Screening for Development of Resistance in the African Stem Borer (*Busseola fusca* Fuller) and in the Spotted Stem Borer (*Chilo partellus* Swinhoe) to Bt-Maize δ-endotoxins

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DECLARATION

This thesis is my original work and has not been presented for a degree award in any other university. No part of this thesis may be produced without prior permission of the author, University of Nairobi and/or International Maize and Wheat Improvement Center (CIMMYT).

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DEDICATION

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ABSTRACT

The African stem borer, *Busseola fusca* Fuller (Lepidoptera: Noctuidae), and the spotted stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyraridae), are the major stem borers species of great economic importance in Kenya. Studies were carried out in the biosafety level 2 greenhouse to determine the development of resistance to Bt δ-endotoxins by stem borers for five and eight generations of *B. fusca* and *C. partellus*, respectively. Treatments included Bt-maize containing either of the two gene constructs (Event223::*cry1Ab::Ubiquitin* and event10::*cry1Ba::Ubiquitin*), and the non-transgenic CML216 control. *B. fusca* colony was developed from collection from Kitale in Highland Tropical maize growing zone, while *C. partellus* colonies were from the Coast Lowland Tropics and from the Dry Mid-altitude areas of Kenya. A fourth colony (mixed colony) was derived from mating the *C. partellus* from the Humid Coastal Lowland Tropics and the Dry Mid-altitude areas of Kenya.

Sowing of the maize was synchronized to pupae stages of the insects. Three hundred (300) neonates from each colony were infested into maize leaves at the six to eight-leaf stage and allowed to feed for one to three hours, with the exception of *B. fusca*, which was allowed to feed for 48 hours. The surviving larvae were removed and reared in artificial diet up to completion of their life cycles. The subsequent generations were similarly exposed to the δ-endotoxins, and allowed to complete their life cycle in artificial diet. The responses to the δ-endotoxins were assessed over time by counting the number of surviving larvae for each generation cycle, and by measuring pupae weight for each cycle.

Results showed significantly fewer surviving larvae from both Bt-maize events compared to the non-transgenic CML 216 control. There was a significant difference ($P<0.05$) between the pupae weight of the control and those fed on Bt-maize tissues. There were no significant differences between the *C. partellus* colonies from the different maize growing zones in Kenya, in their response to Bt-maize δ-endotoxins. There was also no significant difference among the cycle of selection of the two Bt-maize events ($P<0.05$). There was neither development of resistance to *C. partellus* for eight generations nor increase in resistance in *B. fusca* for five generations the Bt-maize events that were studied. This information could be used as baseline data in developing resistance management and deployment strategies to extend the efficacy of Bt-maize.

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GLOSSARY

Agro-ecological zones: Land characterization for agricultural suitability based on climate, soils and landforms.

Biotechnology: Any technique that uses living organisms, or substances from these organisms, to make or modify a product, to improve plants or animals, or to develop microorganisms.

Biosafety: The safe application of biotechnology and pathology and the policies and procedures adopted to ensure this.

BL2GHC: Biosafety level 2 greenhouse complex.

Bt: *Bacillus thuringiensis*. A soil dwelling gram-positive bacterium, which produces spores. It was discovered in 1900 in the region of Thuringia, Germany.

Bt endotoxins: Crystalline proteins produced by *Bacillus thuringiensis* that are toxic to selected insect orders: lepidoptera, diptera and coleoptera.

Conspecific: Geographically distant populations of insects within the same species.

1).DMA-Dry mid-altitude population of *Chilo partellus*.

2).HCLT- Humid Coastal Lowland tropics population of *Chilo partellus*.

Event: One successful gene transfer.

Genetic Engineering: The technique of removing, modifying or adding genes to a DNA molecule in order to change the information it contains. This leads to changes in the type or amount of proteins an organism is capable of producing.

Genetically engineered/ genetically modified/ transgenic material:

Any living organism whose genome organization has been modified by the addition of genes from other organisms or form DNA synthesized in the laboratory using technologies such as bioengineering or recombinant DNA.

Transgenic: Plant or animal material whose genetic heredity DNA has been transformed through the addition of DNA from a source outside its normal gene pool, using recombinant DNA techniques.

Instar: Developmental stage of insects or arthropods between each molt (ecdysis) until sexual maturity is reached.

CHAPTER ONE

INTRODUCTION

1.1 Importance of Maize

Maize (*Zea mays* L.) is the major staple food for the majority of households in eastern and central African regions, dominating the diets of the rural and urban poor (Johansson and Ives, 2001). It is a source of income for many, both in the rural as well as urban areas. Maize is well adapted to different climatic conditions and can therefore be grown in different environments (James, 2003). In developing countries, it is mainly grown under rain fed conditions, while both irrigation and rainfall are used in the industrialized countries (Pingali, 2001). Maize is one of the most important food sources for much of the human population in Africa (Bonhof, 2000). Maize ranks second in importance to wheat in total tonnage in the world, and this is due to its diverse uses, which include food, feed and industrial uses (James, 2003; FAO, 2003). According to Pingali (2001), a major shift in global cereal demand is underway: by 2020, demand for maize in developing countries will surpass the demand for both wheat and rice. Further, this demand will be reflected in a 50% increase in global maize demand from its 1995 level of 558 million tons to 837 million tons by 2020.

1.2 Maize production

Although developing countries grow a large acreage of the maize (two-thirds of global maize production) (Pingali, 2001), imported maize is still estimated to increase from around 600 million MT today to about 850 million MT in the year 2020

(James, 2003), with major new shifts in favor of maize for food and cash. The shortage is partly contributed by low average maize yields in Africa due to biophysical constraints, which include both biotic factors and abiotic factors (DeVries and Toenniessen, 2001). The current global acreage of maize is over 142 million hectares, and production records are over 637 million metric tons (MT), with USA as the leading producer at an average yield of 3.41MT per hectare (FAO, 2003).

Maize is by far the most important food crop in Kenya, being grown as both a subsistence and commercial crop (De Groote *et al.*, 2004). Per capita maize consumption in Kenya is approximately 125 kg/year (Pingali, 2001). It is planted on 1.5 million ha, which is more than 30% of the arable land, and is widely distributed throughout the six major agro-ecological zones. The average annual production of the last 5 years is estimated at 2.4 million tons (FAO, 2003).

There are six maize growing agro-ecological zones in Kenya, which are defined by elevation, total rainfall and length of the growing season and maturity period of the adapted maize cultivars (FAO, 2000; Hassan *et al.*, 1998). Moving from east to west, are the Humid Coastal Lowland Tropics zone (HCLT), at the coast, the Dry Mid-altitude (DMA) and the Dry Transitional (DT) zones which are found between the Mid-altitude (MAT) and highland Tropics (HT) zones. These zones are characterized by low grain yields (below 1.5 ton/ha) and although they cover 29% of maize growing area in Kenya, they produce only 11% of the total maize production in Kenya annually. In central and western Kenya, there are the Highlands Tropics (HT), bordered at the west and east by the Mid-Altitude Moist (MAM) and the Mid-Altitude Transitional (MAT) zones. These zones cover about 30% of the maize area, have

average grain yields of more than 2.5t/ha, and produce about 80% of the maize annually (De Groote *et al.*, 2003).

1.3 Uses of maize

Maize is mostly used for food, feed and for industrial purposes. As food, it is consumed green, either boiled or roasted, or milled when dry and processed to other foods and products (DeVries and Toenniessen, 2001), which include flour, processed meals and /or oils. When used as animal feed, maize is used in silage making for livestock, as well as processed to other feed for farm animals including poultry, pigs and horses. In industries, maize is used for production of starch and starch products, sweeteners and fermented to alcohol and other fermentation products like ethanol (James, 2003).

1.4 Constraints to maize production

Production constraints in maize include both abiotic and biotic stress factors. Abiotic stress factors include soil moisture stress, unreliable rainfall, and declining soil nutrients (DeVries and Toenniessen, 2001). According to DeVries and Toenniessen (2001), drought and low soil fertility contribute greatly to the low yields realized and losses of up to 13% have been recorded in eastern and southern Africa. Among the biotic constraints, arthropod pests are a major contributing factor to low maize yields. Insect pests are more damaging in the tropical than temperate environments, because the climatic conditions are conducive for accelerated insect development with multiple and overlapping generations leading to high infestation

levels (Songa *et al.*, 2001, Mugo 2006). Stem borers form the major pest complex in maize fields causing not only direct damage, but also creating entry points for secondary infections. Quite often, they can reduce the photosynthetic area of the leaves, cause dead hearts or bring about lodging, which lower maize yields substantially (Nderitu, 1999; Mugo *et al.*, 2001). Weeds, especially the parasitic weed *Striga*, and lack of improved seed along side diseases (fungal, viral and bacterial), are other constraints to maize production (Pingali, 2001; Mugo, 2006).

1.5 Importance of stem borers

Stem borers have been known to attack maize from seedling stage through to maturity (Appert and Deuse, 1982), and up to 100% infestation levels have been recorded on maize fields as well as in other cereals like sorghum. These stem borers not only lower yields, but also lower grain quality due to contamination through webbing and faecal waste, as well as wounds, which further act as entry points to secondary pathogens. This endangers the life of the consumers (Nderitu, 1999). De Groote (2002) reported average grain yield losses of 13.5% in Kenya. More than 50 million metric tones (MT) of global maize production are estimated lost due to stem borer annually (James, 2003). James (2003) further reported that stem borers form the key pest complex in maize production globally with estimated grain yield losses of between 15-40%. In Kenya the two major stem borers species that are most prevalent are the spotted stem borer (*Chilo partellus* Swinhoe; Lepidoptera, Pyralidae), and the African stem borer, (*Busseola fusca* Fuller; Lepidoptera, Noctuidae) (Songa *et al.*, 2001).

1.6 Control options for stem borers in Kenya

The control measures used for the management of stem borers are chemical, biological, cultural, and host plant resistance (HPR) through conventional breeding and through genetic engineering. Chemical control methods are most effective, but pesticides are expensive to smallholder farmers, may be harmful to humans and livestock, and may degrade the environment. They also affect non-target and beneficial insects, including pollinators, decomposers and biological control agents. Biological control plays an important role in regulating the populations of lepidopterous pests in Africa (Polaszek and Walker, 1991). Naturally occurring populations of natural enemies are however not usually able to maintain stem borer populations below damaging levels (Overholt *et al.*, 1994). Cultural control practices such as destruction of crop residues, early planting and intercropping have been used for stem borer control. However, cultural control practices are best when used in combination with other control measures and rarely stand alone (Mugo *et al.*, 2002). HPR has been used to develop crop varieties resistant to insect pests for several years (Smith, 1989). However, stem borers have not yielded to conventional resistance breeding approaches, since most existing maize cultivars are still highly susceptible (Gethi *et al.*, 2001).

Farmers can easily adapt host plant resistance as the technology is encapsulated in the seed, which they know how to handle. However, developing insect resistance using conventional means is difficult due to the quantitative nature of inheritance, and the fact that the breeding procedure involves two organisms, the pest and the host. Genetically engineered host plant resistance holds great potential as a

method of stem borer control. This option has been successfully used in other countries, and it is being explored for implementation into the Kenyan farming systems (Mugo *et al.*, 2002).

1.7 Bt-maize

Bacillus thuringiensis (Bt) is a gram positive soil bacterium that produces insecticidal proteins during its sporulation. It was first discovered in Japan in 1907 by Ishawatta, and then in 1911 in Germany by Berliner (Baum *et al.*, 1999). It was subsequently found that thousands of strains of *B. thuringiensis* exists (Lerechus *et al.*, 1993). Each strain produces its own unique insecticidal crystal proteins, or δ-endotoxin, which is encoded by a single gene on a plasmid in the bacterium (Whalon and McGaughey, 1998). Bt-maize is ordinary maize that has had a gene from the bacterium, inserted into the maize genome, through genetic engineering. The inserted gene 'instructs' the plant to produce Bt-proteins upon predation by target insect pests. The neither changes the agronomic performance of the maize, such as, yield potential of the maize; nor the consumer qualities such as taste of the crop. In Bt-maize insecticidal proteins (δ-endotoxins) are produced in tissues where stem borers feed, until after flowering when the concentration levels of the proteins decrease. The insecticidal proteins are specific in action and only affect the target pests (Dutton *et al.*, 2002).

1. 8 Introduction of Bt maize in Kenya

Applications to introduce Bt maize events in Kenya were made to the National Biosafety Committee (NBC) by scientists in the KARI/CIMMYT Insect Resistant Maize for Africa (IRMA) project. Bt transformed maize cut leaves were introduced in 2001 and leaf bioassays done on them to identify the effective gene/s against the major stem borer species in Kenya (Mugo *et al.*, 2005). An application to introduce Bt maize seeds was made to the NBC in 2003, approved in May 2004, and seeds introduced the same month (KARI/CIMMYT, 2003). The results showed that the Bt-maize events were effective in controlling *C. partellus*, the coastal stem borer, *Chilo orichalcociliellus* Strand (Lepidoptera:Pyralidae), the pink stem borer, *Sesamia calamistis* Hampson (Lepidoptera:Noctuidae), and the sugarcane borer, *Eldana saccharina* Walker (Lepidoptera:Crambidae), the major stem borers found in the maize growing regions of Kenya. *B. fusca* was not effectively controlled by the tested events (Mugo *et al.*, 2005).

1.9 Justification for resistance studies

The Kenyan population is ever increasing; with the current population growth at 2.5%per annum (Pingali, 2001; Population of Kenya, 2004). Increase in food production is therefore a national priority. One of the methods of addressing the need is by reduction of the losses associated with stem borers. Bt-maize has been used successfully in other countries (James, 2005), with positive results. Consequently there is need to incorporate it in our agricultural farming systems. Studies have been initiated in Kenya to address this need. While Bt-maize offers solution to the menace

of stem borers, it is important that work is done to implement strategies to ensure that insects do not develop resistance (Shelton *et al.*, 1999).

Part of the information required for commercial release of Bt-maize is the activity of Cry proteins against the different African stem borer species and the durability of this control against the development of resistant biotypes. Such information is useful for the development of deployment strategies and as baseline data for use in the development of insect resistance management strategies, in order to ensure the extension of efficiency of the Bt-technology. It is also important to determine if resistance is a problem in a specific location or within a specific population in order to improve the management and deployment strategies of Bt-maize. Smith (1970) suggested that it is an essential part of pest control procedure to monitor continuously the levels of resistance in major pests. This research aimed at determining the possibility of the stem borers developing resistance, while at the same time considering the different levels of Bt toxins that will effectively control the insect pests and delay the on-set of resistance.

1.10 Overall Objective

To screen generations of the major stem borers, *C. partellus* and *B. fusca*, for the development of resistance to Bt-maize δ-endotoxins.

1.11 Specific Objectives

1. Evaluation of the response of *C. partellus* colonies from the Lowland Tropics (HCLT) and the Dry Mid-altitude (DMA), to cry1Ab and cry1Ba Bt-maize δ-endotoxins.
2. To monitor changes in tolerance to cry toxins by *B. fusca* populations over cycles of selection using Bt-maize containing cry1Ab and cry1Ba δ-endotoxins.

1.12 References:

Appert J. and J. Deuse. 1982. Pests of food and market garden crops in the tropics. Les ravageurs des cultures vivrière et maraîchères sous Les tropiques. G-P, Maisonneuve & Larose Paris, France, 420pp.

Bonhof M.J. 2000. The impacts of predators on maize stem borers in coastal Kenya. Thesis Wagenigen University. Pp 1-18

De Groote H. 2002. Maize yield losses from stem borers in Kenya. Insect Science Applications. 22(2): 89-96.

De Groote H., W. Overholt, J.O. Ouma and S. Mugo. 2003. Assessing the potential Impact of Bt maize in Kenya using a G.I.S. based model. Paper presented at the international Agricultural Economics Conference, Durban, South Africa, August 2003.

De Groote H., S. Mugo, D. Bergvinson, and B. Odhiambo. 2004. Debunking the myths of GM crops in Kenya. Extended Abstract of a Presentation at the

Annual Meeting of the American Agricultural Economics Association (AAEA), Denver, Colorado, 4 August 2004.

De Vries J. and G. Toenniessen. 2001. Securing the harvest: Biotechnology, breeding, and seed systems for African crops. CABI publishing, New York, USA. pp 99-108.

Dutton A., H. Klein, J. Romeis, and F. Bigler. 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea.* Ecological Entomology 27, 441-447.

FAO. 2000. Global Agroecological zones (GAEZ).

http://www.iiasa.ac.at/Research/LUC/GAEZ/index.htm

FAO. 2003. World wheat, Corn, and Rice production. http://www.nue.okstate.edu/.

Gethi M., S. Mugo, O. Odongo, G. Ombakho, K. Njoroge, W. Muasya, and W. Chivatsi. 2001. Evaluating commercial and elite experimental varieties for resistance to stem borers. In: Insect Resistance Maize for Africa Annual Report 2001 KARI/CIMMYT IRMA Project. IRMA Project Document No.4.KARI and CIMMYT (Kenya Agricultural Research Institute and International Maize and Wheat Improvement Centre), Nairobi.

Hassan R.M., R. Onyango, and J. K. Rutto. 1998. Relevance of maize research in Kenya to maize production problems perceived by farmers. In: Maize Technology Development and Transfer: A GIS Application for Research Planning in Kenya (ed. Hassan, R.M) CAB International, Wallingford, UK. Pp 137-161.

James C. 2003. Global Review of Commercialized Transgenic crops: 2002 Feature: Bt Maize. ISAAA Briefs No. 29. ISAAA: Ithaca, NY.

James C. 2005. Preview: Global Status of Commercialized Biotech/GM Crops: 2005. ISAAA Briefs No. 34. ISAAA: Ithaca, NY.

Johansson A. and C. L. Ives. 2001. An Inventory of Agricultural biotechnology for the eastern and central African region. Prepared for ASARECA and the Africa Bureau of the United States Agency for International Development. March 2001. Agricultural Biotechnology Support Project. Michigan State University. Pp16-19.

KARI and CIMMYT. 2003. Insect Resistant Maize for Africa: IRMA IN 2003 Briefs. KARI/CIMMYT IRMA PROJECT. IRMA Project Document No. 12 Nairobi, Kenya: KARI and CIMMYT. Pp 3-6.

Mugo S.N., D. Bergvinson, and D. Hoisington. 2001. Options in Developing Stem borer-Resistant Maize: CIMMYT's Approaches and Experiences. Insect Science Applications. Vol.21, No. 4, pp 409-415.

Mugo S.N., J.M. Songa, H., De Groote, and D. Hoisington. 2002. Insect Resistant Maize for Africa (IRMA) Project: An overview. Syngenta Symposium, June 25, 2005 Washington DC.

Mugo S., H. DeGroote, D. Bergvinson, M. Mulaa, J. Songa, and S. Gichuki. 2005. Developing Bt maize for resource poor farmers- Recent advances in the IRMA project. Africa Journal of Biotechnology. Vol. 4 (13), pp. 1490-1504.

Mugo S.N. 2006. Maize in sub-Saharan Africa: Production and Improvement. Book Chapter In: The status of Biotechnology in Sub-Saharan Africa. (ABSF) In press.

Nderitu C.G. 1999. Biotechnology in Africa; Why the controversy?. "In: G.J. Presley and M.M. Lantin, Editors. 2000. Agricultural Biotechnology and the poor: Proceedings of an International Conference, Washington, D.C., 21-22 October 1999. Consultative Group on International Agricultural Research, Washington, D.C. pp 109-114.

Overholt W.A., A.J. Ngi-Song, S.W. Kimani, J. Mbapila, P.M. Lammers, and E. Kioko. 1994. Ecological considerations of the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for biological control of *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) in Africa. Biocontrol News Information 15, 19N-24N.

Pingali P.L. (Ed.). 2001. CIMMYT. 1999-2000. World Maize Facts and Trends. Meeting world maize needs: Technological opportunities and priorities for the public sector. Mexico, D.F.: CIMMYT. Pp 1-57.

Population of Kenya. 2004. http://www.kenya.info/subsection.asp?ID=2

Polaszek A. and A.K. Walker. 1991. The *Cotesia flavipes* species complex: parasitoids of cereal stem borers in the tropics. Redia 74, 335-341.

Shelton A. M., J. D. Tang, R.T. Roush, T. D. Metz, and E.D. Earle. 1999. Field-tests on managing resistance to Bt- engineered plants. Nature Biotechnology 18:339- 342.

Smith R.F. 1970. Pesticides; their use and limitations in pest management, pp. 103-113 In: Rabb, R.L. and Guthrie, F.E. (eds): Concepts of pest management. North Carolina State Univ., Raleigh, N.C.

Smith C.M. 1989. Plant Resistance to insects: A Fundamental Approach. John Wiley and Sons, New York.

Songa J.M., G. Zhou, and W.A. Overholt. 2001. Relationships of stem borer damage and plant physical conditions to maize yield in a semi-arid zone of eastern Kenya. Insect Science and its Application 21(3), 243-249.

CHAPTER TWO

LITERATURE REVIEW

2.1 Maize Stem Borers

Stem borers are a key pest complex and are the most prevalent insect pests of maize globally (Pingali, 2001, James, 2003). According to Pingali (2001), maize stem borers are most serious especially in the tropics and sub- tropical environments than in the temperate climatic regions. In Africa, the most common stem borer species include *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae), *Busseola fusca* Fuller (Lepidoptera: Noctuidae), *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), *Chilo orichalcociliellus* Strand (Lepidoptera: Crambidae), *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (Overholt *et al.*, 2001). *C. partellus* is native of Asia, but became established in east Africa in the 1950's (Nye, 1960), and has spread to southern and central Africa (Overholt *et al.*, 2001). *B. fusca* is an indigenous pest of the mainland Africa south of the Sahara and is not known to occur outside this area (Inter Africa Phytosanitary Council, 1985; Harris and Nwanze, 1992).

Larvae are the longest and most destructive stage in the life cycle of stem borers and attack maize from seedling to maturity (Appert and Deuse, 1982). Up to 100% infestation, levels have been recorded with loss due to damage and due to contamination with aflatoxins in damaged kernels. In Kenya, *B. fusca* and *C. partellus,* are of greater economic importance than the other species (Songa *et al.*, 2001a, Songa *et al.*, 2002b).

2.1.1 *Busseola fusca*: Biology and Ecology

According to Harris and Nwanze (1992), the life cycle of *B. fusca* varies mainly because of climatic and seasonal differences of temperature, moisture and photoperiodism. The adult moths emerge from pupae in the stems and stubble in the late afternoon and early evening, are active during the night and rest on plant debris during the day and can only be seen upon disturbance. The females release pheromones on the night of emergence to attract males for mating. The female lays eggs in batches of 30-100 for, three to four days after mating and can lay an average of 200 eggs. The eggs hatch after seven days, into larvae, which disperse all over plants before entering leaf whorls to feed.

Once established they burrow into the stem and feed for three to five weeks during which they excavate a hole to the exterior before pupae stage sets. The adult moth uses the hole for exit after emergence, 9-14 days later. The whole life cycle takes seven to eight weeks, if environmental conditions are favorable. If the conditions are unfavorable, the larvae undergo diapause for six months or more in stems, stubbles and other plant residues (Overholt *et al.*, 2001).

2.1.2 *Chilo partellus*: Biology and Ecology

The newly emerged adult moths rest on the plant and plant debris during the day and are seldom seen unless disturbed. Females release pheromones to attract males (Nesbitt *et al.*, 1979; Lwande *et al.,* 1993), and mating occurs soon after emergence. Each female lays up to a total of 200-600 scale- like, overlapping eggs, two to three nights later in 10-80 batches mostly near mid-ribs on the under sides of

the leaves mostly near mid-ribs. The larvae hatch four to eight days later and initially feed in the leaf whorl. Subsequent larval instars tunnel into stems, eating out extensive galleries. Larval development usually takes two to four weeks after which pupae stage begins in damaged stems and adults' emerge 5-12 days later. The whole life cycle is completed in 25-50 days when conditions are favorable. Five or more generations may develop in a growing season in favorable climatic conditions. Larval diapause may set in if the conditions are not favorable.

2.2 Distribution of Maize Stem Borers

The pyralids; *C. partellus* and *C. orichalcociliellus* are found in the low altitude, warmer zones below 1200m above sea level. *C. partellus* is a native of India and became established in East Africa in the 1950s (Nye, 1960). It is prevalent in east, south, and central Africa. It occurs throughout mainland Africa south of the Sahara and has been formally recorded from West Africa, Eastern Africa, and Southern Africa.

B. fusca occurs from sea level to altitudes in excess of 2000m in West Africa, but is most abundant in the wetter parts of the true savannah in Ghana, and Burkina Faso (Nwanze, 1988), and in the drier regions of the tree savannah and the thorn scrub savannah in Nigeria (Harris, 1962; Overholt *et al.*, 2001). In southern Africa, *B. fusca* is the dominant stem borer at elevations above 900m but also occurs at lower altitudes. In east Africa it occurs between 600 and 2700m above sea level and is absent from the coastal areas of Kenya and Tanzania.

In Kenya Stem borers are distributed in the entire maize growing environment in Kenya. *C. partellus* is most found in the lowland tropics and the mid altitude tropics maize growing zones (Overholt *et al.*, 2001). *E. saccharina* is found around the lake region, while the noctuid, *S. calamistis* is found in all the zones (Mugo *et al.*, 2001). *B. fusca* is found in cooler areas above 1500m above sea level. Other regions (Moist Transitional, MT, and Moist Mid-altitude, MM) have an overlap, with the two borer species being found in these maize growing zones.

2.3 Economic Importance

Stem borers are of great economic importance because they are widely distributed and affect a significant portion of the 96 million hectares of maize grown in developing countries (Pingali, 2001). James (2003) reported that the stem borers form the key pest complex in maize production globally with estimated losses of between 15-40%.

Stem borers damage maize plants in three main ways: 1) through foliar damage, 2) stem tunneling and 3) kernel damage (Overholt *et al.*, 2001). Foliar damage is caused by larval feeding (1^{st} and 2^{nd} instars) on the young leaves, often causing lesions that are seen as 'windows' and sometimes as 'shot holes'. This reduces photosynthetic area of the leaves, consequently interfering with the plant growth and production. In young maize plants, dead-hearts can result, leading to either tillering or complete death of the plant. At later stages (3^{rd}-5^{th} instars), the stem borers bore into the maize stems and create tunnels through feeding. This stem tunneling interferes with the movement of water and metabolites through the vascular

system. It can also result in stalk breakage (lodging) and eardrop. Second-generation borers, like *B. fusca*, usually cause damage to the kernel, by feeding on the developing grains and pollen grains. This reduces grain yield by the maize plants (Songa, 1999).

There are two types of losses associated with stem borers; loss in quantity and loss of quality. Loss in quantity results from reduced photosynthetic area hence reduced grain filling, which leads to small or no grain formation. The reduced translocation leads to reduced ear and grain size. Dead hearts lead to total crop loss. A combination of all these factors leads to loss of yield and fodder (Harris and Nwanze, 1992).

Quality loss results from decreased fertility and size of harvested seed. Wounds created through entry and during tunneling, become entry points for secondary pathogens including fungi and bacteria (James, 2003). Fungal pathogens, particularly *Fusarium spp.* colonize the damaged tissue leading to production of mycotoxins like fumonisins and aflatoxins. This leads to ear rots and results in low and toxic grain that contributes to food and feed safety hazards. This can endanger the lives of the consumers (Nderitu, 1999). Grain quality is further lost through contamination, brought about by faecal waste and webbing by the stem borer larvae (Songa *et al.*, 2001b).

2.4 Methods of Control

Various methods for the control of stem borers are common globally including; cultural, chemical, biological, and host plant resistance. Integrated pest management methods have gained popularity in stem borer control.

2.4.1 Cultural Methods of Control

Cultural control is defined as the use of regular farm management practices, which are specifically designed to destroy or prevent pests from causing damage to crops (Omolo and Seshu Reddy, 1985). Cultural control methods include: a) weeding, which reduces competition for nutrients by weeds, some of which are alternate hosts of stem borers, b) avoiding intercropping with alternative hosts such as sugarcane, wheat, barley, sorghum and some wild grasses from maize fields; c) disposing of maize crop residues by using as fodder, burning, deep plowing or harrowing; d) planting early in the season when stem borers population is low, and e) intercropping maize with other crops, preferably legumes that may repel stem borers away from maize fields (Mulaa, 1995). While these methods are less expensive, to the small-scale farmer, they are not commonly used because they are laborious and time consuming. Their use is, therefore, limited.

2.4.2 Chemical Methods of Control

This involves the use of pesticides, which come in various formulations. Contact pesticides , including the organophosphates (that is, chlorpyrifos and diazinon), carbamates (methiocarb), and pyrethroids (bifenthrin, cyfluthrin, fluvalinate, fenpropathrin, and permethrin) work well when the pests are on the plant surface (Cloyd, 2002).They are effective upon direct contact with the pest during spray applications or when the pest comes into contact with wet residues when moving around plant surfaces. Cloyd (2002) reports that contact insecticides generally provide quick knockdown of target pests. Many insecticides from the older chemical

classes including the organophosphates (that is, chlorpyrifos and diazinon), carbamates (methiocarb), and pyrethroids (bifenthrin, cyfluthrin, fluvalinate, fenpropathrin, and permethrin) have contact activity. However, some insecticides that have either *systemic* or *translaminar* (local) properties.

Systemic pesticides, for example (imidacloprid, acephate, and pymetrozine), control stem borers burrowed within in the maize whorl, provide quicker kill of target pests and provide the plant with long-term protection from pest injury. A problem associated with systemic insecticides is that many have a single, or site-specific, mode of activity, which may lead to resistance. The selection pressure placed on pests from the continual use of systemic insecticides may result in the development of resistant genotypes. Due to their high water solubility, they are subject to leaching and may potentially contaminate groundwater. The chemicals are expensive to the smallholder farmer and maybe harmful to humans and livestock. Most pesticides have unfavorable effects on the environment, and are indiscriminate to the target and non-target insects, some of which are beneficial insects including pollinators, decomposers, and the biological control agents (Polaszek, 2001).

2.4.3 Biological Methods of Control

Biological control involves the use of natural enemies such as parasitoids, predators and pathogens for the regulation of population densities of other organisms (van Driesch and Bellows, 1996). A wide range of egg, larval and pupal parasitoids of stem borers has been identified. The microsporidian pathogen *Nosema marucae*, Sp.n (Microspora; Nosematidae) has been studied for adoption by small scale farmer in the

tropics to control *C. partellus* (Odindo *et al.*, 1993). Other control agents, both locally available and exotic biological control agents include *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) and the wasp *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) which have been used to control stem borers in Kenya (Songa, 1999). Songa (1999) found out that there is low percentage of parasitism by indigenous parasitoids and the common pupal parasitoids. Exotic biological control agents, for example, *Cotesia flavipes* already released need time to establish for effective control. Biological control methods are slow and ineffective within the growing season. However, in the long run, these methods are efficient, cost-effective, environmentally safe and safe to the humans and livestock (Songa, *et al.,* 2002a).

2.4.4 The Push-Pull System

This method involves intercropping repellent plants, which 'push' the stem borers out of the maize field, with trap crops that 'pull' the insects outside of the maize field. Napier grass (*Pennisetum purpureum*) and Sudan grass (*Sorghum vulgare Sudanese*) both of which are important fodder crops were found to produce gummy substances that trap stem borers and only 10% of the larvae survive to adulthood (Khan, 2006). Molasses grass (*Melinis minutiflora*) repelled or 'pushed' the stem borers by releasing a complex mixture of volatile substances (terpinoles and nonatrienes among others). Molasses grass also increases stem borer parasitism by harboring a natural enemy, the wasp *Cotesia sesamiae*.

The leguminous silver leaf (*Desmodium uncinatum*) was found to push the insects, fix nitrogen, provide fodder and suppress the notorious weed, *Striga* (Khan, 1997).

2.4.5 Integrated Pest Management (IPM)

IPM has been defined as 'a pest management system that in the context of the associated environment and population dynamics of the pest species, utilizes all available techniques and methods in as compatible a manner as possible and maintains pest population below those causing economic injury' (FAO, 1966). Many small-scale farmers use more than one method of control in order to realize yields. While it is important to incorporate these methods together, IPM calls for a thorough understanding of the pest dynamics and clear knowledge of how these pests interact with the environment they live in. This may be sometimes too much to ask from the farmer and for it to be useful; the farmer may need thorough training, probably through Farmer Field Schools. Time is required and, therefore, the effectiveness of this method may only be realized in the long run.

2.4.6 Conventional Host Plant Resistance

Host plant resistance is defined as the relative amount of heritable qualities possessed by a plant, which influence the ultimate degree of damage done by insect in the field (Painter, 1951). Some maize cultivars grown in lowland areas of Kenya have been identified to have some levels of resistance to *Chilo spp.* CIMMYT has come up with germplasm that is reported to have resistance to the stalk borer complex (Mulaa,

1995). Some of the line developed includes 1CS 1-cm (CIMMYT population 27) and 1CZ2-cm (CIMMYT population 22) among others. Resistant varieties with reduced leaf damage, stalk tunnels increased leaf toughness, and higher grain yields were observed under artificial infestation and protected conditions, as well as by farmer evaluation (Mugo *et al.*, 2006). Superior varieties such as P390 MIRT and ITS1 ST G1XP590B will be taken through National Performance Trial for release and also be used as sources of resistance for the development of new stem borer resistant maize germplasm. Conventional HPR method is both time consuming and involves two organisms, the pest and its host (Mugo *et al.*, 2006).

2.4.7 Host plant resistance through genetic engineering

The deployment of a number of Bt genes for the control of diverse insect pests is one of the promising new technologies for capturing increased yield potential from improved maize germplasm (James, 2003). Using genetic engineering tools, modified novel genes from the soil bacterium *Bacillus thuringiensis* (Bt) have been introduced into maize and this holds great promise in controlling the lepidopteran stem borers (National Academy of Sciences, 2000). These genes encode δ-endotoxin proteins, which, when ingested by the susceptible stem borer, are activated by favorable environments in the insect guts, resulting in larval mortality (Gill *et al.*, 1992). The genes are, therefore, fully incorporated into the genome of the crop, giving it inbuilt resistance to the target pests. Gill *et al.*, (1992) reports that the modified crops require less chemical sprays, and, therefore, farmers will save on costs of production, while at the same time be a reduction in losses associated with stem borers.

2.5 Bt Technology

The insecticidal activity of the toxins from each Bt strain differs, affecting a variety of insects from different orders like Coleoptera (beetles), Lepidoptera (moths and butterflies), and Diptera (flies and mosquitoes) (Gould and Keeton, 1996). Unlike many other pesticides, Bt toxins are very specific to target insects and are therefore safe to most beneficial insects and other animals. They are biodegradable and do not persist in the environment (Van Frankenhuyzen, 1993).

2.5.1 The Bt δ-endotoxins

Whalon and McGaughey, (1998), reported 34 recognized subspecies of *B. thuringiensis*, the most common one being; subspecies *kurstaki* (against lepidopterans), subspecies *tenebrionis* (against Colorado beetle- *Leptinsarsa decemlineata* Say Coleoptera; Chrysomelidae), subspecies *israelensis* (against diptera, primarily against mosquito and black flies). From these subspecies, two general groups of insecticidal crystals proteins have been identified; cytolysins (*cyt*) and crystal δ-endotoxin (*cry*). Crystal δ-endotoxin (*cry*) has four classes of cry genes while the cytolysins have two. The *cryi* and *cryii* toxins are active against lepidopterans, *cryii* and *cryiv* against dipterans, and *cryiii* against coleopteran (Hofte and Whiteley, 1989).

2.5.2 Mode of Action of Bt δ-Endotoxins

The δ-endotoxins cause direct mortality in susceptible target insects. Van Rie *et al.* (1992) reported that the crystalline δ-endotoxins, once ingested, are dissolved in

the insect midgut, liberating protoxins. The protoxins are proteolytically processed into fragments, one of which binds to cells of the midgut epithelium (Hofte and Whitely, 1989). Activated proteins disrupt the osmotic balance of these cells by forming pores in the cell membrane causing the cells to lyse. The gut then becomes perforated and the insect stops feeding, eventually dying within a few hours of ingestion (Marrone and MacIntosh, 1993). However binding does not assure toxicity to the target pest (Whalon and MacGaughey, 1998).

2.5.3 Transgenic Crops

In 1980, commercial interest in *B. thuringiensis* (Bt) grew rapidly as many popular synthetic insecticides became ineffective due to insect resistance or they simply became unusable due to environmental restrictions. Van Frankenhuyzen, (1993) reported insertion of genes encoding for Bt δ-endotoxins into plants that led to the production of the first tobacco and tomato transgenic plants to express Bt toxins, which were tobacco and tomato plants. James (2006) reported the progress made in growing transgenic crops. Today a number of crops have been transformed and grown in several countries both in the industrial and the developing counties. The major Bt transgenic crops by 2006 were; soybean occupying 58.6 million hectares (57% of global area of transgenic crops), and transgenic maize, (yellow and white maize) whose global area increased in 2006 to 25.2 million hectares (25% of global biotech area) (James, 2006). Genetically modified cotton has also gained popularity especially in India, now being the largest cotton growing country in the world (with 3.8 million hectares), followed by China and the USA. Australia and South Africa are currently

also growing the crop and it covers 9.8 million hectares at 11% biotech crop area. Transgenic canola is grown in estimated 4.6 million hectares (5% of biotech crop area) as of 2005, Canada and the USA being the leading countries growing the crop. The global area planted with transgenic crops continues to increase currently being 577 million hectares (or 1.4 billion acres) (James, 2006). The technology is widely used to confer useful traits, to other crops like rice, bananas, cowpeas and sorghum among others. The currently preferred traits are herbicide tolerance and insect resistance (James, 2005; James, 2006). Neppl (2000) observed that the engineering of plants to express Bt δ–endotoxins has been especially helpful against pests that attack parts of the plants. These pests usually are well protected by parts of the crop from the conventional insecticide application. Stem borer larvae are examples, because they bore into the maize stalk and destroy its structural integrity, while, relatively safe from pesticide application. With toxins engineered into the plant, stem borers are exposed and their control easier. Therefore, plants expressing Bt genes overcome many of the disadvantages related to the use of the synthetic sprays like environmental damage and the health hazards (Ferre and Rie, 2002). Bt has become a major presence in agriculture and the development of transgenic crop plants for pest management represents one of the most significant developments in pest management in the last 40 years because of such benefits (Alstad and Andow, 1995). Alstad and Andow (1995), summarizes the benefits of Bt crops. These include significant enhancement of long-term production of higher quality and greater stability of agricultural production, because they offer insurance against the sporadic effects of

severe pest damage, otherwise not manageable with conventional pest management technique.

2.5.4 Bt Maize

In the year 2006, the global area planted with Bt-maize occupied 25.2 million hectares equivalent of 25% of the global biotech crop area (James, 2006). The two main traits considered for engineering into the maize crop were herbicide tolerance and insect resistance Bt-maize engineered with insect resistance occupied 11.3 million hectares (13% of biotech crop area). The crop was grown commercially in the USA, Argentina, Canada, South Africa, the Philippines, Spain, Uruguay, Honduras, Portugal, Germany, France, and Czech Republic (James, 2006). Bt/Herbicide tolerance maize occupied 6.5 million hectares (7% of biotech crop area). It was grown in the USA and Canada. James (2003) said, 'the experience of the past is often the best guide for the future'. Kenya needs to learn from the experience of these countries that in the past have grown Bt-maize and found it to be beneficial. Use of Bt-maize has reduced pesticides application dramatically in countries where it is grown (James, 2003; James 2006)). This in return lowers the cost of production of maize. Use of Bt-maize may prove to be part of the solution in addressing hunger and food security along side poverty eradication. The technology is convenient to use and hopefully will be cost effective and allow farmers to produce maize even in the years when pest infestations are high.

2.5.4.1 Transformation with Bt cry1 genes

Freshly isolated embryos from hybrid CML216 X CML72 were osmotically pre-treated for 4 hours. Gold particles (1.0um in diameter) were coated with plasmid DNA, mixed well and centrifuged (Bohovora *et al.*, 1999). The supernatant was discarded and the solution re-suspended in absolute ethanol. Small aliquots of the suspension were pipetted and microprojectile bombardment of immature embryos done (Bohovora *et al.*, 1999). The transformed cells were selected by incorporating the herbicide phosphinothricin into the culture media. Regenerated shoots were transferred to shooting media and the resulting plantlets transferred to soil. At six to eight-leaf-stage, the putative transgenic plants were screened for resistance to the southwestern corn borer (*Diatraea grandiosella* Dyar: Lepidoptera, Pyralidae). The plants that killed the larvae were considered to contain a functioning cry gene, which was further conclusively confirmed using southern blot procedure. The transgenic plants were backcrossed to the non-transgenic CML 216 recurrent parent to the next generation and studies for inheritance and expression of the transgene done (Gay, 2001). This transformation work was done at CIMMYT-Mexico. The stable ones were selfed and the Seeds from back cross of inbred lines were introduced in KARI Biosafety level 2 Green house complex. Self-pollinating increased the seeds of each event, while crosses to adapted Kenyan maize were made through cross-pollination.

2.5.4.2 The Maize Genotypes

Seeds of two Bt transgenic maize inbred lines namely Event223 *cry1Ab::Ubiquitin* and Event10 *cry1Ba::Ubiquitin* were used. The Bt maize Event223

of CIMMYT Maize Line CML216 was transformed with a vector containing a full-length *Cry1Ab* coding sequence driven by an enhanced maize ubiquitin, a constitutive promoter that enables the production of cry1Ab protein in almost all parts of the plant. The Bt maize Event10 of CML216 was similarly transformed with a vector containing *Cry1Ba* coding sequence using the same promoter. The protein expression however is high in the whorl compared to other parts of the plant. The non-transformed version of the same maize inbred line CML216 was used as the control experiment.

2.5 Concerns in the Use of Transgenic Crops

The major concerns with use of biotechnology-derived crops can be grouped into three categories (Conner *et al.*, 2003; Mugo *et al.*, 2005).

a) Risks to human health include the risk of unintentionally introducing allergens and other anti-nutrition factors in foods (Mugo *et al.*, 2000; Conner *et al.*, 2003). There is concern that presence of foreign genes may affect the nutritive value of the transgenic crop. Toxicity to human and animals or non-target organisms is also a great concern with use of biotech crops (Frietema de Vries, 1996).

b) Ecological and environmental concerns include the possibility of vertical gene flow. This involves the likelihood of a given species to hybridize with wild relative through dispersal of pollen, dispersal of reproductive plant parts such as seeds or fruits (diaspores), and the distribution frequency of wild relatives (Frietema de Vries, 1996; Conner *et al.*, 2003). Environmental concerns include the effects on non-target organisms like predators and parasitoids, and effects on beneficial insects like the pollinators. Invasion of natural habitats could compromise

biodiversity (Conner *et al.*, 2003). Horizontal gene transfer involves transfer of genetic material from one organism (the donor) to another organism (the recipient) that is not sexually compatible with the donor (Gay, 2001).

c) The potential for pests to evolve resistance to the toxins produced by the genes that have been engineered into the crops is another environmental concern. Resistant insects differ from susceptible ones in the way they withstand insecticides (Fenemore, 1964). There is no change in susceptibility of an individual insect during its lifetime. There are several resistant mechanisms that have been identified: 1) Detoxification; which involves the ability of an insect to chemically modify or detoxify the insecticide, probably by use of specific enzymes; 2) Insensitive target; when the target site of action of the insecticide is no longer affected; 3) slower rate of penetration, this gives the insect time to detoxify the chemical rapidly and prevent poisoning, 4) storage of the chemical by certain parts of the body in the insect, such as fat body, 5) avoidance, when the insect avoids the treated surfaces, also known as behavioral resistance. Quite often, there is more than one resistance mechanism operating within the same insect.

2.6 Insect Resistance to Pesticides

Insecticide resistance develops due to genetic variation in large insect population, when a few individuals in the original insect population, remain unaffected by a given insecticide (Michaud, 1997). This is due to either the nature of the insecticide's target molecules in the insect, or in the method the insect uses to break down toxin molecules. When the insecticide is applied, individuals who are

unaffected are the ones, which survive to pass on their genes to the following generations. Over time, greater and greater proportions of the insect population is unaffected by the insecticide, and therefore consists mostly of resistant individuals (Fenemore, 1984).

Wearing and Hokkanen (1995) listed some of the factors related to the development of resistance as, the rate of reproduction, shorter generation cycles, greater number of progeny, and larger, more genetically varied population. The more persistent an insecticide, the more likely it is for insects to develop resistance, because the susceptible ones die faster without passing their susceptibility genes to the next generation, and therefore only the resistant insects survive. Woods (1981) stated that frequent applications of non-persistent insecticides have the same effects.

2.7 Resistance to Bt

The fact that insects have developed resistance to other insecticides is an indication that they are likely to develop resistance to Bt. The first evidence of resistance developing in the field against Bt δ-endotoxin was reported in the Indianmeal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), in storage bins of Bt- treated grain (McGaughey, 1985). Laboratory studies showed that in such conditions, like in grain storage areas, Bt resistance could develop in the pest in less than one storage season.

Liu and Tabashnik (1997) reported that the diamondback moth, *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae), populations from Hawaii, Florida and New York in the United States, Japan, China, the Philippines and Thailand treated

with spray formulations of Bt toxins were loosing susceptibility to the insecticide. Iqbal *et al.* (1996) reported similar incidences in Malaysia through farmers' personal experiences.

Over the last few years Bt has been selected in laboratory populations of 13 insect species, 11 of which have developed resistance to various strains of the Bt toxins, but not in the field conditions (Tabashnik, 1994; Whalon and McGaughey, 1998; Liu *et al.*, 1999).

2.8 Resistance management strategies

Some insect resistance management strategies can be employed to control resistance development, which include, avoiding resistance where and if possible, delaying resistance as long as possible and making resistances revert to susceptibility (Croft, 1990). There are proposed insect resistance management strategies for Africa that would be appropriate for the farming systems (Mugo *et al.*, 2002); i). Use of other untreated alternate host plants, such as, *Sorghum bicolor* (L) Moench, as refugia crops (Khan *et al.*, 1997). This may increase the probability of adoption because it is economically viable and socially acceptable. ii) Bt-maize source lines can be crossed with germplasm that has conventional host plant resistance, iii) developing varieties that carry multiple forms of resistance, for example, multiple Bt genes. This high dose toxin would kill most resistant biotypes, and combination of Bt genes and conventional resistance, and, iv) Gene pyramiding which involves combining Bt genes with different resistance mechanism; v) rotation of Bt-crops with non-Bt crops can also be used as a method of resistance management (Khan *et al.*, 1997; Mulaa *et al.*, 2005).

2.9 References:

Altad D.N. and D.A. Andow. 1995. Managing the evolution of insect resistance to transgenic plants. Science. 268: 1894-1896.

Appert J. and J. Deuse. 1982. Pests of food and market garden crops in the tropics. Les ravageurs des cultures vivrière et maraîchères sous Les tropiques. G-P, Maisonneuve & Larose Paris, France, 420pp.

Baum J.A., T.B. Johnson, and B.C. Carlton. 1999. *Bacillus thuringiensis*: Natural and recombinant bioinsecticide products. In: Biopesticides: Use and delivery. Hall. F.R. and Menn, J.J., Eds., Humana Press, Totowa, N.J, 189-210.

Bohorova N., W. Zhang, P. Julstrum, S. McLean, B. Luna, R.M. Brito, L. Diaz, M.E. Ramos, P. Estanol, M. Pacheco, M. Salgado, and D. Hoisington. 1999. Production of transgenic tropical maize with *cryIAb* and *cryIAc* genes via microprojectile bombardment of immature embryos. Theor. Appl. Genet. 99: 437–444.

Cloyd R. A. 2002. Systemic, local systematic, or tramslaminar: What is the difference?. Illinois pesticide review. Vol. 2002. No. 4.

Conner J.A., T.R. Glare, and J. Nap. 2003. The release of genetically modified crops into the environment. The Plant Journal (2003) 33, 19-46.

Croft B. A. 1990. Developing a philosophy and program of pesticides resistance management. In: Pesticide resistance in Arthropods, Ruosh, R.T., and Tabashnik, B.E., Eds., Chapman and Hall, New York, USA.

FAO. 1966. www. Fao.org. FAOSTAT Database.

Ferre J. and J. Rie. 2002. Biochemistry and Genetics of Insect Resistance to *Bacillus thuringiensis*. Annual Review of Entomology. 47: 501-33.

Fenemore P.G. 1984. Plant Pests and their Control. Rev.UK ED. Butterworth & co. New Zealand. Pp 207-210.

Frietema de Vries F.T. 1996. Cultivated plants and the wild flora: Effect Analysis by dispersal codes. PhD. Thesis. Leiden, the Netherlands: Hortus Botanicus.

Gay P. 2001. The biosafety of antibiotic resistant markers in plant transformation and the dissemination of genes through horizontal gene flow. In: Custers, R. (ed.). 2001. Safety of genetically engineered crops. Zwijnaarde, Belgium: Flanders Interuniversity Institute for Biotechnology, pp.135-159.

Gill S.S., E.A. Cowles, and F.V. Pietrantonio. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Annual Review of Entomology. 37: 615-636.

Gould J. L. and W.T. Keeton. 1996. Biological science (6^{th} Ed.) New York: W.W. Norton and Company.

Harris K.M. 1962. Lepidopterous stem borers of cereals in Nigeria. Bulletin of entomological research, 53:139-171.

Harris K.M. and K.F. Nwanze. 1992. *Busseola fusca* (Fuller); The African maize stalk borer: A handbook of information. ICRISAT Information Bulletin, no. 33: pp 1-18.

Hofte H. and H.R. Whitely. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiology Review. 53: 242-255.

Inter-African Phytosanitary Council. 1985. *Chilo partellus* (Swinhoe). Distribution Maps of major crop pests and Diseases in Africa map no. 199, Yaoundé, Cameroon; Inter-African Phytosanitary council. Pp 199.

Iqbal M., R.H.J. Verkerk, M.J. Furlong, P.C. Ong, S.A. Rahman, and D.J. Wright. 1996. Evidence for resistance to *Bacillus thuringiensis* (Bt) Subsp. Kurstaki HD-1, Bt Subspecies aizawai and Abamectin in field populations of *Plutella xylostella* from Malaysia. Pesticide science 48:89-97.

James C. 2003. Global Review of Commercialized Transgenic crops: 2002 Feature: Bt Maize. ISAAA Briefs No. 29. ISAAA: Ithaca, NY.

James C. 2005. Preview: Global Status of Commercialized Biotech/GM Crops: 2005. ISAAA Briefs No. 34. ISAAA: Ithaca, NY.

James C. 2006. Preview: Global Status of Commercialized Biotech/GM Crops: 2006. ISAAA Briefs No. 35. ISAAA: Ithaca, NY

Khan Z.R., K. Ampong-Nyarko, P. Chiliswa, A. Hassanali, S. Kimani, W. Lwande, W.A. Overholt, J.A. Pickett, Smart, L.J. Wadhams, and C.M. Woodcock. 1997. Intercropping Increases Parasitism of Pests. Nature 388: 631-632.

Khan Z.R., Chiliswa P., Ampong-Nyarko K., L.E. Smart, A. Polaszek, J. Wandera, and M.A. Mulaa. 1997. Utilization of wild gramineous plants for management of cereals stem borers in Africa. Insect Sci. Appl. (17) (1): 143-150.

Khan Z.R., C.A.O. Midiega, N.J. Hutter, R.M. Wilkims, and L.J. Wadhams. 2006. Assessment of the Potential 0f Napier grass (*Pennisetum purpureum*)

varieties as trap plants for management of *Chilo partellus*. Entomologia Experimentalis et Applicata. 119: 15-22.

Lereclus D., A. Delecluse, and M.M. Lecadet. 1993. Diversity of *Bacillus thuringiensis* toxins and genesis. In: Entwistle, P.F., Cory, J.S., Bailey, M.J. and Higgs, S., (Eds.); *Bacillus thuringiensis*, An Environmental Biopesticides: Theory and practice, John Wiley & sons, Chichester, UK, 37-69.

Liu Y.B., B.E. Tabashnik, T.J. Denhehy, A.L. Patin, and A.C. Barlett. 1999. Development time and resistance to Bt crops. Nature 400. Pp519.

Liu Y.B. and B.E. Tabashnik. 1997. Experimental evidence that refuges delay insect adaptation to *Bacillus thuringiensis*. Proc. R. Soc. Lond. B 264:605-10.

Lwande W., N. Gikonyo, S. Lux, A. Hassanali, J. Lofquist. 1993. Periodicity in the frequency of calling and quality of pheromone in volatile emissions of the spotted stalk borer, *Chilo partellus*. Bulletin OILB/SROP, 16(10): 174.

Marrone P.G and S.C. MacIntosh. 1993. Resistance to *Bacillus thuringiensis* and Resistance Management. In: Entwistle. P.F., Cory. J.S., Bailey, M.J. and Higgs, S., (Eds); *Bacillus thuringiensis,* An Environmental Biopesticides: Theory and practice, John Wiley & sons, Chichester, UK, 221-235.

McGaughey W.H. 1985. Insect Resistance to the Biological Insecticide. *Bacillus thuringiensis.* Science 229: 193-195.

Michaud D. 1997. Avoiding protease-mediated resistance in herbivorous pests. Trends Biotechnology. 15:4-6.

Mugo S., D. Poland, H. DeGroote, and D. Hoisington (eds.). 2000. Stakeholders Meeting: Insect Resistant Maize for Africa (IRMA) Project. IRMA Project Document No.3.Nairobi, Kenya: KARI and CIMMYT.

Mugo S., M. Siambi, G. Birigwa and F. Kanampiu. 2001. Strengthening Maize seed supply systems for small-scale farmers in western Kenya and Uganda: CIMMYT 2001. CIMMYT-Kenya Annual Report Nairobi, Kenya: CIMMYT. PP 49-56.

Mugo S.N., J. Songa, M. Mulaa, D. Bergvinson , C. Taracha, D. Hoisington, and H. De Groote. 2002. Developing Insect Resistant Maize Varieties for Food Security in Kenya. Paper presented at the 8th KARI Biennial scientific conference 11-15 November 2002, KARI Headquarters, Nairobi Kenya.

Mugo S., H. DeGroote, D Bergvinson, M. Mulaa, J. Songa and S. Gichuki. 2005. Developing Bt maize for resource poor farmers- Recent advances in the IRMA project. Africa Journal of Biotechnology. Vol. 4 (13), pp. 1490-1504.

Mugo S., M. Oyoo, D. Bergvinson, H. De Groote, and J. Songa. 2006. Evaluation of Open Pollinated Maize Varieties for Resistance to *Chilo partellus* in Dry Land Mid-Altitudes and Coastal Lowlands. Paper presented at the 10th KARI Biennial Scientific conference 12-17 November 2006.

Mulaa M.A.O.A. 1995. Evaluation of factors leading to rational pesticide use for the control of the maize stalk borer, *Busseola fusca* in Trans-Nzoia District, Kenya. PhD Thesis, University of Wales, Cardiff.19-26 pp.

Mulaa M., S. Mugo, B. Muli, and D. Poland (Eds.). 2005. Report Of A Workshop On Integrating Pastures, Fodders and Cereal Crops as Refugia for Stem Borers

in The Farming Systems of the Humid Coastal Kenya, 26-29 July 2004: IRMA Project Document No.17 Nairobi Kenya and Mexico D.F: KARI and CIMMYT. PP 6-10.

National Academy of Sciences. 2000. Transgenic Plants & World Agriculture. www.nap.edu/html/transgenic.

Nderitu C.G. 1999. Biotechnology in Africa; Why the controversy?. "In: G.J. Persely and M.M. Lantin (Eds.). 2000. Agricultural Biotechnology and the poor: Proceedings of an International Conference, Washington, D.C., 21-22 October 1999. Consultative Group on International Agricultural Research, Washington, D.C. pp 109-114.

Neppl C.C. 2000. Management of Resistance to *Bacillus thuringiensis* toxins. Environmental Studies Program, May 26, 2000. The University of Chicago. Pp. 2.

Nesbitt B.F., P.S. Beevor, D.R. Hall, R. Lester, J.C. Davies, and K.V.S. Reddy. 1979. Components of the sex pheromone of the female spotted stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae): identification and preliminary field trials. Journal of Chemical Ecology, 5(1): 153-163;

Nwanze K.F. 1988. Distribution and seasonal incidence of some major insect pests of sorghum in Burkina Faso. Insect science and its application, 9(3):313-321.

Nye I.W.B. 1960. The insect pests of graminaceous crops in East Africa. Colonial Research Study. Her Majesty's Stationary Office. Pp 48.

Ondindo M.O., P.A. Amutalla, M. Opondo-Mbai, and T.A. Odero. 1993. Production of Nosema marucae for biological control of cereals stem borers. Entomol. Exp. Appl. Vol. 67. No. 2. Pp. 143-148.

Omolo E.O. and K.V. Seshu Reddy. 1985. Effects of different sorghum-based cropping systems on insect pests in Kenya. In: Proceedings of the International Sorghum Entomology Workshop15-21 July1984.Texas A & M University, College Station, Texas USA, ICRISAT, Patancheru, India. Pp 395-401.

Overholt W.A., K.V.N. Maes, and F.R. Goebel. 2001. Field Guide to the Stem Borer larvae of Maize, Sorghum and Sugarcane in Eastern and Southern Africa. ICIPE Science Press, Nairobi, Kenya.

Painter R.H. 1951. Insect Resistance in crop plants. The university press of Kansas, Lawrence. Rochstein, M., Ed. 1964-1965. The physiology of Insecta. 3 vols. Academic press. New York and London. Pp 715-718.

Pingali P.L. (Ed.). 2001. CIMMYT. 1999-2000. World Maize Facts and Trends. Meeting world maize needs: Technological opportunities and priorities for the public sector. Mexico, D.F.: CIMMYT. Pp 1-57.

Polaszek A. (Ed.). 2001. African cereal stem borer: economic importance, taxonomy, natural enemies and control. CABI Publishing, Wallingford, Oxon, UK. PP 21-24.

Songa J.M. 1999. Distribution importance and management of stem borers (Lepidoptera) in maize production systems of semi-arid Eastern Kenya with emphasis on biological control. PhD dissertation, Kenyatta University, Nairobi, Kenya. Pp. 1-20.

Songa J.M., G. Zhou, and W.A. Overholt. 2001a. Relationship of stem borer damage and plant physical conditions to yield in a semi-arid zone of eastern Kenya. *Insect Science and its Application. 21:243-249.*

Songa J.M., D. Bergvinson, and S. Mugo. 2001b. Mass rearing of the maize stem borers *Chilo partellus, Busseola fusca, Sesamia calamistis, Chilo orichalcociliellus* and *Eldana saccharina* at KARI, Katumani: In Friesen D.K. and A.F.E. Palmer (eds.). 2004. Integrated Approaches to Higher Maize Productivity in the New Millennium: Proceedings of the Seventh Eastern and Southern Africa Regional Maize Conference, 5-11 February, 2002, Nairobi, Kenya: CIMMYT (International Maize and Wheat Improvement Centre) and KARI (Kenya Agricultural Research Institute).

Songa J. M., W.A. Overholt, R.O. Okello and J. M. Mueke. 2002a. Regional distribution of Lepidopteran stem borers and their parasitoids among wild grasses in the semi-arid Eastern Kenya. *African Crop Science Journal. 10 (2):183-194.*

Songa J. M., W.A. Overholt, R.O. Okello, and J. M. Mueke. 2002b. Control of lepidopteran stem borers in maize by indigenous parasitoids in the semi-arid areas of Eastern Kenya. *Biological Agriculture and Horticulture. Volume 20: 77-90.*

Tabashnik B.E. 1994. Evolution of resistance to *Bacillus thuringiensis*. Annual Review of Entomology. 39. 47-49.

Van Driesch R.G. and T.S. Bellows. 1996. Biological Control. Chapman and Hall, New York, 539pp.

Van Frankenhuyzen K. 1993. The challenge of *Bacillus thuringiensis*. In: *Bacillus thuringiensis*, An Environmental Biopesticide: Theory and practice, Entwistle P.E., Cory J.S., Bailey M.J. and Higgs S., Eds., John Wiley & sons, Chichester, UK, 1-35.

Van Rie J., H. Van Mellart, and M. Peferoen. 1992. Mechanism of insect resistance to *Bacillus thuringiensis* in *Plodia interpunctella.* In: Molecular mechanisms of insecticide resistance: diversity among insects, Mullin, C.A. and Scott, J.G., Eds., American Chemical Society, Washington, D.C. pp.72-74.

Wearing C.H. and H.M. Hokkanen. 1995. Pest resistance to *Bacillus thuringiensis*: Ecological crop assessment for Bt gene incorporation and strategies of management. In: Biological control: benefits and risks, Hokkanen H.M.T. and Lynch J.M., Eds., Cambridge University press, Cambridge, UK, 236-252.

Whalon M. E. and W. H. McGaughey. 1998. *Bacillus thuringiensis*: Use and Resistance management. In: Insecticides with novel modes of action: Mechanism and application, Ishaaya I. and Degheele D. (Eds.), Springer, Berlin. 106-137.

Woods R. J. 1981. Strategies for conserving susceptibility to insecticides. Parasitology 82: 69-80.

CHAPTER THREE

EVALUATION OF THE RESPONSES OF *Chilo partellus* AND *Busseola fusca* TO Bt MAIZE δ-ENDOTOXINS FOR RESISTANCE DEVELOPMENT

3.1 Abstract

A biosafety greenhouse based study was carried out to determine the reaction of *Chilo partellus* Swinhoe; (Lepidoptera: Pyralidae) from different maize growing agro-ecological zones in Kenya, to Bt maize δ-endotoxins. The two *C. partellus* populations were screened for development of resistance to two Bt cry proteins for eight-generation cycles of selection. The cry proteins were *cry1Ab::Ubiquitin*, from Bt-maize Event223 (transformed with Bt *cry1Ab* gene), and *cry1Ba::Ubiquitin* from Bt-maize Event10 (transformed with Bt *cry1Ba* gene). Planting of the maize was done in synchrony to pupae stage of the pest. The pots were laid in a randomized complete block design in the bio-containment facility. The proportion of surviving larvae from the maize plants and the corresponding pupae weights for each population were lower for the Bt-maize. Both maize events, Event223 and Event10, were effective in controlling the stem borers. While surviving number of insects varied in the control experiment for the populations, the stem borers that fed on Bt cry proteins showed no statistical difference in the mean number of surviving larvae, nor any difference in the pupae weights for the larvae between the study populations. The two cry proteins showed stability in control over the generations and no resistant biotypes of the pest were observed.

In Kenya *B. fusca* is known to be multivoltine, completing two generations within one growing season, therefore only five generations were studied. Scoring for

resistance development was based on the reaction of the insects to the cry proteins in the Bt-maize plants. The trend of susceptibility for these generations was recorded. The results showed neither difference between the weights from the different maize genotypes used, nor any change within the cycles of selection. The original resistance levels were maintained and no gradual trends towards higher tolerance were observed.

3.2 Introduction

Spotted stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) accounts for more than 90% of borers in the Lowland Tropics, Mid-altitude and the Moist Transitional areas, but is almost absent in the high potential areas (Highland Tropics) (De Groote *et al.*, 2003). This pest has acquired greater importance than the other stem borer species since it is proving more successful in causing damage and loss (Ofomata *et al.*, 2000). Kfir (1997) showed that *C. partellus* is becoming a pest even at higher altitudes and is the most widely distributed of the stem borer species in the maize growing zones (Kfir 1997; Overholt *et al.*, 2001).

The African stem borer, *Busseola fusca* Fuller (Lepidoptera: Noctuidae) is known to cause high economic damage to maize in Kenya and elsewhere in Africa (DeGroote *et al.*, 2002). It is estimated to cause crop losses of 82% of all stem borer losses in Kenya. *B. fusca*, is dominant in the high potential areas (transitional zone and highlands), (Songa *et al.*, 2001a; De Groote *et al.*, 2003) and can have more than one generation within the same growing season (Overholt *et al.*, 2001). Frequent use of pesticides often results in loss of susceptibility by the target pest to the chemicals. While climatic variations play a major role in pest and species distribution, genetic

makeup of the individuals may affect the reaction of the pest to different treatments. Studies done using conspecific populations of diamondback moth *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae), obtained from different regions in Hawaii (Tabashnick *et al.*, 1990), revealed differences in susceptibility to Bt sprays (Tabashnik, 1994). The farming systems however differ from one ecological zone to another, with changing patterns of chemical usage over the past years (Mulaa, 2004). Differentially treated insect populations may develop various means or mechanisms to overcome effects of toxins (Oppernoorth, 1976). Over the last few years Bt has been selected in laboratory populations of 13 insect species, 11 of which have developed resistance to various strains of the Bt toxins, but not in the field conditions (Tabashnik, 1994; Whalon and McGaughey, 1998; Liu *et al.*, 1999). Some insect resistance management strategies can be employed to control resistance development, which include, avoiding resistance where and if possible, delaying resistance as long as possible and making resistances revert to susceptibility (Croft, 1990).

The cry proteins in Bt-maize Event10, and cry protein in Bt-maize Event223 were found not completely effective against *B. fusca* (Mugo *et al.*, 2001; De Groote *et al.*, 2003). Studies done elsewhere (Dutton *et al.*, 2004) revealed that the expression levels of Bt-maize δ-endotoxins are known to decrease with the age of the plant. The decrease of cry protein concentrations with plant age can contribute to resistance development. *B. fusca* is already known to have some level of resistance to δ-endotoxins in the Bt maize events that were used in this experiment (Mugo *et al.*, 2001; Mugo *et al.*, 2005). The current study was therefore aimed at finding the difference in reaction to cry proteins found in Bt-maize (cry1Ab in Event223 and

cry1Ba in Event10) by *C. partellus* collected from different maize growing regions in Kenya, and compare which of the populations would be more likely to develop resistance to cry proteins found in Bt-maize. It was also designed to find out whether level of resistance to Bt toxins by *B. fusca* would increase with exposure to the cry proteins.

3.3 Materials and methods

3.3.1 Experiment site

The experiments were carried out at the Biosafety Level 2 Greenhouse Complex (1^{o} 15.409′S, 36^{o}46.410′E, 1806m, above sea level) approved by the Kenya Standing Technical Committee on Imports and Exports (KSTCIE) for research on transgenic plants. This facility is situated at the KARI, National Agricultural Research Laboratories (NARL) at Kabete in Nairobi, Kenya. It is very similar to a normal greenhouse except that it has special features to prevent the transfer of pollen, seed, or other plant material from transgenic plants to the outside environment. These special features include restricted access to only trained and authorized personnel, and fine screen mesh that does not allow pollen to pass. Other features are double-entry doors to the greenhouse rooms to prevent inadvertent movement of pollen, soil traps to prevent plant materials from being carried off through the drainage system to the city grid. Special facilities and procedures to properly dispose off all plant and insect tissues and trained staff in proscribed protocols for operation of the facility are included as well.

3.3.2 Insect culture

3.3.2.1 *Chilo partellus*

Third to sixth instar larvae were collected from farmers' fields in Coast province at Mavueni (39° 48'E, 3° 40.8'S), Chonyi (39° 50.4'E, 3° 52.2'S), Vipingo (39° 48'E, 3° 49.2'S) and Kikambala (39° 46.2'E, 3° 52.2'S), which are situated at the humid coastal lowland tropics (HCLT) maize-growing zone. This collection constituted the HCLT-population of *C. partellus*. The DMA-population was made up of individuals collected from farmers' fields in Gatuanyaga (37° 12'E, 1° 03'S) in Thika district (Central province), Kwa vonza (37° 54'E, 1° 21'S) in Kitui, Iveti (37° 18'E, 1° 30'S) in Machakos and from Kaiti (37° 30.6'E, 1° 46'S) in Makueni districts (Eastern province), which lie within the Dry Mid-altitude and Moist transitional maize growing zones. A third population (mixed *C. partellus* population) was established by mating the *C. partellus* from Coast, Eastern and Central provinces of Kenya, which gave an adequate sampling of alleles (and used as the check-population). The original populations of each colony consisted of 300-500 individuals, established during the July 2004 and October 2004 period. The stem borers were reared using artificial diet and confined within an insectary, in large cages for oviposition (Odindo and Onyango, 1998; Songa *et al.*, 2001), at KARI-Katumani (37° 14'E, 1° 35' 24") in Machakos district, Eastern province of Kenya, at room temperature (28.0±20 C), and relative humidity 70±10% under L12:D12 photoperiodism.

3.3.2.2 *Busseola fusca*

B. fusca colony was obtained from the moist transitional (MT) and highland tropics (HT) situated at Kitale, in the Rift-valley province of Kenya. Fourth to sixth instar larvae were collected from farmers fields and transported in sections of maize stems to the same insectary at (KARI) - Katumani, This original population of *B. fusca* was established between February 2004 and August 2004. The same rearing methods and artificial diet used for *C. partellus* colony were applied (Onyango *et al.*, 1994; Odindo and Onyango, 1998; Songa *et al.*, 2001) within the insectary.

3.3.3 Bioassays

Bt maize seeds were planted into small square pots (7.5cm x 7.5cm x 9cm), in the biosafety greenhouse. The media used consisted of equal mixture of sand, soil and coconut peat. Routine management practices including watering, weeding, and fertilizer application were employed at frequent intervals, as required. Maize planting was synchronized to the pupae stage of the pests such that at emergence of neonates, the plants were at the four to six-leaf stage and ready for infestation. The potted plants were placed on benches in a Randomized Complete Block Design (RCBD).

Once the plants were at four-leaf stage, leaf bioassays were carried out, by introducing 20 neonates into the whorl of each transgenic maize plant and allowing them to feed for two to three days (IRMA, 2001) to ascertain the presence of the gene in the Bt-maize plants. Complete mortality of the neonates was used as an indication of the presence of the genes. The plants expressing cry genes survived the infestation and were used in the final screening experiment, while those without the gene were

destroyed. *B. fusca* neonates were not used to determine the presence of the genes, because the pest was not effectively controlled by the cry proteins.

The plants which survived the initial infestation were cut above the growing point and transferred to plastic jars for infestation. Four replicates (plants) were used for each maize genotype (event) per insect population. Each plant was infested with 300 neonates using camel hairbrush. The larvae were allowed to feed on the plants for three to four hours. *B. fusca* larvae were allowed to feed for 48 hours, before being removed from the plants and transferred to artificial diet. Up to 95% (Table1) of the neonates were recovered from the plants before being transferred to artificial diet and allowed to develop. Ten days later, the larvae were removed from the diet; the surviving individuals were counted and transferred to fresh diet, and allowed to develop until pupae stage. The pupae were harvested and weighed, then transferred to cages laid with oviposition media (butter paper), to develop to adult stage. The adults, upon emergence, oviposited, and the media (butter paper) was changed every three days to avoid egg masses being laid on top of each other. Each *C. partellus* population was kept separate. The eggs were disinfected using 10% formaldehyde for 10-15 minutes then rinsed with distilled water five times and incubated. The neonates emerging were used for infestation in the subsequent experiment. The same protocol was applied for all the subsequent generations up to the eighth cycle of selection for each of the three *C. partellus* colonies, and up to the fifth cycle of selection for *B. fusca*. This is because *B. fusca* has a longer life cycle, which would take between 60-70 days to be complete.

3.4 Data analysis

Percent mortality and insect counts data were subjected to arc-sin and logarithmic transformations respectively before analysis. Correction for control mortality was done using Abbott's formula (1925).

Pt = Po-Pc/ 100-Pc x100, where;

Pt = corrected mortality,

Po = observed mortality and

Pc = control mortality. (All in percentages).

Pupae weight ratios (event/control) were obtained and subjected to arc-sin (square root) transformation. Natural logarithm for count data, (x+1) were used to avoid infinite results.

The data was then subjected to analysis of variance (ANOVA), and the means were computed and separated using Tukey's Studentized Range (HSD) Test (SAS, 2000), for each experimental data set at (P=0.05).

3.5 Results

3.5.1 Insect recovery

3.5.1.1 *C. partellus* populations

Insect recovery from experimental plant materials was 97-98% (Table1). There was no marked difference between the recovered insects from the control experiments (CML216) and the Bt-maize treated fed insects. A few individuals of the neonates were not recovered from the experimental maize plants. The recovered insects were transferred to artificial diet for development up to pupae stage.

Table1: Percentage of larvae from conspecific populations of *C. partellus* which were recovered from infested plants.

	Chilo partellus Populations								
	HCLT			DMA			Mixed		
Treatment	Live	Dead	Missin	Live	Dead	Missi	Live	Dead	Missing
CML	97.7aA	0.9aB	1.0aB	97.3aA	1.2aB	1.1aB	98.1aA	1.8aB	1.0aB
Event 10	97.5aA	0.8aB	1.8aB	97.8aA	0.9aB	1.3aB	98.3aA	0.5aB	1.3aB
Event 223	97.8aA	0.6aB	1.5aB	98.0aA	0.6aB	0.8aB	98.0aA	0.7aB	1.5aB
Average	97.7aA	0.8aB	1.1aB	97.7aA	0.9aB	1.1aB	98.1aA	1.0aB	1.2aB

Means within columns bearing the same lower case letter are not significantly different. Means within rows bearing the same upper case letter are not significantly different. HSD-Test. $P<0.05$.

3.5.1.2 Surviving larvae

Reduced surviving insects were observed after infestation into artificial diet (Table 2). High mortality was observed in the Bt-maize treated insects compared to the control. Many insects did not reach adult stage even from the non-transgenic control CML216.

Table 2: Percentage of stem borers from the conspecific populations of *C. partellus* that reached adult stage.

	Populations			
Treatments	HCLT	DMA	Mixed	Means
CML216	42.0aA	33.3aAB	30.0abA	35.1aA
Event 10	2.6bA	3.3bA	2.5bA	2.8bA
Event 223	2.1bA	2.4aA	2.2aA	2.2bA

Means within columns bearing the same lower case letter are not significantly different. Means within rows bearing the same upper case letter are not significantly different. HSD-Test. $P<0.05$.

3.5.1.3 Cry1Ab on *C. partellus*

Mortality due to cry1Ab proteins were high, therefore very few surviving larvae completed the life cycle to adult stage. Throughout the eight cycles of the selection, surviving larvae counts remained very low (Table 3). The cry protein showed similar control efficacy the different *C. partellus* populations.

Table 3: Mortality (%) of eight successive generations of *Chilo partellus* populations treated with Bt-maize Event223 *Cry1Ab*.

	Populations		
Generations	**HCLT**	**DMA**	**Mixed**
1	94.5 a	85.0 bc	88.4 b
2	95.3 a	83.5 bc	98.2 b
3	94.6 a	87.0 bc	88.0 b
4	95.3 a	92.1 b	96.1 a
5	95.3 a	93.1 b	92.4 b
6	94.5 a	96.0 a	93.5 b
7	94.4 a	94.8 a	93.8 b
8	95.1 a	95.8 a	93.8 b
Mean	94.9 A	90.9 AB	93.0 A

Means within columns bearing the same lower case letter are not significantly different. Means within rows bearing the same upper case letter are not significantly different. HSD-Test. $P<0.05$.

3.5.1.4 Cry1Ba on *C. partellus*

The efficacy of Event10 to control the spotted stem borer was assessed by larvae counts from the *C. partellus* populations. The results showed similar level of efficacy of cry1Ba on eight successive generations of *C. partellus* that were treated (Table 4). While cry1Ab seemed to have higher mortality rate of *C. partellus*, the

differences between the cry1Ab and cry1Ba proteins were not statistically significant (P = 0.05, HSD-Test).

Table 4. Mortality (%) of eight successive generations of *Chilo partellus* populations treated with Bt-maize Event10 *Cry1Ba*.

	Populations		
Generations	**HCLT**	**DMA**	**Mixed**
1	91.2 b	55.6 c	85.4 b
2	93.6 a	80 b	88.9 ab
3	92.9 ab	88.3 ab	86.7 ab
4	94.6 a	92.1 a	92.8 ab
5	94.6 a	93.1 a	93.4 a
6	94.5 a	95.4 a	93 ab
7	93.4 a	94.8 a	93.3 a
8	93.4 a	95.2 a	93 ab
Mean	93.5 A	86.8 B	90.8 AB

Means within columns bearing the same lower case letter are not significantly different. Means within rows bearing the same upper case letter are not significantly different. HSD-Test. P<0.05.

3.5.1.5 Pupae weights

There was a marked reduction in pupae weights of the insects that were exposed to the cry proteins, with the control experiments having relatively higher pupae weights (Tables 5 and 6) compared to the pupae weights of the insects that fed on Bt-maize cry proteins. A comparison of the pupae weights across eight generations revealed some increase in weight for the non-transgenic control (CML216), while some decrease was observed on the individuals that fed on Bt-maize cry proteins.

Table 5: Effects of cry1Ab protein on pupae weights (mg) ±SE of conspecific populations of *C. partellus*.

	Populations					
	HCLT		DMA		Mixed	
Generations	CML216	Event223	CML216	Event223	CML216	Event223
1	95.4±1.1bc	39.7±2.0a	96.1±3.1bc	34.1±1.7a	89.8±2.0bc	31.5±1.9ab
2	93.1±1.1c	37.7±1.9ab	103.6±1.8ab	31.3±1.3a	83.3±1.4c	31.6±1.7ab
3	99.1±1.2a	32.7±1.8bc	95.8±1.6abc	35.2±2.3a	104.3±2.0a	27.3±1.9b
4	98.0±1.2ab	35.5±1.8a	86.2±1.6c	33.3±1.8a	94.1±1.4abc	31.1±1.9ab
5	98.9±1.1ab	32.9±1.6bc	89.3±1.4bc	35.3±2.2a	99.2±1.4ab	31.6±1.9ab
6	100.2±1.1a	34.1±1.7bc	108.1±0.8a	31.7±1.8a	104.2±1.2a	32.4±1.9a
7	101.5±1.2a	35.2±2.0abc	101.7±1.2abc	33.0±2.0a	96.6±1.3ab	34.5±1.9a
8	101.9±1.3a	30.5±2.1c	102.5±1.0ab	31.2±1.6a	92.0±1.1abc	35.2±2.0a

Means within columns bearing the same lower case letter are not significantly different. (Tukey's Studentized Range (HSD) Test, P = 0.05). Bt-maize; Event223 cry1Ab::Ubiquitin.

Table 6: Effects of cry1Ba protein on pupae weights (mg) ±SE of conspecific populations of *C. partellus*.

	Populations					
	HCLT		DMA		Mixed	
Generations	CML 216	Event 10	CML 216	Event 10	CML 216	Event 10
1	95.4±1.1bc	36.4±1.8a	96.1±3.1bc	44.5±1.7a	89.8±2.0bc	36.5±2.1ab
2	93.1±1.1c	38.9±2.2a	103.6±1.8ab	33.5±1.4bc	83.3±1.4c	29.8±2.1c
3	99.1±1.2a	35.2±1.8a	95.8±1.6abc	33.9±2.0bc	104.3±2.0a	31.5±2.1bc
4	98.0±1.2ab	38.1±1.6a	86.2±1.6c	29.2±1.4c	94.1±1.4abc	32.5±1.4abc
5	98.9±1.1ab	34.2±1.6a	89.3±1.4bc	32.5±1.8bc	99.2±1.4ab	39.8±2.5a
6	100.2±1.1a	35.8±1.8a	108.1±0.8a	32.3±1.9bc	104.2±1.2a	34.1±1.6ab
7	101.5±1.2a	36.3±1.7a	101.7±1.2abc	34.1±2.3bc	96.6±1.3ab	34.8±2.1ab
8	101.9±1.3a	39.5±1.6a	102.5±1.0ab	37.0±2.1b	92.0±1.1abc	36.2±1.7a

Means within columns bearing the same lower case letter are not significantly different. (Tukey's Studentized Range (HSD) Test, P = 0.05). Bt-maize; Event10 cry1Ba::Ubiquitin.

3.5.1.5.1 Cry1Ab δ-endotoxins effects on pupae weights

The results obtained showed 65% weight reduction for both the DMA-colony and the HCLT-colony. There was no significant difference in the recorded weights from the study populations (HCLT =34.8 and DMA= 33.1). The cycle means of HCLT-colony, showed some differences (Table 3), with cycle 1 and 2 having higher mean weights (39.7 and 37.7 respectively). There was no difference between the cycles exposed to the protein from the DMA-colony (Table 3).

3.5.1.5.2 Effects of cry1Ba proteins on pupae weights of C. partellus populations

Sixty-three percent weight losses were recorded from the DMA-colony of *C. partellus* after exposure to cry1Ba proteins for the eight selected generation (using the control experiment mean weights as the standard measure). The HCLT-colony differed from DMA-colony by 1.8%, hence 61.2% loss from the HCLT-colony, which is not statistically significant. There was an observed decrease in weight for DMA-colony cycle four, however, after analysis, the difference was not significant from the other generations (HSD-Test, P=0.05).

3.5.2 Monitoring changes in tolerance to cry1Ab and cry1Ba δ-endotoxins by *B. fusca*

3.5.2.1 *B. fusca* Surviving larvae

The percentage of larvae reared to maturity was high for the two Bt-maize events. The mortality was 34.9-49.6%. The surviving larvae remained fairly

stable from the first generation to the fifth (Table 7). Event223 had relatively high mortality than Event10; however no complete control was achieved.

Table 7: Comparison of the surviving larvae (%) ±SE of *B. fusca* which were recovered from infested plants.

Generations	CML216	Event 10	Event 223
1	100.0±0.0aA	65.2±0.9aBC	52. 2±0.8abCD
2	100.0±0.0aA	67.1±1.0aBC	55.2±0.8abCD
3	100.0±0.0aA	74.4±1.0aB	62.2±0.9aBC
4	100.0±0.0aA	64.2±0.9aBC	41.0±0.7bD
5	100.0±0.0aA	54.9±0.8abC	41.2±0.7bD
Mean	100.0±0.0aA	65.2±0.9aBC	50.4±0.8aCD

Means within columns bearing the same lower case letter are not significantly different. Means within rows bearing the same upper case letter are not significantly different. HSD-Test. $P<0.05$.

3.5.2.2 Pupae weights

The CML216 control recorded high pupae weights in generation 1. There was an observed decrease in pupae weight in cycle 3, in the control. Bt-maize Event10 recorded relatively high pupae weights with the control in generation 3. Bt-maize Event223 was noted to have greater effect on the development of the *B. fusca* population, which led to reduced pupae weights compared to control, in cycles 1, 3 and 5. However, generations 2 and 4 showed no statistical difference between the pupae weight means for the three maize germplasm.

Table 8: Pupae weights (mg) ± SE of *Busseola fusca* recorded for five generations of selection after 48 hours of larvae feeding on Bt-maize, Event10 and Event223. CML216 was used as control.

Generations	Control	Event10	Event223
1	195.9±0.06aA	174.6±0.06aB	172.4±0.06aB
2	169.4±0.06aA	169.1±0.11aA	175.4±0.11aA
3	208.7±0.07aA	201.9±0.08aA	165.9±0.06aB
4	189.9±0.06aA	191.8 ±0.07aA	193.8±0.08aA
5	210.4±0.11aA	191.4±0.06aA	170.3±0.06aB
Average	194.9±0.06aA	185.8±0.06aA	175.6±0.06aB

Means within columns bearing the same lower case letter are not significantly different. Means within rows bearing the same upper case letter are not significantly different. HSD-Test. $P<0.05$.

3.6 Discussion

It was not possible to obtain 100% surviving *C. partellus* insects develop up to adult stage. The initial mortality records for *C. partellus* may have been as a result due to damage of larvae during infestation. The missing insects could have been trapped within vascular tissues of the plant material, as observed during the recovery of the insect from plant tissues. While most of the insects were recovered (>97%), not all of then reached adult stage. Trapping of neonates, by cotton wool used to plug the diet vials, reduced the insect counts after infestation into artificial diet. This led to loss of insects within the artificial diet. Other losses were due infections by fungal and bacterial pathogen in the diet. There was a marked difference in the mean count of surviving larvae from the control (CML216) experiments of the *C. partellus*

populations (HCLT, DMA and Mixed), in the first four-generation cycles. The HCLT-colony had more larvae compared to DMA-colony. The population built-up in the control colonies is an indication of the absence of δ-endotoxins, which reduced the number of the surviving insects in the exposed populations. The pupae weights from the control experiments, of the colonies remained stable. The growth and development of the insects was not affected. Studies by Heinrichs *et al.*, (1985) indicate that population increase and growth index of an insect can give information on antibiosis type of resistance. No negative observations were made on the control, indicating that there were no notable factors or inhibiting chemicals interfered with the insects' metabolic activities. This agrees with the findings of Panda and Khush (1995) that in the absence of toxins or inhibiting factors, developmental process goes on uninterrupted through the insects' life cycle.

There was significant difference in the number of surviving larvae and their corresponding pupae weights between the transgenic Bt-maize used in the experiment, in comparison to the CML216 control. Slight increase in mean counts observed in Event10 DMA-colony might not be a reflection of differences in genetic variation.

The high mortalities observed from the Bt-maize were due to the effects of the δ-endotoxins to the target insects. The larvae means of DMA-colony cycle 1 from Event10 plants were relatively higher compared to the means of the insect from the rest of the cycles. However, no decrease in susceptibility to the cry proteins was observed in any of the colonies.

The pupae weight of insects that were exposed to the cry proteins was less, compared to the insects which were fed on the non-transgenic maize. This could mean that the δ-endotoxins interfered with the insects' metabolism, either by interfering with feed intake or its assimilation within the insects' mid-gut. This agrees with the findings of Fenemore (1984) that, a pest, which has fed on resistant plant, does not develop properly, an antibiosis phenomenon, which due to the presence of toxic substances in a plant. However no resistant biotypes of the insects were observed for the generations of insects that were exposed to the Bt-maize δ-endotoxins. Heinrichs *et al.*, (1985) indicated that toxicity of a chemical is a function of the insects' body weight. The pupae weights for *C. partellus* colonies exposed to cry proteins agree with these findings. The low pupae weight recorded is an indication of toxicity by the δ-endotoxins. The subsequent generations of insects maintained low pupae weights for up to eight generations of exposure. This can be used as an indicator that no resistance developed for these generations to cry proteins in Bt-maize.

No increase in population growth and weight gain in generations of insects that were exposed to the δ-endotoxins. The reverse could have meant a possibility of resistance developing. However, susceptibility was maintained within the generations of these individuals.

Exposure of the insects to the Bt-maize plants with cry proteins is not a guarantee that the insects actually fed on the tissues before recovery and transfer into artificial diet for development to maturity. Chances would be that some did not feed on the maize plant, but were recovered and developed to adult. This could have led to

the significant difference observed in the Event10 surviving larvae and pupae weights of DMA-colony recorded in cycle one.

Observations on the conspecific *C. partellus* populations indicate that the Bt-maize cry proteins did not affect the colonies differently. Susceptibility was maintained in the colonies after exposure to the cry proteins throughout the generations of this experiment.

The three colonies showed little variation in the mean number of surviving larvae from each event. This may imply that the populations do not have different genetic makeup. Event10, cry1Ba and Event223, cry1Ab, showed no statistical difference in the surviving larvae counts and their corresponding pupae weights. An increase in the mean count of surviving larvae from the control experiment (CML216) of the DMA-colony may be due to adaptation to the hot conditions within the biosafety greenhouse. Considering that these insects were not exposed to the cry proteins, the differences can only be attributed to other factors other than δ-endotoxins. One such factor, and of great interest could be the high temperatures recorded in the green house (>30°C). Possible explanation could be that the individuals from the HCLT-region were well adapted to the high temperatures found in the coastal region, while the individuals from the DMA-region required time to acclimatize to the high temperatures within the biosafety greenhouse complex.

B. fusca colony showed marked difference in the pupae weights recorded from the three maize types used. Event223 maintained relatively low pupae weights throughout the five cycles of selection. Lack of a clear marked difference in weights between the Bt-maize Event10 and the control (CML216) could be interpreted to

means that the cry proteins cry1Ba had no effect on *B. fusca*. These findings confirm the work reported earlier, which showed that the events used in this research work were not 100% effective in controlling *B. fusca* (Mugo *et al.*, 2001, Mugo *et al.*, 2005). The cycle-to-cycle variation in weight gain may reflect a non-genetic cause like changes in environmental conditions or management procedures, rather than indications of genetic changes occurring within the insect. Robertson *et al.*, (1995), and Marcon *et al.*, (1999) obtained similar findings when working with European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae).

The failure of a chemical treatment in the field to control a pest is not adequate proof of the existence of resistance by the insect pest (Fenemore, 1984). The fact that Bt-maize Event10::Ubiquitin and Event223 cry1Ab::Ubiquitin, did not adequately control *B. fusca* is not proof that there was resistant development by *B. fusca* to δ-endotoxins. However, this could be a pointer that, there is already some resistance mechanism that may be interfering with the activity of these proteins in controlling *B. fusca*.

This is an indication that the effects of δ-endotoxins within the midgut of *B. fusca* were minimal. Tabashnik *et al.*, (1997), reported that reduced binding of the toxins to the insects mid-gut is the only known resistance mechanism to Bt-toxins. However, from the results obtained from this research there is no indication of the increase of weights across generations of insects which were exposed to the cry proteins.

3.7 References:

Abbott W.S. 1925. Method of Computing the effectiveness of an insecticide. Journal of Econ. Entomol. 18: 265-267.

De Groote H. 2002. Maize yield losses from stem borers in Kenya. Insect Science Applications. 22(2): 89-96.

De Groote H., W. Overholt, J.O. Ouma, and S. Mugo. 2003. Assessing the potential Impact of Bt maize in Kenya using a G.I.S. based model. Paper presented at the international Agricultural Economics Conference, Durban, South Africa, August 2003.

Dutton A., M. D'Alessandro, J. Romeis, and F. Bigler. 2004. Assessing expression of Bt-toxin (Cry1Ab) in transgenic maize under different environmental conditions. IOBC/WPRS Bulletin 27: 49-55.

Farinos G.P., M. la Poza, P.H. Crespo, F. Ortego, and P. Castanera. 2003. Resistance monitoring of field populations of the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis* after 5 years of Bt-maize cultivation in Spain. *Entomologia Experimentalis et Applicata*. 110: 23-30

Fenemore P.G. 1984. Plant Pests and their Control. Rev.UK ED. Butterworth & co. New Zealand. Pp 207-210.

Heinrichs E.A., F.G. Medrano, and H.R. Rapusas. 1985. Genetic Evaluation for Insect Resistance in Rice. International Rice Research Institute, Los Banos, Philippines. Pp 2-6

Kfir R.1997. Competitive displacement of *Busseola fusca* (Lepidoptera: Noctuidae) by *Chilo partellus* (Lepidoptera: Pyraridae). Annals of the Entomological Society of America, 90: 619-624.

Marcon P.C.R.G., L.J. Young, K.L. Steffey, and B.D. Siegfried. 1999. Baseline susceptibility of European corn borer (Lepidoptera: Crambidae) to *Bacillus thuringiensis* toxins. Journal of Economic Entomology 92: 279-285

Mugo S and H. De Groote. 2001. Insect Resistant Maize for Africa Project: In CIMMYT-Kenya annual report 2001. Pp 27.

Mugo S., C. Taracha, D. Bergvinson, D. Odhiambo, J. Songa, D. Hoisington, S. Mclean, I. Ngatia, and M. Gethi. 2004a. Screening cry proteins produced by Bt-maize leaves for activity against Kenyan stem borers. Pp 102-105. In: Friesen D.K. and A.F.E. Palmer (Eds). 2004. Integrated approaches to higher maize productivity in the new millennium: Proceedings of the seventh Eastern and Southern Africa regional maize conference. 5-11 February 2002. Nairobi, Kenya. CIMMYT and KARI.

Mugo S., G. Murenga, S. Mclean, B. Odhiambo, C. Taracha, and J. Songa. 2004b. Insect Resistant Maize for Africa (IRMA). Report of BGHC activities 28th May 2004-3rdDec. 2004: A report to the Kenya National Biosafety Committee (NBC) on the progress following introduction of Bt-maize seeds. Pp 30-38.

Mulaa M.A.O.A. 1995. Evaluation of factors leading to rational pesticide use for the control of the maize stalk borer, *Busseola fusca* in Trans-Nzoia District, Kenya. PhD Thesis, University of Wales, Cardiff. 19-26 pp.

Mulaa M.S. Mugo, B. Muli, and D. Poland (Eds.). 2005. Report of a Workshop on Integrating Pastures, Fodders, and Cereals Crops as Refugia for Stem Borers in the Farming Systems of the Humid Coastal Kenya, 26-29 July 2004. IRMA

Project Document No. 17. Nairobi, Kenya and Mexico D.F.: KARI and CIMMYT. Pp 6-10..

Odindo M.O. and F.O. Onyango. 1998. Rearing maize and sorghum stem borers. In: Polaszek, A. (ed). African Cereal stem borers. Economic importance, Taxonomy, Natural enemies and Control. CAB International. Wallingford, Oxon, United Kingdom, pp59-72.

Ofomata V.C., W.A. Overholt, S.A. Lux, A. van Huis, and R.A. Egwuatu. 2000. Comparative studies on the fecundity, egg survival, larval feeding, and development of *Chilo partellus* and *Chilo orichalcociliellus* (Lepidoptera: Crambidae) on five grasses. Annals of the Entomological Society of America, 93: 492-499.

Onyango F.O. and J.P.R. Ochieng-Odero. 1994. Continuous rearing of the maize stem borer *Busseola fusca* on an artificial diet. *Entomologia Experimentalis Applicata*. 73:139-144.

Oppernoorth F.J. 1976. Biochemical Genetics of Insecticide Resistance. Annual Review of Entomology. 10: 185-206.

Panda N. and G.S. Khush. 1995. Host plant resistance to insects. CAB International. Wallingford. Oxon, UK. Pp 151-269.

Robertson J.L., H.K. Preisler, S.S. Ng, L.A. Hickle, and W.D. Gelerter. 1995. Natural variation: A complicating factor in bioassays with chemical and microbial pesticides. Journal of Economic Entomology 88: 1-10

SAS Institute Inc. 2000. SAS/STAT™ Guide for personal computers. SAS Institute, North Carolina, USA.

Songa J.M., D. Bergvinson, and S. Mugo. 2001. Mass rearing of the maize stem borers *Chilo partellus, Busseola fusca, Sesamia calamistis, Chilo orichalcociliellus* and *Eldana saccharina* at KARI, Katumani: In Friesen D.K. and A.F.E. Palmer (Eds.). 2004. Integrated Approaches to Higher Maize Productivity in the New Millennium: Proceedings of the Seventh Eastern and Southern Africa Regional Maize Conference, 5-11 February, 2002, Nairobi, Kenya: CIMMYT (International Maize and Wheat Improvement Centre) and KARI (Kenya Agricultural Research Institute).

Tabashnik B.E., N.L. Cushing, N. Finson, and M.W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). Journal of Economic Entomology. 83:1671-76.

Tabashnik B.E. 1994. Evolution of resistance to *Bacillus thuringiensis*. Annual Review of Entomology. 39. 47-49.

Tabashnik B.E., Y. B. Liu, N. Finson, L. Mason, and D.G. Heckel. 1997. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. Proc. Natl. Acad. SCI. USA 94: 1640-1644.

Traynor P.L., D. Adair, and R. Irwin. 2001. A practical guide to containment: Greenhouse research with transgenic plants and microbes. Information systems for Biotechnology. Virginia Tech

CHAPTER FOUR

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The findings of this study confirm that, cry proteins are effective in controlling the major stem borer species in the maize growing agroecological zones in Kenya. The durability of the control was assessed for eight stem borer generations, and it was found to be effective and stable.

The difference in the pupae weight means between the control and the treatments that were exposed was due to the effects of the δ-endotoxins. There was noticeable reduction in pupae weights between the individuals that were exposed to cry proteins and those that were not exposed. The observed trend across the generations was stability in mean survivors from Event10 and Event223, by the different populations. This implies that no resistant biotypes of *C. partellus* from either the HCLT-region or the DMA-region, evolved during the period this experiment was carried out.

There was no marked difference observed between the conspecific populations of *C. partellus* and also no resistance developed to the cry proteins found in the Bt-maize event10 and Event223 within the eight generations of *C. partellus* and five generations of *B. fusca* that were exposed to δ-endotoxins.

The study further revealed that there were no differences in the response of *C. partellus* populations from different maize growing regions and that *B. fusca* was not fully controlled. This agrees with findings by Mugo *et al.,* 2005, that the events imported into Kenya were not effective in controlling *B. fusca.* This is a pointer that there is already some resistance mechanism that could be interfering with the activity

of these proteins in the control of *B. fusca.* However, results from this work revealed that the tolerance levels did not increase with continued exposure to the cry proteins. From this research work, the following recommendations can be made:

a). This work need to be carried out for more generations of *C. partellus* and *B. fusca* stem borer species and similar work also, can be carried out, using other Bt-maize events and see whether the results differ from the findings obtained from this study.

b). Further research could be carried out to determine whether other stem borer species like the pink stem borer (*Sesamia calamistis* Hampson) and the sugarcane stem borer (*Eldana saccharina* Walker) would respond to cry proteins in a similar manner.

c). *B. fusca* is known to have more than one-generation cycle within the same growing season. Research work could be done to test the effects of the second-generation individuals on the rate of resistance development.

d). This research work should be carried out in field conditions, and at different farming systems, where climate and other constraint, like abiotic and biotic factors, operate, in order to compare the findings under field conditions with those in laboratory,

e). Further molecular work can be carried out to verify if there exists differences in the genetic variations between the conspecific *C. partellus* population.