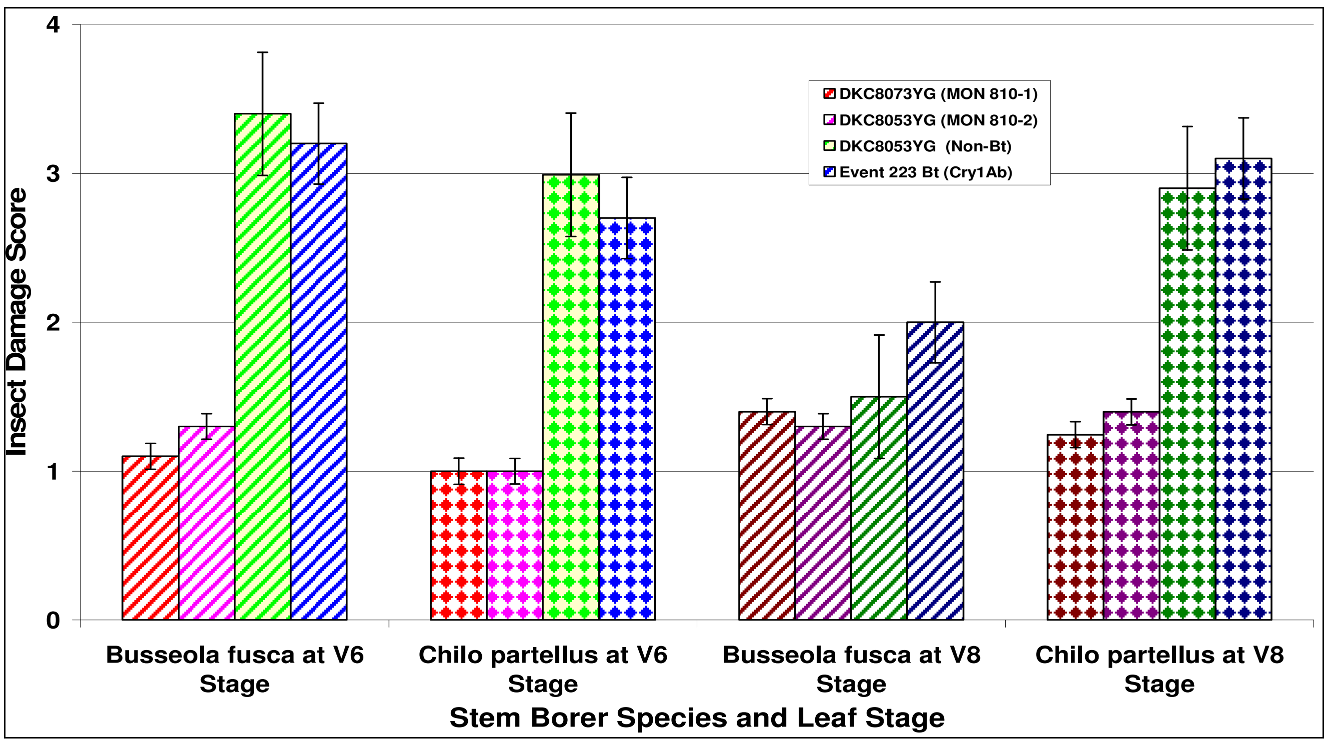

Figure 1. Leaf damage scores from Bt and non-Bt maize plants infested with larvae of *Busseola fusca* and *Chilo partellus* at V6 and V8 stages of growth.

for *C. partellus*. Possible reasons could be because the seeds of Event 223 used were BC2F1; bringing the possibility that the Bt-gene was not stable at that stage.

There were significant differences in larval weight of both insect species at the V6 leaf stage and V8 stage. The Bt hybrids suppressed larval weight accumulation compared to the non-Bt hybrid and the public Bt Event 223. These results agree with studies by Fenemore (1984), that toxic chemical substances ingested by insects can interfere with the metabolic processes and eventually reduce the growth of the insect (Mugo et al., 2011). Plant height with the public Bt Event 223 Bt were the shortest in both the *B. fusca* and *C. partellus* infested plants in both V6 and V8 leaf stages, while the MON810 hybrids were the same height as their non-Bt equivalent. It is highly likely that the shorter Bt Event 223 plants were due to genotype characteristics and effects of second generation of backcrossing (BC2F1) rather than effects of exposure to the Cry proteins from Bt-maize. There was only a 10-day interval between larvae infestation and recovery from experimental material. This period was not adequate for the stem borers to cause significant damage on plants, resulting in adverse effects on plant height.

The live larvae recovered from non Bt hybrid checks and Event 223 advanced to third instar were more than those in the Bt-maize hybridsfor both *C. partellus* and *B. fusca*. This was in agreement with the findings of Fenemore (1984), and Tende et al. (2010).

Conclusions

This study demonstrated the efficacy of Bt-maize Event MON810 against the two major stem borer species in Kenya, the spotted stem borer, *C. partellus* and the African stem borer *B. fusca*. Bt-maize Event MON810 was effective in the two genetic backgrounds represented by the two different hybrids. Bt-maize Event MON810 appears to act in as a chronic toxin, whereby the larvae are killed soon after ingesting the plant tissue. This has the potential of reducing the probability of pests developing resistance from consuming sub-lethal doses of cry1Ab δ-endotoxins.

Since MON810 has been effective in control of pests, it can be expedited for release and control of stem borers in Kenya which are distributed in all maize growing areas of Kenya. Bt- maize Event MON810 needs to be evaluated under field environments, where the plants and the pests can be subjected to natural conditions during the plant growth.

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