Bt-transgenic maize does not deter oviposition by two important African cereal stem borers, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae)

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ABSTRACT

Objective: To assess *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) ovipositional responses on Bt (Event 216, containing the *Cry1Ab* gene) and isogenic non-Bt (CML 216) maize plants.

Methodology and results: Stem borer moths were allowed to oviposit on maize plants in oviposition cages under both choice (cages containing both Bt and non-Bt plants) and non-choice conditions (cages containing either only Bt or non-Bt plants). There were no significant differences in the mean numbers of: egg batches per plant, eggs per batch, eggs laid per plant and egg hatchability between Bt and non-Bt plants in both choice and non-choice tests.

Conclusion and application of findings: Event 216 did not deter oviposition by *Chilo partellus* and *Sesamia calamistis*. This factor should be taken into consideration when designing suitable refuge arrangements for managing resistance. Although most of the larvae exposed to the Bt plants would be killed, thus reducing chances of the pests developing resistance, an effective resistance management strategy would require that the Bt plants are further engineered to incorporate additional genes that would make them unattractive for oviposition by stem borer moths.

Key words: *Bacillus thuringiensis*, environmental biosafety, natural enemies, GM maize

INTRODUCTION

Stem borers are a major limiting factor to the production of maize, *Zea mays* L. (Poaceae), in tropical Africa (Kfir *et al.*, 2002). The spotted stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and the pink stem borer *Sesamia* *calamistis* Hampson (Lepidoptera: Noctuidae) are amongst the most important maize pests (Overholt *et al.*, 1994), and in combination with other stem borer species, can cause yield losses ranging from 10% to total crop loss (Kfir *et al.*, 2002).

C. partellus is the dominant and most important species in the lowland and mid-altitude areas in East Africa (Setamou *et al.*, 2005). In Africa, *C. partellus*, which is of Asian origin (Zhou *et al.*, 2001), was first reported in Malawi in the early 1930s (Tams, 1932). Since then, it has spread to nearly all countries in Eastern and Southern Africa, with the first reports in Kenya appearing in the early 1950s (Nye, 1960). The pest has now spread throughout the maize growing areas of Kenya that lie below 1500m asl (but in some cases even at higher elevations) (Overholt *et al.*, 1994; Zhou *et al.*, 2001; Songa *et al.*, 2002). It is the most widely distributed and abundant stem borer species in Kenya and is often the most damaging pest, particularly in the warmer lowland areas (Songa, 1999). Evidence over a period of 30 years indicates that *C. partellus* is gradually replacing the indigenous stem borer, *Chilo orichalcociliellus*, Strand (Lepidoptera: Crambidae) in some regions in Kenya (Ofomata *et al.*, 2003).

S. calamistis is a native stem borer species that occurs in low densities in all areas in Kenya up to 2400m above sea level (Muhammad & Underwood, 2004). Studies in eastern Kenya found *S. calamistis* to be the second most widely distributed stem borer species, after *C. partellus* (Songa *et al.*, 2002).

Globally, chemical insecticides have been used widely for stem borer control (Muhammad & Underwood, 2004). These synthetic pesticides are too expensive and unaffordable to many farmers (Bonhof *et al.*, 2001). In addition, widespread use of synthetic pesticides causes environmental pollution. While the use of botanical insecticides has advantages of cost over synthetic insecticides, it is hampered by difficulties such as the logistics of production and preparation (Morse *et al.*, 2002).To effectively manage insect pests, transgenic crops present a potential and viable alternative to synthetic and natural insecticides. The cultivation of crops that are genetically modified to resist insect pests would lead to reduced application of conventional pesticides, which would potentially reduce production costs, environmental pollution and risks to human health. Reduced use of synthetic pesticides would also allow natural enemies of the insect pests to flourish, thus supporting natural control of the target pests as well as other pests that are not affected by the toxin produced by the transgene (Groot & Dicke, 2002).

Maize expressing the synthetically modified *Cry1Ab* gene from the soil bacterium *Bacillus thuringiensis* ssp. kurstaki strain HD-1 (Bt) was the first insect-resistant transgenic crop to be commercialised in 1996 (EPA, 2000). Although it was initially developed to control the European corn borer, *Ostrinia nubilalis*, Hubner (Lepidoptera: Crambidae) (Ostlie *et al.*, 1997), Bt maize has the potential to control other stem boring Lepidoptera including the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) (Lynch *et al.*, 1999); the southwestern corn borer, *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae) (Archer *et al.*, 2000; Castro *et al.*, 2004; Huang *et al.*, 2006); the corn earworm, *Helicoverpa zeae* Boddie (Lepidoptera: Noctuidae) (Lynch *et al.*, 1999, Storer *et al.*, 2001; Horner *et al.*, 2003), the common stalk borer, *Papaipema nebris* Guenee (Lepidoptera: Noctuidae) (Binning & Rice, 2002) and the pink maize borer, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) (Van den Berg & Van Wyk, 2007).

To control maize stem borers in Kenya, the Insect Resistant Maize for Africa (IRMA) project has been considering the introduction of the transgenic maize, Event 216, which expresses the gene *Cry1Ab*. A major risk associated with the use of insect-resistant crops is that pests can develop resistance which could reduce the efficacy of such crops as plant protection tools (Wolfenbarger & Phifer, 2000; Conner *et al.*, 2003). The high dose/refuge strategy has been proposed to delay development of resistance by insects against transgenic plants (Bates *et al.*, 2005). This strategy emphasises the presence of susceptible insect populations so as to slow down the evolution of resistance (Andow *et al.*, 2000; Bentur *et al.*, 2000; Davis & Onstad, 2000; Shelton *et al.*, 2000;Tang *et al.*, 2001; Stodola *et al.*, 2006). The premise is that susceptible insects, if present in

sufficient numbers, will mate with resistant insects and dilute resistance genes. However, in order for this strategy to work, several conditions have to be fulfilled. Several biological factors that influence the number of insects exposed to Bt toxin could substantially affect the success of the high dose/refuge strategy (Ives & Andow, 2002). One such factor is oviposition preference.

Preference for Bt maize would require more refuge plants to counter an increased selection pressure. However, preference for refuge plants could have the opposite effect. From a resistance management perspective, an ideal plant, in addition to killing larvae, should repel adult oviposition (Hellmich *et al.*, 1999). This would reduce selection for resistance because fewer larvae would be exposed to plant toxins. Bt transgenic maize could also impact on natural enemies of stem borers, with the effect partly depending on the oviposition preferences of stem borers, either for Bt or non-Bt maize. Furthermore, if larvae developed resistance to the Bt toxin, there could be greater chances of natural enemies getting host-mediated exposure to the toxin. In light of the foregoing, it is necessary to determine stem borer oviposition preferences for Bt transgenic maize. Such information could also be very useful in developing models that can be used to forecast the potential evolution of pest resistance to Bt transgenic crops (Qureshi *et al.*, 2005). Also, there have been concerns over the possibility of widespread movement of pests from toxic Bt crops to non-Bt crops (Kumar, 2004). The information obtained through this study would also help to address this concern.

Even though some studies suggest that changes in plant attributes during genetic transformation could affect pest oviposition preferences (Bernal & Setamou, 2003), relatively little is known about the effects of Bt transgenic plants on *C. partellus* and *S. calamistis* oviposition behaviour. The main objective of this study was to assess *C. partellus* and *S. calamistis* ovipositional responses on Bt (Event 216, containing the *Cry1Ab* gene) and isogenic non-Bt (CML 216) maize plants under non-choice and choice conditions. The two stem borer species were chosen for this study because *Cry1Ab* has only shown sufficient efficacy against them (Andow *et al.*, 2004).

MATERIALS AND METHODS

Experimental plants: The maize lines used in this study were provided by the joint Kenya Agricultural Research Institute (KARI)/International Maize and Wheat Improvement Centre (CIMMYT) IRMA project. The Bt maize line, Event 216, expresses the *Cry1Ab* gene (Andow *et al.*, 2004) and was produced by co-transformation of a ubi:*Cry1Ab* construct and a separate *bar* selectable marker construct. The marker was eliminated by selection on the progeny for independent assortment of *Cry1Ab* and bar. The selectable marker genes and the *Cry1Ab* gene were under the control of the maize ubiquitin promoter (*SN Mugo, personal comm.., Sept. 9. 2008*). Untransformed plants of the parent cultivar CML 216 were used as control. Plants were grown in 15 cm diameter pots in a greenhouse at KARI, Nairobi, Kenya, at day and night temperatures averaging 26 and 15°C, respectively, and natural light conditions of approximately 12/12 h light: darkness photoperiod.

Insects: The *C. partellus* and *S. calamistis* originated from colonies maintained on artificial diet according to the procedure of Ochieng *et al.* (1985). The insects were obtained from the insectary at KARI, Katumani, and the Animal Rearing and Quarantine Unit of the International Centre for Insect Physiology and Ecology (ICIPE).

Responses under non-choice conditions

Experiments with pupae: The experiments were conducted in oviposition cages within the greenhouse at KARI. The cages measured 40 cm long x 40 cm wide x 60 cm high with a wire mesh wall over three sides and galvanized iron on the top, bottom and a top-down sliding door on the front.

Ten male and ten blackened female pupae per cage were placed in open Petri dishes (9cm diameter), at the centre of separate cages (Figure 1). Each cage contained either 8 potted Bt or 8 potted non-Bt maize plants (1 plant/pot). The plants were used three weeks after emergence, the age at which maize is most susceptible to stem borer damage (Kumar & Asino, 1993). Pupae, rather than adults,

were used so that adults could emerge and disperse as they eclosed over a few days. Having high densities of insects at the release site can affect their dispersal behaviour (Qureshi *et al.*, 2005), and the responses of a large number of moths confined in a small area might not reflect behaviour of moths in the field (Liu *et al.*, 2002). Using pupae rather than adults could therefore avoid the unnatural interactions that could occur when large numbers of adults are released at the same time. A 9 cm diameter wad of cotton wool, moistened with water, was placed in a Petri dish at the centre of each cage. Providing only water gives the best oviposition results (Taneja & Nwanze, 1990). After 8 days, the plants were removed and the number of egg batches per plant counted. Subsequently, the sections on which the moths had oviposited on each plant were cut off and the eggs counted under a microscope (x64 magnification). The eggs were then incubated in the laboratory at a temperature of 25 ± 1 °C in Petri dishes lined with moist filter paper for 8 days (by which time it was assumed all fertile eggs had hatched). Upon hatching, the neonates were counted and expressed as percentage emergence.

Experiments with moths: Male and female moths that emerged on the same morning were introduced into cages within the biosafety greenhouse which had average day and night temperatures averaging 26 and 15°C, respectively, and natural light conditions of approximately 12/12 h light: darkness photoperiod. Following the procedure described by Khan *et al.* (2006), 15 male and 12 female moths per cage were placed in Petri dishes (9 cm diameter), which were then placed at the centre of separate cages. The rest

of the setup was as described above for the experiment with pupae.

Figure 1: Experimental cages used for oviposition preference studies.

Responses under choice conditions: These were evaluated in a similar set up as described above except that the cages had 4 Bt and 4 non-Bt plants each. In these cages the plants were arranged such that Bt plants alternated with non-Bt plants, with the leaves intermingled, to allow the moths to choose any leaf from any plant for oviposition.

All the experiments were arranged in a randomised complete block design and replicated four times.

Statistical analysis: Data were subjected to analysis using the Student's *t*-test (Sokal & Rohlf, 1995) at α = 0.05. Count data were square root transformed while percentage data were arc sine transformed to correct for heterogeneity of variances prior to analysis (Sokal & Rohlf, 1995).

RESULTS AND DISCUSSION

There were no significant differences between Bt and non-Bt maize plants with regard to all parameters assessed for both *C. partellus* and *S. calamistis* under both non-choice (Table 1) and choice (Table 2) conditions. *S. calamistis* and *C. partellus* moths did not seem to discriminate between Bt and non-Bt maize for egg laying under both non-choice and choice conditions, implying that the presence of Bt toxin was either not perceived by the moths or it did not deter oviposition. These findings are consistent with what has been observed with other lepidopteran pests when exposed to Bt crops. In field tests, the

number of eggs laid by susceptible European corn borer females did not differ between Bt corn (containing *Cry1Ab*) and non-Bt corn (Orr & Landis, 1997). Pilcher & Rice (2001) observed that *O. nubilalis* females did not show any oviposition preference towards non-Bt or Bt maize (using Event 176 and Bt11). In the laboratory, the number of eggs laid by diamond back moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) females did not differ between Bt and non-Bt canola (Ramachandran *et al.*, 1998), broccoli (Tang *et al.*, 1999) and cabbage (Kumar, 2004). Kumar (2004) further observed that

the transgenic plants had no adverse effects on the hatchability of *P. xylostella* eggs. In four out of five cage experiments and in two field experiments Hellmich *et al.* (1999) found that various Bt events did not influence *O. nubilalis* oviposition. In cage experiments in the greenhouse, Liu *et al.* (2002) found that the pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) did not discriminate between Bt (containing *Cry1Ac*) and non-Bt cotton for oviposition. Van den Berg & Van Wyk (2007) reported that *S. calamistis* adults did not differentiate between Bt and non-Bt maize plants in oviposition choice experiments.

Table 1: Number egg batches per plant, eggs per batch, eggs laid per plant and mean percentage of eggs hatched by *Chilo partellus* and *Sesamia calamistis* on Bt (Event 216) and non-Bt (CML 216) maize following introduction of pupae or moths into oviposition cages under non-choice conditions.

	No. of egg batches per plant	No. of eggs per batch	No. of eggs laid per plant	% eggs hatched
C. partellus introduced as pupae				
Non-Bt maize	3.6±1.3(8)[a]	38.0±12.5(114)	98.3 ±39.1(8)	82.8±15.0(114)
Bt maize	2.2±1.2(8)	39.9±13.6(70)	77.7 ±28.4(8)	85.4±9.3(70)
Students *t*-value	0.87	0.09	0.69	0.48
p-value	0.42	0.93	0.51	0.65
C. partellus introduced as moths				
Non-Bt maize	3.4±2.5 (8)	43.0±20.2(109)	115.6±78.8 (8)	84.4±10.1(109)
Bt maize	3.8±2.8 (8)	46.7±17.8(122)	122.8±83.3 (8)	89.0±2.5 (122)
Students *t*-value	0.23	0.21	0.70	0.67
p-value	0.83	0.84	0.51	0.53
S. calamistis introduced as pupae				
Non-Bt maize	2.7±0.4(8)	54.5±3.9(86)	143.1±27.7(8)	97.7±1.0(86)
Bt maize	3.3±0.5(8)	49.1±9.3(107)	128.9±14.1(8)	98.1±1.3(107)
Students *t*-value	2.05	2.95	0.33	1.85
p-value	0.09	0.05	0.33	0.16
S. calamistis introduced as moths				
Non-Bt maize	4.9±1.1(8)	50.2±7.5(157)	231.4±85.3(8)	92.8±3.5 (157)
Bt maize	5.4±0.4(8)	56.6±6.9(169)	293.6±27.6(8)	90.8±2.9 (169)
Students *t*-value	0.36	0.56	0.82	0.46
p-value	0.73	0.73	0.45	0.63

[a] Data shown is mean ± 1 SD of all observations [n] shown in brackets.

Volatile compounds released by plants can influence the behaviour of herbivores searching for oviposition sites (Anderson & Alborn, 1999; De Moraes *et al.*, 2001). Dean & De Moraes (2006) however observed that genetic modification did not alter the volatile profile of undamaged maize plants while Turlings *et al.* (2005) observed that the ratios of caterpillar-induced odour emissions of Bt maize plants were identical to that of non-Bt plants, a likely reason for the non-discriminatory behaviour of *C. partellus* and *S. calamistis* moths to Bt vs. non-Bt maize plants in this study.

An important limitation in our study is that the numbers of eggs laid were determined at the end of the egg-laying period of the moths and hence it was not possible to evaluate the day-to-day dynamics of egg laying. This was however unavoidable since at times the eggs were concealed (especially those of *S. calamistis*) and could only be accessed by destroying the plants. It was therefore not possible to determine

if the presence of previously laid eggs had any effect on subsequent oviposition behaviour. However, in previous studies moth oviposition was not affected by the presence of previously laid eggs (Chadha & Roome, 1980; Pats & Ekboom, 1994; Liu *et al.*,

2002). Moreover, the scenario presented in this experiment is a more holistic situation and closer to what would happen in nature whereby moths could encounter previously laid eggs.

Table 2: Number egg batches per plant, eggs per batch, eggs laid per plant and mean percentage of eggs hatched by *Chilo partellus* and *Sesamia calamistis* on Bt (Event 216) and non-Bt (CML 216) maize following introduction of pupae or moths into oviposition cages under choice conditions.

	No. of egg batches per plant	No. of eggs per batch	No. of eggs laid per plant	% eggs hatched
C. partellus introduced as pupae				
Non-Bt maize	3.6±2.3(4) [a]	35.2±15.4(76)	98.3±49.1(4)	73.0±28.7(76)
Bt maize	2.2±1.2(4)	37.4±15.1(60)	77.7±38.4(4)	73.6±28.3(60)
Students *t*-value	0.34	0.87	0.21	0.17
p-value	0.76	0.45	0.85	0.88
C. partellus introduced as moths				
Non-Bt maize	4.3±2.9(4)	44.2±3.0(68)	201.5±112.7(4)	81.7±14.0(68)
Bt maize	5.4±3.7(4)	45.1±6.0(86)	246.9±162.0(4)	78.3±19.4(86)
Students *t*-value	1.11	0.23	0.58	0.80
p-value	0.35	0.83	0.59	0.80
S. calamistis introduced as pupae				
Non-Bt maize	3.0±1.0(4)	44.0±13.6(48)	125.9±59.5(4)	98.4±0.3(48)
Bt maize	4.2±0.5(4)	37.1±5.8(67)	152.1±21.6(4)	98.6±0.7(67)
Students *t*-value	0.19	1.14	1.67	0.79
p-value	0.19	0.34	0.19	0.49
S. calamistis introduced as moths				
Non-Bt maize	5.3±1.6(4)	61.4±10.8(84)	298.3±48.8(4)	74.5±23.4(84)
Bt maize	5.6±1.9(4)	59.4±16.1(90)	320.9±44.2(4)	83.4±20.0(90)
Students *t*-value	0.33	0.20	0.18	2.81
p-value	0.33	0.85	0.86	0.07

[a] Data shown is mean ± 1 SD of all observations [n] shown in brackets.

The results of this study have important implications for pest resistance management and monitoring. For example, if oviposition is not affected by the Bt toxin, and females are exposed equally to Bt maize and non-Bt maize refuges, it can be assumed that eggs will be distributed equally between Bt and non-Bt maize, hence there will always be a pool of insects on susceptible crops, which is necessary for resistance management. Furthermore, the development of resistance against Bt toxins requires the survival and development of at least two exposed larvae into a male and a female (Kumar, 2004).

Even though Bt maize in this study did not affect the hatchability of stem borer eggs, neonates of both *C. partellus* and *S. calamistis* suffer 100% mortality on the Bt maize plants (Obonyo *et al.*, unpublished data). Feeding initiation by neonates of various lepidopterans including *P. xylostella* (Ramachandran *et al.*, 1998) and *P. gossypiella* (Liu *et al.*, 2002) is not deterred by Bt toxins in transgenic crops. Kumar (2004) observed that Bt cabbage did not emit any inhibitory signals to divert diamondback moth larvae from them. Most of the larvae exposed to Bt plants would initiate feeding on these plants and be

killed (Obonyo *et al.*, unpublished data), hence further restricting the possibility of resistance development to Bt maize. The mortality of Bt exposed stem borer larvae, alongside the limited chances of resistance development, could minimize the likelihood of stem borer natural enemies getting host-mediated exposure to the Bt toxin. Although the Bt maize could affect natural enemies indirectly as a result of host depletion due to death of maize stem borers (Groot & Dicke, 2002), the impact of such a development would most likely be minimal since several other stem borer species occur in wild host plants and they also host the same parasitoid species (Van den Berg & Van Wyk, 2007). Furthermore, stem borers surviving on the non-Bt refuge maize plants could be invaluable in sustaining natural enemy populations.

As the IRMA project further develops this technology it would be necessary, for purposes of resistance management, that the Bt maize plants under development are made unattractive for oviposition by stem borer moths, possibly by further engineering them with additional genes.

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