EFFECTIVENESS OF VARIED REFUGIA CONFIGUARTIONS FOR GENETICALLY MODIFIED MAIZE (*Zea mays* L.) IN KWAZULU-NATAL MIDLANDS

by

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submitted in accordance with the requirements for
the degree of

MASTER OF SCIENCE

in the subject

AGRICULTURE

at the

UNIVERSITY OF SOUTH AFRICA

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NOVEMBER 2013

Declaration

I, Odeshnee Moodley (student no: 4810-971-1) sincerely and solemnly declare that in the work: **EFFECTIVENESS OF VARIED REFUGIA CONFIGURATIONS FOR GENETICALLY MODIFIED MAIZE (*Zea* mays L.) IN KWAZULU-NATAL MIDLANDS:**

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Dedication

The completion of this study is dedicated to my fiancé (Thiren Naidoo) my mum (Vimla Moodley), brother (Seshan Moodley) and to the loving memory of my dad (Morgan “Jones” Moodley) for showing me the meaning of sacrifice and determination.

A very special dedication must also be made to my new family (Devarani Naidoo, Deshini Naidoo and Satchi Naidoo) for their never ending belief and support in both me and my abilities.

To my dearest friend, confidante and mentor (Ravisha Ranjith), for her constant motivation even under the most trying of circumstances.

And lastly to my guru and god for the tests, trials and tribulations that kept me if nothing else grounded and infallible.

‘’Great spirits have always encountered violent opposition from mediocre minds’’ – Albert Einstein

Acknowledgements

I thank the following people and organisations for their efforts in the successful completion of this study:

Prof. David Modise. PhD. Director: School of Agriculture and Life Science (UNISA). My study supervisor for his support and assistance.

Dr. Sandy Snyman. PhD. Senior Researcher: Biotechnology and Variety Improvement Programme Manager (SASRI) and Honorary Lecturer (UKZN). My study co-supervisor for her constant supervision, guidance, expertise and large shoulders.

Neil van Rijj. MSc. Plant scientist (KZN-DAE – Cedara). Division: Mushroom Science. My co-worker for his knowledge on all things related to maize.

Archana Nunkumar. MSc. Plant pathologist (KZN-DAE – Cedara). Cedara Plant Disease Clinic Division: Crop Protection. My project co-worker for her assistance with the field trial.

Cathy Stevens. BSc. Agric. (Biometrics) Biometrician (KZN-DAE – Cedara). For her long hours spent pondering on raw data, seeing method where I saw madness.

The KwaZulu-Natal Department of Agriculture and Environmental Affairs (KZN-DAE) (Cedara), for the use of their trial site, labour, chemicals, materials, staff and for all the consultation provided.

Mr. Grant Pringle. Agronomist for the KwaZulu-Natal and Eastern Cape Regions (Pannar, Greytown), for generously sponsoring the *Bt* and RR maize for the field trial and for his constant advice regarding all aspects of maize agriculture.

Mr. Gavin Robinson. Specialist seed sales representative for the Winterton Region in KwaZulu-Natal (Pannar, Winterton), for sacrificing his time and energy in manual dissemination and collection of the survey questionnaires.

Mr. Nelson Nkomo. Maize production officer (Pannar, Greytown), for his valuable advice and information regarding maize production in KwaZulu-Natal.

Dr. Tlangelani Cedric Baloyi. Researcher and agronomist for the Agricultural Research Council (ARC) Grain Crops Institute (Potchefstroom), for his knowledge and research into maize yields for different varieties under varying conditions.

Mr. Willie Weideman. Factory manager for Pannar (Greytown), for his assistance in forwarding and receiving the survey questionnaires.

Mr. Andries Van Niekerk. Specialist sales representative for the Greytown and KwaZulu-Natal Midlands regions (Link Seed, Greytown), for forwarding and receiving the survey questionnaires.

Abstract

Genetically modified (GM) white and yellow maize, *Zea mays,* has been commercially released and cultivated in South Africa since 1997/1998. The traits expressed are insect resistance and herbicide tolerance conferred by the bacteria *Bacillus thuringiensis* (*Bt*) Cry genes and *Agrobacterium* 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase gene, respectively. The Cry genes have been used widely to control lepidopteran insect pests but insect resistance to GM *Bt* crops has been a concern since the introduction of this technology. A management strategy includes refugia planting of 5% non-*Bt* plants, with no insecticide application, and 20%, where insecticide application is allowed. These refugia are designed to allow the survival of insect pests within restricted planted zones. However, in South Africa there are reports of *Bt*-resistant stem borer (*Busseola fusca*) (Fuller) (Lepidoptera: Noctuidae) and non-compliance with refuge planting. The aims of this study were two-fold: 1. To conduct a survey among KwaZulu-Natal (KZN) GM maize growers to ascertain information such as level of compliance with refuge planting and to determine which refugia were predominantly planted and reasons thereof; 2. To conduct a replicated field trial to determine yield, insect borer damage and economic benefit of the 5% unsprayed and 20% sprayed refuge options (including three configurations namely strip, perimeter and block and a 5 and 20% 'refuge-in-a-bag' option). The survey indicated that 28 out of 29 (96.6%) KZN *Bt* maize growers plant the 5% non-sprayed refuge with 27 (96.4%) of those respondents planting the strip configuration for the purpose of insect management (75%) and ease of planting (32.2%). The survey also showed that 7 (seven) i.e. 21.9% of KZN *Bt* maize growers observed borer damage and although growers are now fully compliant with refugia planting requirements, initially 7 respondents (24.1%) did not comply with or plant refugia correctly. Furthermore, 7 respondents reported insect borer damage in their maize with 4 of the 7 instances (57.1%) likely stemming from incorrectly planted refugia.

No significant differences in yield or insect damage were observed between the 5 and 20% refugia for any of the planting configurations in the field trial. However due to costs involved with insecticide application and labour required for the operation in the 20% option, these treatments were less economically advantageous than the non-*Bt* control. The 20% block and strip configurations had a cost benefit ratio of ZAR 7.21 and ZAR 6.67 respectively, earned per R1 spent by the grower compared with ZAR 7.76 in the sprayed control. The cost-benefit comparison for the 5% block and strip configurations was ZAR 8.48 and ZAR 7.71, respectively compared with ZAR 9.44 in the unsprayed control. In addition, the 20% seed mixture limited borer damage to 4.95% when compared with 15.77% damage in the sprayed control (ANOVA, F pr = 0.124). The seed mixtures are not available commercially and the results from the survey indicated that some education and marketing by the seed companies would be advisable prior to their release to the farming community.

In order to determine which of the refuge options between 5 and 20% would be more advantageous for growers overall, regardless of the planting configuration; data were grouped and analysed. There were no significant differences in either the yield or insect damage for the 5 and 20% refugia, but the cost-benefit calculations indicated that the 5% option was more cost effective – for the 5 and 20% refugia, ZAR 7.97 and ZAR 7.15 respectively, earned per ZAR 1 spent by the grower (ANOVA, F pr. = 0.03). This is because no insecticide was used in the 5% treatments. Mean ear damage comparisons between the 5 and 20% refugia showed that the 20% refuge in the perimeter configuration incurred the least damage (2.65% ear damage) compared with 5% perimeter (10.86% ear damage), although the reasons for this are not clear.

While the results of the field trials showed no significant differences in insect damage and yield with regard to choice of refuge configuration, monitoring insect resistance management remains an integral part of *Bt* maize crops in South

Africa, in order to delay further resistance development and to prolong the viability of *Bt* technology.

Keywords: Genetically modified, refugia, *Bt* maize, yield, cost-benefit ratio, farmer compliance, seed mixture, insect damage, refuge planting requirements, refuge configuration, refuge option

List of abbreviations

ANOVA	Analysis of variance
Bt	*Bacillus thuringiensis*
CV%	coefficient of variation percentage (ANOVA)
DAFF	Department of Agriculture, Forestry and Fisheries (RSA)
d.f.	Degrees of freedom (Pearson chi-square test)
DNA	Deoxyribose nucleic acid
EPA	Environmental Protection Agency
ECB	European corn borer
EU	European Union
F pr.	Probability factor (ANOVA) also called P value
GM	Genetically modified
Ha	Hectares
HDR	High-dose refuge
HT	Herbicide tolerant
IRM	Insect resistance management

ISAAA	International Service for the Acquisition of Agri-biotech Application
KZN	KwaZulu-Natal
KZN-DAE	KwaZulu-Natal Department of Agriculture and Environmental Affairs
LSD	Least significant difference (of means) (ANOVA)
MSV	*Maize Streak Virus*
n	Number of replicates for the field trial and the number of respondents for the survey questionnaire
RSA	Republic of South Africa
RCBD	Randomised complete block design
RR	RoundUp Ready
Sp.	Species
Ssp.	Sub species
USA or US	United States of America
v.r.	Variance ratio (ANOVA) also called F test statistic
WCR or CRM	Western corn root worm or corn root worm

ZAR	South African Rand

Definitions of words and phrases used in the study

Biosynthesis	production of chemical compounds by a living organism
Biotech crops (GM crops)	genetically modified crops (GMCs), genetically modified crops, or biotech crops are plants, the DNA of which has been modified using genetic engineering techniques. In most cases the aim is to introduce a new trait to the plant which does not occur naturally in the species
Bollgard™	insect resistant (*Bt*) cotton developed by Monsanto
Chlorosis	yellowing of plant tissues due to partial failure to develop chlorophyll which can be caused by a nutrient deficiency or the activities of a pathogen
Commercial farmers	farmers farming on a larger scale for commercial purposes
Communal farmers	farmers farming on a smaller or subsistence scale
Deadheart	necrosis of the primary growing shoot of maize causing the lodging and death of the plant

Endotoxin	a heat stable toxin located inside the bacterial cell that is released when the bacterial cell lyses. Also called delta-endotoxin
Entomopathogenic	the parasitic action of an organism able to kill or seriously disable insects
Gene stacking/gene pyramiding	strategy developed for example to delay insect resistance in transgenic crops in the field, in this case multiple and different Cry genes intended to target different insect pests can be combined in the same transgenic plant. Gene stacking can also be used for other traits and events in GM crops
Glyphosate	active ingredient of a commonly used herbicide and is marketed as RoundUp
Herbicide tolerance	the acquired ability of a crop to survive a herbicide application that could have once controlled the population
Host specific	organism or chemical compound capable of survival or causing infection in or on one species of host
Insect resistant	ability of a plant to resist the attack of insects due to genetic modification or manipulation
Mycotoxin	the toxin or poison produced by a fungus
Necrosis	death of a portion of animal or plant tissue

Parasporal crystals	tightly packed insect toxins produced naturally by the bacterium *Bacillus thuringiensis*
Plant lodging	bending of a stem (stem lodging) or the entire plant (root lodging). Lodging can be caused by nutrient deficiency or inclement weather
Pupa(e)	growth stage in insect development between the larvae and adult stages
Precautionary principle	an environmental management rule that states if there is a threat to human health or the environment then a lack of complete scientific understanding of the threat should not stop containment of the threat or remedial action
Purposive sampling	is a type of non-probability sampling that is used in quantitative research. Purposive sampling is used when the researcher has specific selection criteria for choosing participants or respondents
Refuge	refugia are non-*Bt* areas/zones planted in GM fields and have been designed to delay the evolution of insect resistance. Refugia allow for the production of susceptible insects that can randomly mate with rare resistant homozygous insects thereby creating a dilution of resistant alleles i.e. creation of heterozygous offspring that will be susceptible to the *Bt* crop.

Respondent	person or individual answering the survey questionnaire
Selective pressure	any phenomena which alters the behaviour and fitness of living organism within a given environment. It is the driving force of evolution and natural selection
Septicaemia	the state of putrefaction or decay caused by the presence of a pathogen
Sporulation	the process of producing spores or reproductive bodies
Synthase	an enzyme that catalyzes the synthesis of a substance without the use of a high energy source
Transformation (genetic)	the change or modification of genetic material in an organism
Transgenic	an organism containing a gene or genes transferred from another species through genetic manipulation techniques
Treatment	chemical and design strategies applied to the plots in the field trial
YieldGard™	insect resistant (*Bt*) maize developed by Monsanto

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Chapter One

1 Introduction and background

Genetically modified (GM) crops have proved successful in terms of yield, economics and insect management strategy, with 29 countries worldwide having adopted this technology (James, 2011; Kaphengst *et al*., 2011). Of the GM traits available commercially, the two most commonly used are herbicide tolerance and insect resistance (Christou, 2005). Insect resistant GM *Zea mays* L. (maize) technology involved the introduction of an insecticidal gene derived from the soil bacterium *Bacillus thuringiensis* (Berliner) (*Bt*) (Ambec and Desquilbet, 2011). Genetically modified maize was developed as an insect management strategy to control lepidopteran insect populations; the result was significant yield increases and economic gains (Picher *et al*., 2002). The use of GM crops has also led to a decrease in the amount of harmful pesticides used by farmers to control insect pests (Cannon, 2000; Vacher *et al*., 2003; Frisvold and Reeves, 2010). Some of these pesticides persist in the environment and were shown to be dangerous to both humans and animals (de Maagd *et al*., 2000; Christou, 2005; de Groote *et al*., 2005).

Since their deployment one of the major concerns surrounding GM crops has been the emergence of unintended insect resistance and herbicide resistant weeds. The emphasis of current research is finding possible ways to slow down this inevitable phenomenon (Gould, 2000; Bourguet *et al*., 2005). Christou (2005) suggested that transgenic crops which have a single *Bt* gene may cause emergence of secondary pests which are unaffected by the insecticidal gene. Gatehouse (2008) further intimated that, in addition to the excess damage by secondary pests, resistance to a single gene is more likely to occur as opposed to multiple *Bt* genes in the same transgenic crop. Other researchers such as Garcia and Altieri (2005) observed that the excessive and overuse of herbicides such as glyphosate can allow the development of resistance in some weed species.

However, the inclusion of alternative herbicides is strongly recommended as a conventional management strategy (Garcia and Altieri, 2005; Jones, 2011).

The Environmental Protection Agency (EPA) USA developed a planting policy for transgenic crops in order to slow down the possible emergence of insect resistance in the field (Livingston *et al*., 2004). Livingston *et al*. (2004) as well as Ambec and Desquilbet (2011) stated that planting of a non-GM refuge area became mandatory in 1995 for *Bt* cotton and in 2000 for *Bt* maize for all farmers in the USA. The refuge designs had two possible options: the first being a 5% unsprayed and the second a 20% sprayed (with insecticide) type on each field/farm. Included in this design strategy were four refuge configurations from which to choose, namely the within field (block and split planter/strip) and separate field patterns (perimeter and separate field/adjacent) (Kruger *et al*., 2009; Kruger *et al*., 2011; Kruger *et al*., 2012). Reports of insect resistance in *Bt* maize and cotton fields in countries such as South Africa, Puerto Rico, India and the USA emerged (Van Rensburg, 1999; Van Rensburg, 2001; Van Rensburg, 2007; Tabashnik *et al*., 2009; Kruger *et al*., 2011; Van den Berg *et al*., 2013). One possible reason given for the emergence of insect resistance is farmer non-compliance in terms of refuge planting requirements (Kruger *et al*., 2009; Kunert, 2011; Kruger *et al*., 2012). Andow *et al*. (2010) and Kruger *et al*. (2012) proposed that farmers had deviated from the prescribed planting requirements due to financial, spatial and temporal limitations.

In South Africa, the maize stem borer (*Busseola fusca*) (Fuller) (Lepidoptera: Noctuidae) has shown resistance to *Bt* maize in the regions of Christiana and Vaalharts (North-West Province, South Africa) (Figure 1), Hoopstad, Bothville and Reitz (Free State Province, South Africa) and Delmas and Standerton (Mpumalanga Province, South Africa) (Kruger *et al*., 2012). Kruger *et al*. (2012) also noted that there have been no reports of insect resistance in other provinces in South Africa nor has there been sufficient research conducted to determine whether non-compliance with refuge planting mandates, exists in other areas of the country. Emphasis in South Africa has also been placed on the 5% unsprayed refuge option with no documented research on the benefit or shortcomings of the 20% sprayed type (Kruger *et al*., 2012).

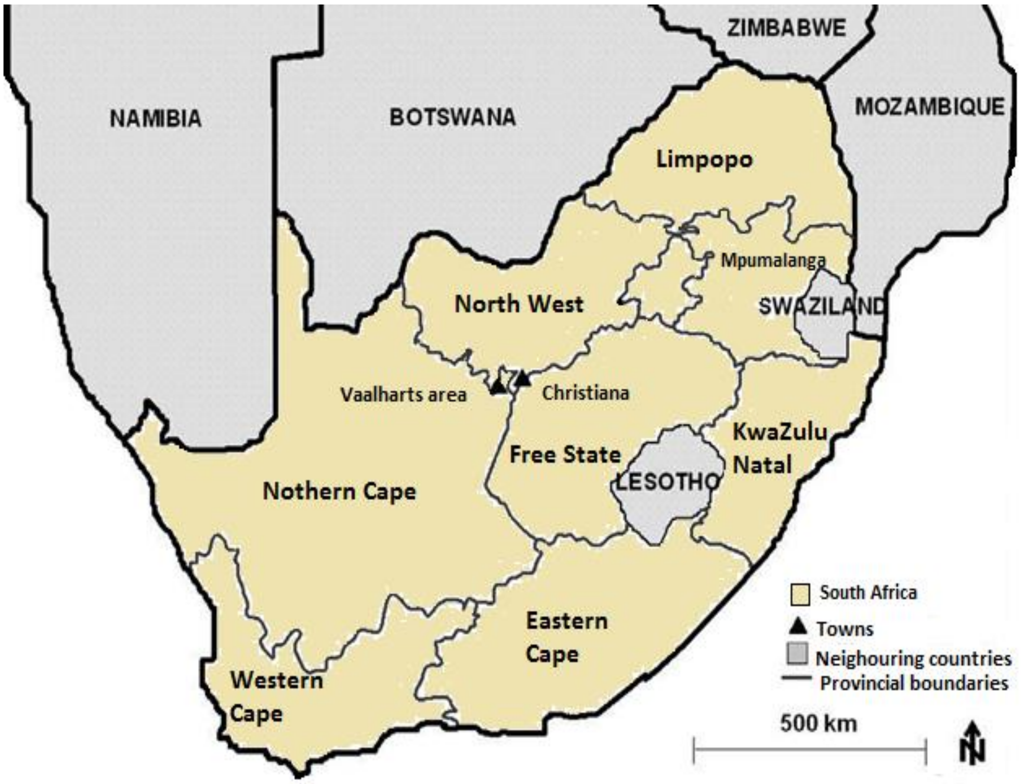


Figure 1: Map of Southern Africa showing the areas of Vaalharts and Christiana where insect resistance to *Bt* maize was observed (Source: Kruger *et al*., 2011)

1.1 Problem statement

In other parts of the world, insect resistance emergence in *Bt* crops has been relatively low, however, in South Africa, there have been reports on this phenomenon in several places (Kruger *et al*., 2009; De Villiers and Hoisington, 2011; Kruger *et al*., 2011; Kruger *et al*., 2012). One reason attributed to this emergence has been non-compliance by farmers in terms of refuge planting (Vacher *et al*., 2005; Christou *et al*., 2006; Kunert, 2011). There are still reports of *Bt* maize being planted without corresponding non-*Bt* refuge plots in South Africa (Kruger *et al*., 2009; Kruger *et al*., 2012). The 5% refuge option includes a 95% *Bt* area and 5% non-*Bt* zone which may not be sprayed with insecticide. Conversely, the 20% refuge option consists of an area of 80% *Bt* and 20% non-*Bt* which may be sprayed with insecticides (Livingston *et al*., 2004). It has been found that the majority of farmers opt for the 5% unsprayed refuge without fully considering the 20% sprayed alternative (Kruger *et al*., 2012) especially in terms of yield, insect damage and cost-benefit ratio. This therefore causes the problem of insect resistance as alluded to, above.

1.2 Motivation

It is proposed that insect resistance emergence in South Africa could be slowed down considerably with stricter adherence to proper refuge requirements (Kruger *et al*., 2012). Previous studies on GM crops in South Africa conducted by Kruger *et al*. (2009; 2012) have focused the 5% unsprayed refuge option while very little has been done to address the benefit or effectiveness of the 20% sprayed option (Kruger *et al*., 2012). In addition, there is no information concerning insect resistance and farmer compliance with refuge requirements in the KwaZulu-Natal Province of South Africa where GM *Bt* maize is grown (Kruger *et al*., 2012). The most important factor influencing grower decision making is profit, which comprises of two parts namely the input costs (for example cost of herbicides and insecticides) and the yield obtained (Hurley *et al*., 2009). The scope of this study therefore was to provide communal (i.e. small scale or subsistence growers) and commercial scale growers information on the 5% unsprayed and 20% sprayed refuge options (including three configurations) on the basis of yield, implication of cost and insect damage as well as to investigate farmer compliance with refuge planting requirements. Five percent and 20% seed mixtures (to represent the commercially unreleased 'refuge-in-a-bag' planting formulations) were also planted in the field to determine if there was a significant difference in insect damage, yield and the cost incurred.

1.3 Hypothesis

It has been hypothesized that the use of certain refugia configurations may be more beneficial than others in the planting of GM *Bt* maize especially in terms of crop yield, limiting insect damage and cost implication.

1.4 Objectives

The objectives of this investigation were therefore to:

- determine which refuge type was the most widely implemented in GM *Bt* maize plantings in KwaZulu-Natal (KZN) and to establish reasons for the prevailing scenario.

- establish which of the refugia configurations was most effective in terms of yield, limiting insect damage and cost-benefit ratio.

1.5 Specific objectives

The specific objectives of this study were to:

- establish, by means of a survey, which refugia configurations were most commonly implemented by the farmers.

- investigate the compliance of farmers with refuge planting requirements using the survey.

- conduct field trials with GM *Bt Zea mays* to determine which type of planted refugia (i.e. strip, perimeter and block) was optimum in terms of insect damage (in terms of stem, leaf and ear damage), yield and cost.

Chapter Two

2 Literature review

2.1 Origin and significance of maize in agriculture

According to Johannessen (1982), domestication is a process and not a single act. It stems from farmers' visualising the benefit of a defined crop and all its possible crosses, in this case maize. The farmer will then establish that maize can be reproduced, he or she then selects the seed for planting and reproduces the seed in a manner that generates the highest sustained quality of maize and a method to deliver this crop to others (Johannessen, 1982). Domestication of maize and other agricultural crops began approximately six to ten thousand years ago (Eyre-Walker *et al*., 1998; Wang *et al*., 1999; Doebley, 2004).

The adoption of modern agricultural practices as well as the establishment of genetic databases has inspired scientists to trace the origins of domesticated crops (Matsuoka, 2005). Although *Zea mays* L. (Poales: Poaceae) is considered one of the most important domesticated grain crops in the world and is the staple food crop in many countries the true maize progenitor remained a mystery for centuries (Piperno and Flannery, 2001). Doebley (1990) remarked that crops such as *Triticum spp*. L. (Poales: Poaceae) (wheat), *Hordeum vulgare* L. Poales: Poaceae (barley) and *Secale cereale* L. (Poales: Poaceae) (rye), had wild types which could be easily genetically and morphologically linked as their progenitors, while Doebley (1990) also found that this was untrue for maize. The subject of maize evolution has been covered extensively by different branches of science such as botany, genetics, taxonomy, cytology and anthropology with limited success until recently (Doebley, 1990).

In 1939, George Beadle proposed that the Mexican annual teosinte was the ancestor to domesticated maize (Bennetzen *et al*., 2001; Doebley, 2001). Beadles' teosinte hypothesis was scientifically viable since maize and teosinte could be crossed easily and the subsequent hybrids exhibited fertility (Doebley, 1990). Teosintes were initially thought to be related to *Oryza sativa* L. (Poales: Poaceae) (rice) rather than maize due to the difference in morphologies between maize and teosinte (Doebley, 1990; Doebley, 2004). The most prominent difference is the long branched tasseled tips of teosinte and the small branched ears of maize (Figure 2) (Wang *et al*., 1999). These differences can best be explained as the result of human selection during maize domestication (Doebley, 2004). During the process of domestication favourable teosinte genes were selected while less favourable genes were lost and this subsequently led to a reduction in genetic diversity in maize compared with teosinte (Eyre-Walker *et al*., 1998; Wang *et al*., 1999).

Figure 2: Proposed maize ancestor teosinte ear (left), teosinte-maize hybrid ear (centre) and modern maize ear (right) (Source: Doebley, 2001)

The origin of maize may have sparked debate in the scientific community, however the significance of maize in agricultural practices remain undisputed. Maize is a cereal crop and is grown throughout the world for grain, silage production and human consumption purposes (Idikut *et al*., 2009). These crops are endemic to warmer climates with suitable soil moisture levels (Okweche and Umoetok, 2012). In terms of world importance for food production maize is surpassed only by wheat and rice (Minorsky, 2001). Cereal grains are collectively responsible for more than half of the total agricultural crop production in the world and therefore they command the majority of applied and basic plant research (Minorsky, 2001). Rice, wheat and maize account for at least 30% of the total food calorie supplied to approximately 450 hundred million people in 94 developing countries (Shiferaw *et al*., 2011; Hellin *et al*., 2012). The dietary carbohydrate content found in sorghum and wheat is exceeded by maize and additionally maize is a good source of phosphorus and has trace amounts of calcium, iron, niacin and fat and are essential constituents of a healthy diet (Mboya *et al*., 2011). Maize has always been an important crop for human consumption purposes however over the past decade the demand for maize for animal feed purposes has increased. In addition, maize is used in industrial products especially in the production of biofuels (Demissie *et al*., 2008; Shiferaw *et al*. 2011) and according to Kelleman *et al*. (2009), the people of Meso-America view the maize plant as the origin of life.

In 2008, countries such as the USA, European Union, China, India, Mexico and Brazil were the largest producers of maize in the world. The total mass of maize produced in 2008 was 750 000 million kilograms (Nuss and Tanumihardjo, 2010). On the African continent maize is the most important grain crop and has been hailed as the miracle seed shaping Nigeria's agricultural and economic development (Onuk *et al*., 2010). In Ethiopia maize is highest ranking agricultural crop and is the most important calorie producing staple food crop (Demissie *et al*., 2008). Tanzania considers maize an important dietary household food security crop (Mboya *et al*., 2011) especially since maize can be grown by small holder farmers on dry land (non-irrigated) without having expensive irrigation infrastructure (Hellin *et al*., 2012).

In South Africa, the basic staple food crop is white-grain maize, with yellow-grain maize being grown primarily for animal feed purposes (Gouse *et al*., 2005). Maize is grown throughout South Africa under varying climatic conditions and KZN produces high yields even under dry land conditions (Agricultural Research Council – Grain Crops Institute, ARC – GCI, 2013). The majority of maize produced in South Africa is from the North West Province, the Free State, Mpumalanga Highveld and the KZN midlands by approximately 9 000 commercial growers (Department of Agriculture, Forestry and Fisheries, 2013). South Africa's grain industry is responsible for production of between 25 to 33% of all agricultural products and maize is the largest produced crop in the country (Department of Agriculture, Forestry and Fisheries, 2013). The widespread intensive cultivation of maize in South Africa and around the world means that pressures due to pests and diseases can significantly affect yields and food security (Okweche and Umoetok, 2012). The following section highlights pests and diseases of economic concern.

2.2 Pests and diseases of economic concern in maize

Diseases caused by insect, viral and fungal pests are a concern for food security across the world; therefore extensive research has been focused on possible methods of eradication and control of these pests (Ali and Yan, 2012). Ali and Yan (2012) reported that despite some measure of control being applied the average loss of crops due to disease each year is 91 000 million US dollars.

2.2.1 Insect pests

The most important insect pests infesting maize in Europe are the European corn borer (ECB) (causal agent *Ostrinia nubilalis*) (Hübner) (Lepidoptera: Crambidae) and the Mediterranean corn borer (causal agent *Sesamia nonagrioides*) (Lefèbvre)

(Lepidoptera: Noctuidae) (Meissle *et al*., 2011) (Table 1). During the larval phase of growth these insect pests tunnel through and feed on maize stems and ears causing degradation of the maize plant with eventual breakage of the ears and stems due to the uptake of nutrients and water (Meissle *et al*., 2011). The borings can also serve as a source of secondary fungal infections (Keetch *et al*., 2005). The collective damage caused by *O. nubilalis* larvae causes a reduction in yield of approximately 20% in the US and Europe each year (Siegfried and Hellmich, 2012). The fall armyworm (causal agent *Spodoptera frugiperda*) (J. E. Smith) (Lepidoptera: Noctuidae) is responsible for reduction in yield for both smallholder and commercial maize growers in North and Central America (Takahashi *et al*., 2012). The western corn rootworm (causal agent: *Diabrotica virgifera virgifera*) (Le Conte) (Coleoptera: Chrysomelidae) is responsible for highest usage of insecticides in the USA (Murphy *et al*., 2010).

Chippendale and Sorenson (2007), determined that the Southwestern corn borer (causal agent *Diatraea grandiosella*) (Dyar) (Lepidoptera: Crambidae) was first observed in maize crops in Mexico and Central America (Table 1). However over recent years the borer has rapidly spread into the southern parts of North America. *Diatraea grandiosella* limits the growth of maize by stopping the development of ears (Chippendale and Sorenson, 2007).

Table 1: Important maize insect pests and diseases, the causal insect and their distribution

Disease/Infection	Causal Insect	Distribution	References
Western corn rootworm (WCR) or (CRM)	*Diabrotica virgifera virgifera* (Le Conte) (Coleoptera: Chrysomelidae)	North America and Europe	Spencer *et al*., 2009; Wesseler and Fall, 2010; Meissle *et al*., 2011
Northern corn rootworm	*Diabrotica barberi* (Smith and Lawrence) (Coleoptera: Chrysomelidae)	North America and Europe	Spencer *et al*., 2009; Wesseler and Fall, 2010
European corn borer (ECB)	*Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae)	North America, Europe and Northern Africa	Dowd, 2001; Meissle *et al*., 2010; Meissle *et al*., 2011
South western corn borer	*Diatraea grandiosella* (Dyar) (Lepidoptera: Crambidae)	North and Central America	Chippendale and Sorenson, 2007
Mediterranean corn borer	*Sesamia nonagrioides* (Lefèbvre) (Lepidoptera: Noctuidae)	Mediterranean region and Central Africa	Meissle *et al*., 2010; Meissle *et al*., 2011
Fall armyworm	*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)	North and Central America	Takahashi *et al*., 2012
Corn earworm or cotton bollworm	*Helicoverpa zea* also called *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae)	North America	Onstad *et al*., 2011
Maize stem borer	*Busseola fusca* (Fuller) (Lepidoptera: Noctuidae)	Africa	Van Rensburg, 1999; Van Rensburg, 2001; Van Rensburg, 2007
Spotted stem borer/Sorghum stem borer/chilo worm	*Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae)	Asia and Africa	Van Rensburg, 1999; Keetch *et al*., 2005; Van Wyk *et al*., 2008
Maize weevil	*Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae)	Tropical regions	Demissie *et al*., 2008

In regions of the world such as Ethiopia, maize can be infected with *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) (maize weevil), a field-to-storage insect pest responsible for infestation of the ripened maize ear prior to harvest and during storage (Demissie *et al*., 2008). The authors further reported that the weevil enters the maize husk while the maize is still in active growth and the insect reproduces during maize storage (Demissie *et al*., 2008).

The maize stem borer (*B. fusca*) (Figure 3) and the spotted stem borer/sorghum stem borer/chilo larvae (*Chilo partellus*) (Swinhoe) (Lepidoptera: Pyralidae) (Figure 4) are found in South Africa and KZN and together account for maize yield losses of between ten to 45% per year (Van Rensburg, 1999; Keetch *et al*., 2005; Van den Berg *et al*., 2013). The chilo larvae are spotted, yellow in colour and upon maturation develop stripes along the entire length of the body (Gunewardena and Madugalla, 2011). Duration of the stem borer larval periods range between 25 to 58 days and vary between the different species; the pupal stage lasts for approximately five to 14 days after which the moths appear (Mailafiya *et al*., 2010). The stem borer's first point of infestation are the leaves followed by burrowing through the stem and in extreme cases eventually infesting the ears (Bamaiyi and Joan, 2011). After hatching, the stem borers are able to tunnel deep into maize stems rendering chemical control methods ineffective (Mugo *et al*., 2011). Prolonged feeding by the borers can lead to necrosis of the primary growing shoot (producing a 'deadheart') followed by ear losses, and the eventual loss of structural integrity and crop lodging (Mugo *et al*., 2011). Keetch *et al*. (2005) proposed that severe borer infestation of maize ears leads to secondary infection by fungal pests which further decreases yield projections.

Figure 3: Image showing larval stage of *Busseola fusca* (maize stem borer) (Source: Stringa, 2009)

Figure 4: Image showing larval stage *Chilo partellus* (spotted stem borer) (Source: Agricultural Research Council, 2013)

2.2.2 Diseases of maize

Foliar wilt diseases such as gray leaf spot (causal agent: *Cercospora zeae-maydis*) (Tehon and E. Y. Daniels) and Northern leaf blight (ascomycete causal agent: *Setosphaeria turcica*) (Luttrell) and the fungal conidial state called *Exserohilum turcicum* (Passerini) (Table 2) are ubiquitous fungal diseases and are responsible for decreases in maize yield on a global scale (Welz and Geiger, 2000; Asea *et al*., 2012).

Table 2: Important maize fungal pests and diseases, their causal agents and their distribution

Disease/Infection	Fungal Causal agent	Distribution	References
Gray leaf spot	*Cercospora zeae-maydis* (Tehon and E. Y. Daniels)	Ubiquitous	Asea *et al*., 2012
Northern corn leaf blight	Non-reproductive state: *Setosphaeria turcica* (Luttrell) Conidial state: *Exserohilum turcicum* (Passerini)	Ubiquitous	Welz and Geiger, 2000; Fininsa and Yuen, 2001; Asea *et al*., 2012
Downy mildews	*Peronosclerospora* (Weston and Uppal) and *Sclerospora* species (Payak and Renfro)	Asia	Rashid *et al*., 2012
Common rust	*Puccinia sorghi* (Schwartz)	Ubiquitous	Fininsa and Yuen, 2001
Maize ear, root and stem rot	*Fusarium* sp.	Ubiquitous	Dowd, 2001; Logrieco *et al*., 2002; Mazzoni *et al*., 2010

There are several viral diseases affecting maize yield in Africa but the most economically significant viral disease is *Maize Streak Virus* (MSV) caused by maize streak *Mastrevirus*. *Mastrevirus* is transmitted by leafhopper insects and the virus pathogenesis begins with leaf chlorosis and in severe cases plant necrosis (Bosque-Pèrez, 2000).

2.3 History and importance of genetically modified crops from an international perspective

A study by Celec *et al*. (2005) noted that in the past, food found naturally growing was sufficient to sustain human populations, later growers turned to crop domestication for enhanced cultivation purposes. The author further noted that hybridization of plants using similar plant varieties, classic selection (for crop species improvement) and eventually gene identification, splicing and modification were all methods explored by scientists attempting to increase and enhance crop production (Celec *et al*., 2005). Jones (2011) postulated that in the year 2030, the global agricultural community needs to produce 50% more food to meet the increasing demand as the population rises. There has also been a decreasing trend in number of practicing commercial and subsistence farmers in the world and the production of cereal grains currently is inadequate (Ali and Yan, 2012). Taking into account the limited supply of water, the lack of new agricultural land, energy expenses and the effects of climate change, genetically modified crop methods may provide the answer to increased crop yields (Jones, 2011; Campagne *et al*., 2013).

Genetic modification is defined as the introduction of genes into genomes using synthetic methods as opposed to natural crossing and recombination methods (Bruce, 2011). Thus far there are two important commercially released GM traits globally, namely the *Bt* endotoxins for control of lepidopteran insects and herbicide tolerance (glyphosate and glufosinate ammonium) (Christou, 2005; Garcia and Altieri, 2005; James, 2011).

Weeds are a consistent problem in farming practices (Jones, 2011). Conventional methods for weed eradication include manual removal, which is both time consuming and economically unviable (Garcia and Altieri, 2005; Jones, 2011). Chemical pesticides are widely used however this method often kills both the weed and the crop of interest (Jones, 2011). Glyphosate is a commonly used herbicide and is marketed as RoundUp (active ingredient glyphosate; Monsanto). RoundUp limits the activity of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase which is needed for the biosynthesis of aromatic amino acids (Jones, 2011), thereby causing mortality of the plant to which the chemical is applied. The GM trait for herbicide tolerance (HT) is important since the RoundUp Ready (RR) trait promotes the production of a type of EPSP synthase that is unaffected by glyphosate. In addition, glyphosate is not known to cause toxicity in mammals and is not as harmful to the environment as other herbicides (Jones, 2011).

Herbicide tolerance has been successfully used in various crops such as maize, cotton (*Gossypium hirsutum* Malvales: Malvaceae), soybean (*Glycine max* L. Merr. Fabales: Fabaceae) and oil seed rape (Hurley *et al*., 2009; Jones, 2011). However, studies indicate that excessive usage of glyphosate allows for the selection of certain resistant weed types (Garcia and Altieri, 2005) and it has been thus proposed that, the addition of other HT traits be considered to curb the problem (Andow, 2008; Jones, 2011). Resistance to glyphosate was noted in HT soybean fields in both Argentina and the USA (Andow, 2008). The use of HT techniques in farming practices have resulted in a reduction in tillage which promotes a higher carbon uptake in RR soybean crops compared with non-GM soybean crops (Frisvold and Reeves, 2010; Jones, 2011).

The next most commonly used GM trait is insect resistance obtained through the modification of crops using the bacterium *B. thuringiensis* (*Bt*) (Tabashnik *et al*., 2001; Jones, 2011). *Bacillus thuringiensis* is ubiquitous, gram positive and spore forming with entomopathogenic activity (Bravo *et al*., 2007). During the sporulation phase *Bt* produces parasporal crystals which are insecticidal proteins; these crystals are composed of delta-endotoxins, also called Cry and Cyt toxins (Bravo *et al*., 2007). The *Bt* bacterium was first discovered in Japanese silkworms early in the 20th century (Gryspeirt and Grégoire, 2012) and was revered for its insecticidal properties (Tyutyunov *et al*., 2007). The bacterium was initially isolated in Thuringia, Germany (Kunert, 2011). *Bacillus thuringiensis* sprays released in the 1950's were developed by incorporating the *Bt* bacterial spores and retrieved toxins and utilised as natural pesticides in organic farming practices (Tyutyunov *et al*., 2007; Kunert, 2011). *Bacillus thuringiensis* and other field-sprayed agents have consistent limitations in that the spray doesn't cover the full extent of the plant surface, there is a breakdown of the active ingredients under ultraviolet radiation and heating and drying decreases the effectiveness of the active ingredients (Tyutyunov *et al*., 2007).

The use of recombinant DNA techniques have aided in the insertion of *Bt* genes coding for insecticidal properties directly into the maize genome (Mwangi and Ely, 2001). This resulted in the entire maize plant expressing the insect toxin (Mwangi and Ely, 2001). Tyutyunov *et al*. (2007) also noted that GM *Bt* crops usually do not require any field application of insecticide and should result in reduced chemical insecticide application and therefore are favoured. The other important advantage of *Bt* crops is that this expression is maintained throughout the life cycle of the maize plant and for the whole growing season (Mwangi and Ely, 2001; Goldberger *et al*., 2005). The mode of action of *Bt* maize entails the insect larva feeding on plant tissue and ingesting the *Bt* toxin produced by the maize plant cells (Manyangarirwa *et al*., 2006; Andow, 2008). The *Bt* toxin is activated by the Cry proteins being solubilized under the high alkalinity of the insect gut, where gut proteases cleave the proteins and the *Bt* toxin binds to the gut receptors (Manyangarirwa *et al*., 2006; Andow, 2008). This paralyses the insect and stops it from further feeding. The gut membrane leaks out its contents and the bacteria begin to multiply which then causes septicaemia, ultimately killing the larva (Mwangi and Ely, 2001; Kunert, 2011).

The added benefit of using *Bt* technology is the fact that the Cry protein is host specific and is only toxic to a limited number of insect species (de Maagd *et al*., 2000; Fabrick and Tabashnik, 2012). Different strains of *Bt* produce different proteins which have unique specificities to insects such as the coleopteran beetle larvae (e.g. corn rootworm) commonly found in maize, the lepidopteran larvae which infests cotton and the maize stem borer which destroys maize crops (Gatehouse, 2008; Kaphengst *et al*., 2011). Thus far ten Cry genes have used to transform 26 crop and tree species (Cannon, 2000). The Cry1 and Cry2 proteins target Lepidoptera while the Cry3 protein targets Coleoptera (Hellmich and Hellmich, 2012). Apart from the benefit of crop protection, GM crops limit yield loss and reduce the cost of production due to lowered pesticide use, labour and fuel (Jones, 2011; Kaphengst *et al*., 2011).

Soybean, cotton, maize and canola (composed of either rapeseed: *Brassica napus* L. Brassicales: Brassicaceae or field mustard: *Brassica campestris* L. Brassicales: Brassicaceae) are the four major GM crops commercially available worldwide (Garcia and Altieri, 2005; Kaphengst *et al*., 2011). Soybean is the most cultivated of the GM crops, and in 2009, 77% of the total 90 million Ha of soybean planted were GM (Kaphengst *et al*., 2011). The first commercially available GM crop was *Bt* cotton released in the USA in 1995 (Gatehouse, 2008). This insecticidal GM plant was then adopted by other countries such as Australia and China and South Africa (Shelton *et al*., 2000).

Genetically modified crops have been introduced to developing countries such as South Africa with great success and increases in both crop yields and profit without recorded negative health or environmental effects having been observed (Kaphengst *et al*., 2011). De Groote *et al.* (2005) suggested that the benefit of increased crop yield deliverable by GM technologies is not of particular concern to Europe since they have issues with consistent overproduction and any other possible benefits were deemed small. The authors also confirmed that Europe has employed the precautionary principle which uses strict criteria in the risk assessment of GM crops, resulting in an initial banning of cultivation of GM crops (Kaphengst *et al*., 2011). However, in 2004, Europe approved the importation of GM maize for food and feed although the cultivation of GM maize seeds was still not allowed (De Groote *et al*., 2005). Spain, Czech Republic, Romania, Portugal, Germany, Poland, and Slovakia in the European Union (EU) began growing *Bt* maize for commercial release in 2008 (Kaphengst *et al*., 2011).

The year 2012 was the 17th anniversary of the commercialization of biotech crops (James, 2012). Since 1996, the total amount of GM or biotech crops has exceeded 100 million Ha globally (James, 2011), this is a measurable increase of 100-fold in Ha between 1996 and 2012 (James, 2012). It was established that an excess of 15 million farmers in 29 countries planted 148 million Ha of GM and biotech crops in 2010. Nineteen of the 29 countries involved with GM and biotech crops in 2011 were developing countries and the remaining ten were industrial counties however in the 2012 growing season 28 countries grew GM crops with 20 countries being developing and eight industrial (James, 2012). James (2011) additionally reported that the five leading developing countries to grow and produce GM and biotech crops in the 2010/2011 growing season were China, India, Brazil, Argentina and South Africa (Figure 5). Since developing countries produced 48% of the international biotech and GM crops in 2011, it has been suggested that in 2015 the total amount of biotech crops grown by developing countries will surpass that of the developed countries (James, 2011). James (2012) reported that in 2012 two countries Sudan and Cuba planted GM crops for the first time, *Bt* cotton and *Bt* maize respectively. Furthermore, James (2012) reported that in 2012 approximately 60% of all people live in the 28 countries currently growing GM crops.

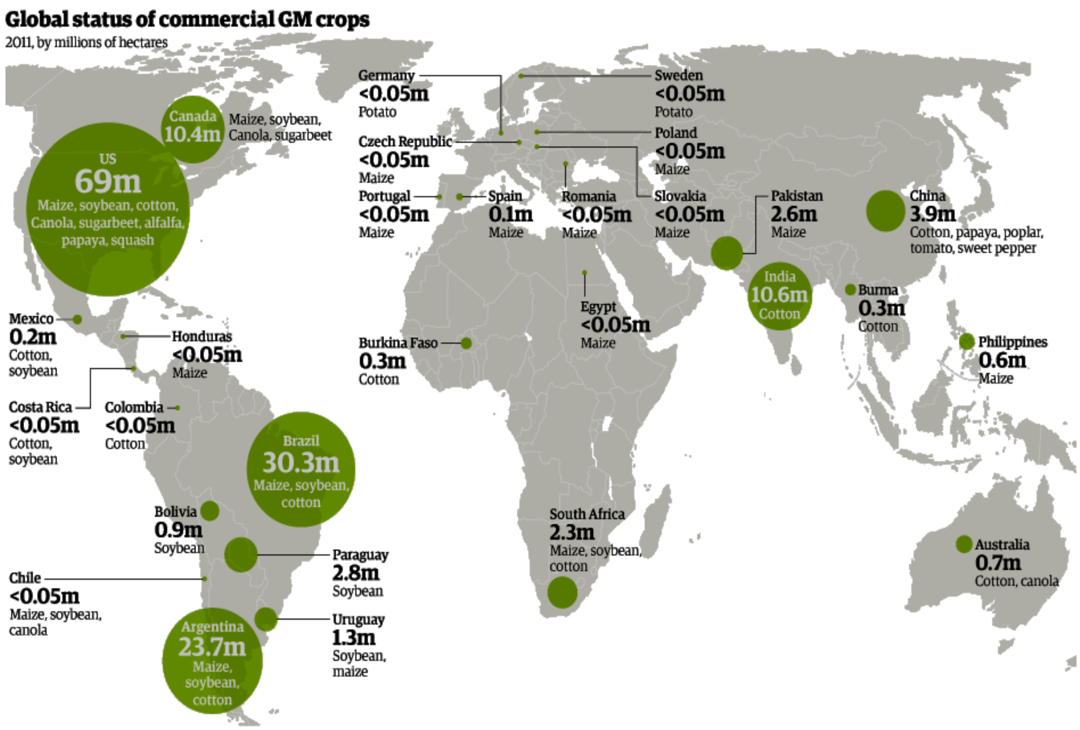


Figure 5: The 2011 global status and types of commercial GM crops represented as millions of hectares (Source: The Guardian Mail, UK, 2013)

2.4 Overview of genetically modified crops in South Africa

South Africa was the first developing country to grow GM varieties of the basic staple food crop (Gouse *et al*., 2005) and was the first African country to produce commercial *Bt* crops (Gouse *et al*., 2005; Van den Berg *et al*., 2013). The first *Bt* crop introduced into South Africa and approved for commercial usage by the Department of Agriculture, Forestry and Fisheries (DAFF) was *Bt* cotton in 1997 (Table 3) (Gouse, 2012). In the 1999/2000 growing season an area of 50 000 Ha of GM cotton was planted in South Africa by 1 530 commercial scale and 3 000 communal growers in the Northern Province, Free State and KwaZulu-Natal (Ismael *et al*., 2002). Ismael *et al*. (2002) further reported that communal growers in the Makhathini Flats area in KwaZulu-Natal have been planting the GM cotton variety NuCOTN 37-B with Bollgard™ (Monsanto) since 1998 and Morse *et al*.

(2008) highlighted the livelihood benefits of those growers since their adoption of GM cotton. Fok *et al*. (2007) suggested that the success of *Bt* cotton planting in the Makhathini Flats can be applied in other rural farming communities in South Africa and other developing countries provided they have financial and technical support since communal growers are willing to use new technology.

Table 3: A summary of GM crops commercially available in South Africa and the year in which they were approved for cultivation (Source: Department of Agriculture, Forestry & Fisheries, Division: Biosafety, 2012)

Event	Crop	Traits	Company	Year approved
TC1507	Maize	Insect resistance Herbicide tolerant	Pioneer	2012
Bt11 x GA21	Maize	Insect resistance Herbicide tolerant	Syngenta	2010
GA21	Maize	Herbicide tolerant	Syngenta	2010
MON89034 x NK603	Maize	Insect resistance Herbicide tolerant	Monsanto	2010
MON89034	Maize	Insect resistance	Monsanto	2010
Bollgard IIxRR flex (MON15985 x MON88913)	Cotton	Insect resistant Herbicide tolerant	Monsanto	2007
MON88913 (RR flex)	Cotton	Herbicide tolerant	Monsanto	2007
MON810 x NK603	Maize	Insect resistant Herbicide tolerant	Monsanto	2007
Bollgard RR	Cotton	Insect resistant Herbicide tolerant	Monsanto	2005
Bollgard II, line 15985	Cotton	Insect resistant	Monsanto	2003
Bt11	Maize	Insect resistant	Syngenta	2003
NK603	Maize	Herbicide tolerant	Monsanto	2002
GTS40-3-2	Soybean	Herbicide tolerant	Monsanto	2001
RR lines 1445 & 1698	Cotton	Herbicide tolerant	Monsanto	2000
Line 531/Bollgard	Cotton	Insect resistant	Monsanto	1997
MON810/Yieldgard	Maize	Insect resistant	Monsanto	1997

Genetically modified maize was first introduced to South African agriculture in the 1997/1998 growing season (Table 3), by modifying local maize hybrids with the *Bt* gene (Gouse *et al*., 2005; Kruger *et al*., 2011). Transformation of maize hybrids conferred resistance against maize stem borers (Kruger *et al*., 2011). This technology was tested and approved for commercial release by the government for yellow-grain maize with a subsequent commercial release for white-grain maize in the 2001/2002 growing season (Gouse *et al*., 2005).

In 2012 South Africa had an area of 2.9 million Ha of GM maize, cotton and soybean (James, 2012). This gave the country an overall ranking of eight out of the 28 countries to grow biotech crops in 2012. The author further surmised that combined over the year approximately 10 million Ha of white and yellow-grain GM maize was grown in South Africa between 2001 and 2010. The reported value of GM crops produced in South Africa over the period from 1999 to 2009 was approximately 675 million US dollars or 5400 million South African Rands (ZAR) (James, 2011).

James (2011) reported that the total area of maize planted in South Africa in 2010/2011 was approximately 2.47 million Ha, of which 1.9 million Ha was GM (about 76.9%). This value was reduced from 2009 which recorded a value of 78%. The reason for this lowered amount was probably due to the smaller market price for maize. However, in 2012 South Africa grew GM crops on 2.9 million hectares of land showing an increase in area of 26% compared with the 2010/2011 growing season (James, 2012). James (2011) further reported that the GM events having a single *Bt* gene amounted to 865, 589 Ha or 45.6% of the total area grown in 2011 while the herbicide tolerant crops equaled 254, 211 Ha or 13.4% of the total planted area. The crops with stacked genes for insect resistance and herbicide tolerance were produced on 777, 820 Ha or 41% of total hectarage in 2011 (James, 2011). In addition, it was noted that small holder and rural farmers produced 19,000 Ha of GM maize in 2009, 55% of which was *Bt*, 23% for HT and a further 25% being stacked genes. James (2011) further noted that in the case of GM cotton in South Africa, 15,000 Ha was produced for the year end 2010, with all the cotton planted being GM. The preferred planting of stacked *Bt*/HT was observed with 5% RR applied in the refuge portion. Soybean hectarage in 2011 amounted to 390,000, this is a 24% increase compared with figures from 2009. Herbicide tolerant soybean was planted in 331, 500 Ha and of the 66 varieties of soybean planted in South Africa for 2011, 27% were GM (James, 2011).

2.5 Importance of insect resistant maize

Genetically modified insect resistant maize is currently grown in Argentina, Canada, the Philippines, Spain, South Africa, Brazil, Canada, Paraguay, Uruguay, Chile, Honduras, Portugal, Czech Republic, Cuba, Egypt, Romania, Slovakia and the USA (James, 2012) on an estimated area of six million hectares (Van den Berg *et al*., 2013) and is regarded as one of the most universally used GM crops (Alexander, 2007). According to Kaphengst *et al*. (2011), *Bt* maize has shown an increase in economic value of between ten and 17% compared with non-*Bt* maize on a worldwide scale. Although the seed costs of *Bt* maize are approximately ten to 36% higher in comparison to non-*Bt* maize, there was lower pesticide usage of 25 to 60% reported (Kaphengst *et al*., 2011). There was also a significant increase observed in terms of yield benefits in *Bt* maize of five to 25% (Kaphengst *et al*., 2011). Furthermore, the yield advantages of *Bt* maize differed within countries over space and time dictated by the season and region of planting (Kaphengst *et al*., 2011).

In South Africa, growing *Bt* maize proved successful as a method for lowering risks associated with pest pressure (Van Rensburg, 1999; Van Rensburg, 2001; Kaphengst *et al*., 2011; Van den Berg *et al*., 2013). In addition, according to a case study conducted by Keetch *et al*. (2005), *Bt* maize has been introduced to small holder growers in South Africa and increased yield and decreased borer damage was noted. Communal or subsistence farmers in South Africa tend not to spray maize fields to control stem borers due to expenses incurred and inadequate spraying equipment, hence *Bt* maize has been well adopted (Keetch *et al*., 2005).

Tabashnik *et al*. (2001) noted that foliar insecticides are used to control ECB (*O. nubilalis*) and southwestern (*D. grandiosella*) corn borer in non-*Bt* maize. However the usage of these foliar insecticides is limited due to the timing of application which is critical - the application needs to occur before the insect larvae bore into the maize plant (Tabashnik *et al*., 2001). The use of insecticides leads to higher cost implications in the non-*Bt* maize compared with *Bt* maize (Tabashnik *et al*., 2001). Since the introduction of *Bt* crops worldwide there has been a decline in the use of insecticides for the control of insect pests (Pilcher *et al*., 2002; Campagne *et al*., 2013). Farmers who planted *Bt* maize for the control of European corn borer in the USA, recorded higher yields and economic gains compared with non-transgenic maize (Pilcher *et al*., 2002; Wilson *et al*., 2005).

2.6 Insect resistance management

A major limitation in crop production is the influence of insect pests which collectively are responsible for agricultural loses of approximately 15% each year (Christou, 2005). It has been suggested by De Villiers and Hoisington (2011) that if the losses due to pest damage is minimised then there would be an increase in crop yields. Christou (2005) stated that chemical insecticides are used by farmers throughout the world to combat crop loss due to insect damage. However, there is also the concern that an increasing number of insect pests are capable of evolving resistance against chemical insecticides (Christou, 2005) therefore; the concept of insect resistance management was implemented to ensure the continued effectiveness of insecticides (Mallet, 1989).

Pesticide resistance describes the decreased susceptibility of a pest population to a chemical that was previously effective at controlling the pest (Tabashnik *et al*., 2009; Campagne *et al*., 2013). Pest species evolve pesticide resistance via natural selection: the most resistant organisms are the ones to survive and pass on their genetic traits to their offspring (Tabashnik *et al*., 2009; Campagne *et al*., 2013). Insects have the ability to evolve rapid resistance to organic insecticides (such as organochlorines and organophosphates) and *Bt* sprays (Mallet, 1989). This is because organic insecticides have single modes of action or target-site specificity (Mallet, 1989). Previously used inorganic insecticides like lime sulphur, had multiple target sites which limited the development of insect resistance, although these insecticides were unsafe for humans and the environment (Mallet, 1989).

Resistance to pesticides occurs due to changes in gene regulation resulting in an increase in the efficiency of the physiological mechanisms used by the insect for detoxification and in rare cases a single gene mutation can create new detoxifying abilities (Heckel, 2012). The most common mechanism of resistance to *Bt* noted in insect pests (both laboratory strains and field insects subjected to *Bt* sprays) has been the mutation of the larval midgut binding sites that prevented the attachment of the *Bt* toxin (Fabrick and Tabashnik, 2012). The process of resistance development can occur either very quickly or extend over many generations taking decades (Tyutyunov *et al*., 2007). Insect resistance to both chemical insecticides and *Bt* crops occurs due to in part the strength of the toxin, the number of resistance producing alleles present in the insect population and the frequency of insect migrations (Tyutyunov *et al*., 2007) and intensifies when there is a lack of resistance management strategies employed in the field (Campagne *et al*., 2013).

2.6.1 Insect resistance management strategies

Insect resistance to *Bt* crops is not only important to *Bt* growers but also to growers who use *Bt* based pesticides especially organic farmers (Alexander, 2007). Therefore insect resistance management (IRM) strategies to delay insect resistance have been proposed and used practically (Vacher *et al*., 2003; Alexander, 2007). Successful IRM strategies to prolong the efficacy of insecticides traditionally involved the correct selection of insecticide with broad modes of action and appropriately timed applications to target insects at their most susceptible life stages by continually scouting and monitoring the fields for insect damage (Cloyd and Cowles, 2010). Other methods used previously and still maintained today are a rotation of different insecticides to treat a single crop as well as a mixture of different insecticides applied simultaneously at lethal doses (Mallet, 1989). Mallet (1989) added that in order for insecticide rotation to be effective as an IRM strategy many different chemicals had to be included into the rotation programme. However two insecticides used in a rotation was more beneficial than the use of a mixture in delaying insect resistance (Mallet, 1989). Using synergists (such as demethylation inhibitors; plant growth regulators and conventionally used piperonyl butoxide) during the application of insecticides is another method employed to slow down insect resistance since the synergists inhibit or block detoxifying enzymes in insects and allow for prolonged usage of the insecticide (Cloyd and Cowles, 2010).

Insect resistant crops have been successfully used as a method of insect pest control in agriculture, which has directly led to a decrease in pesticide usage and studies have indicated that *Bt* crops are environmentally safe (Vacher *et al*., 2003; Christou *et al*. 2006). The emergence of insect resistance due to the global widespread planting of *Bt* crops may be inherently detrimental to the continued usage of GM and *Bt* crops (especially with the single Cry gene) since these transgenic crops were designed to prevent insect damage by applying a selective pressure on the insect pests (Gould, 2000; Bourguet *et al*., 2005). The stem borers *B. fusca* and *C. partellus* are of particular interest in maize in South Africa (Kfir *et al*., 2002; Kruger *et al*., 2009) because an estimated 425 000 Ha of *Bt* maize was planted in 2007 to limit the effect of these insects (Gouse *et al*., 2005; Van Wyk *et al*., 2008).

Biological control agents or natural enemies can also delay insect resistance to *Bt* crops as was evidenced in a study conducted by Liu *et al*. (2014). The study used the diamondback moth (*Plutella xylostella*) (Linnaeus) (Lepidoptera: Plutellidae), *Bt* broccoli containing Cry1Ac, an insecticide (spinosad; active ingredients: Spinosyn A and Spinosyn D; Dow AgroScience) and the spotted ladybird beetle *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae) (which is a natural enemy of *P. xylostella*). The study compared damage caused by *P. xylostella* in two treatments; a sprayed *Bt* treatment and an unsprayed non-*Bt* refuge containing *C. maculata*. The multigenerational (6 generations) study confirmed that *P. xylostella* numbers were consistently lower in the treatments containing *C. maculata* and the unsprayed non-*Bt* refuge plants. The treatment with the *Bt* plants having no refuge area were infested by *P. xylostella* and subsequently destroyed within 4-5 generations (Liu *et al*., 2014).

Other insect resistance management strategies used in *Bt* crops include alterations in cultural practices of farmers, changes in chemical insecticides, planting of refugia (either non-*Bt* plants or other insect-host crops), insecticide mixtures, seed mixtures and gene stacking or pyramiding (Bourguet *et al*., 2005). Bourguet *et al*. (2005) further noted that the effectiveness of IRM strategies depends on its synergy with existing farming systems and practices. In addition, the chosen refugia crops must be acceptable both economically and sociably especially to those involved in the decision making processes on the farms (Mulaa *et al*., 2011). The most relevant of criteria is that the IRM needs to be simple to implement or the farmer may not comply with the steps involved (Mulaa *et al*., 2011). Integrating different IRM strategies simultaneously for the same crop may guard against the emergence of insect resistance in transgenic crops (Bates *et al*., 2005).

Gene stacking or gene pyramiding is a strategy developed to delay insect resistance in transgenic crops in the field (Jurat-Fuentes *et al*., 2003; Bourguet *et al*., 2005). Single *Bt* genes have become less desirable since insects can evolve resistance to a single gene (Christou, 2005). Gene stacking relies on multiple and different Cry genes (and possibly other insecticidal genes) intended to target different modes of action against the insect pests and are combined in the same transgenic plant (Christou, 2005; De Villiers and Hoisington, 2011).

2.6.2 Refugia as an insect resistance management strategy

One essential component of IRM is the planting of a refuge area (Gould, 2000). Consequently refugia have attracted the interest of both regulatory authorities and GMO seed industries (Siegfried and Hellmich, 2012). Refugia have been strategically designed on the hypothesis of delaying insect resistance by allowing a proportion of insects susceptible to the toxin/chemical to survive the treatment and mate with resistant insects thereby creating a dilution of resistant alleles (Manachini, 2006). The planted refuge area was proposed by the EPA, since IRM for *Bt* crops lies within their mandate in the USA (Bourguet *et al*., 2005), and was created to allow insect pests to feed on non-toxic plant material. The concept of the refuge was implemented in the USA in 1995 in the planting of transgenic cotton and in 2000 for *Bt* maize and potato (Bourguet *et al*., 2005). Resistance to *Bt* crops progresses slowly when the size of the non-transgenic planted refuge area is increased and when resistance inherited from prior generations is recessive (Tabashnik *et al*., 2009).

The EPA in 1995 mandated that cotton farmers grow five percent of their total plots with non-transgenic varieties (Livingston *et al*., 2004). The EPA further instructed that this area was not to be sprayed with any insecticides that kill lepidopteran insect pests, as this area was to allow for the survival of these insects (Livingston *et al*., 2004). Another proposed option was the planting of a 20% refuge area (which could be sprayed) as an alternative to farmers who did not want to risk potential losses in planting the 5% unsprayed scenario (Kruger *et al*., 2009; Kruger *et al*., 2012). According to Gould (2000) the hypothesis of the 20% and 5% refuge areas is that common pesticides kill approximately 80% of insect pests hence, the two refuge options were thought to allow for the same number of living *Bt* susceptible insects. The Science Advisory Committee of the EPA advised that a high-dose refuge (HDR) strategy be used in which an excess of 25-fold the amount of the toxin is used to kill the susceptible insects in combination with an unsprayed non-*Bt* refuge (Gould, 2000). The high-dose approach means that the *Bt* plant is modified to produce an amount of toxin which far exceeds the minimum quantity required to kill all susceptible insect pests (Tang *et al*., 2001; Ringland and George, 2010). The difference between gene stacking and HDR as IRM strategies is that HDR targets heterogygotes insects whereas gene stacking kills non-resistant homozygotes insects (Russell, 2005). Bourguet *et al*. (2000) found that the HDR method was the most widely implemented resistance management strategy.

According to the User Guide for the Production of YieldGard, Roundup Ready and YieldGard with Roundup Ready Maize in South Africa (Monsanto, 2007) and RSA DAFF, the planting of a non-*Bt* refuge area is mandatory in order to slow down the development and spread of insect resistance. The refuge must be planted at the same time as the *Bt* maize and the 5% refuge must not be treated with any chemical pesticides (Monsanto, 2007; Canadian Corn Pest Coalition, 2011; Tiwari and Youngman, 2011). Monsanto also stipulated that the refuge must be planted within, close to or adjacent to the *Bt* crops (Wilson *et al*., 2005; Price *et al*., 2006; Monsanto, 2007). Monsanto offered four possible refugia configurations that may be employed at the discretion of the farmer. These include the within field (block and split planter/strip) and separate field patterns (perimeter and separate field/adjacent) (Tiwari and Youngman, 2011). The perimeter option (Figure 6) can be planted with the *Bt* maize completely surrounded by the non-*Bt* refuge (Monsanto, 2007; Canadian Corn Pest Coalition, 2011). The block approach is another alternative and can be an effective refuge however the non-*Bt* portion of the field becomes inaccessible during harvesting, especially since it is closed off by the *Bt* crops (Monsanto, 2007). Alternating strips could also be used in planting refugia (Figure 6) (Monsanto, 2007). Andow *et al*. (2010) posited that in this instance the requirement is that each refuge strip be a minimum of 4 rows wide, however 6 rows is preferred (Price *et al*., 2006). In the adjacent refuge configuration the non-*Bt* maize must be planted as close to the *Bt* portion as possible, with the separation distance being not more than the width of a road, lane or ditch (Price *et al*., 2006; Monsanto, 2007; Canadian Corn Pest Coalition, 2011). However, even with established IRM strategies in place the emergence of insect resistance in maize is possible (Ali and Yan, 2012).

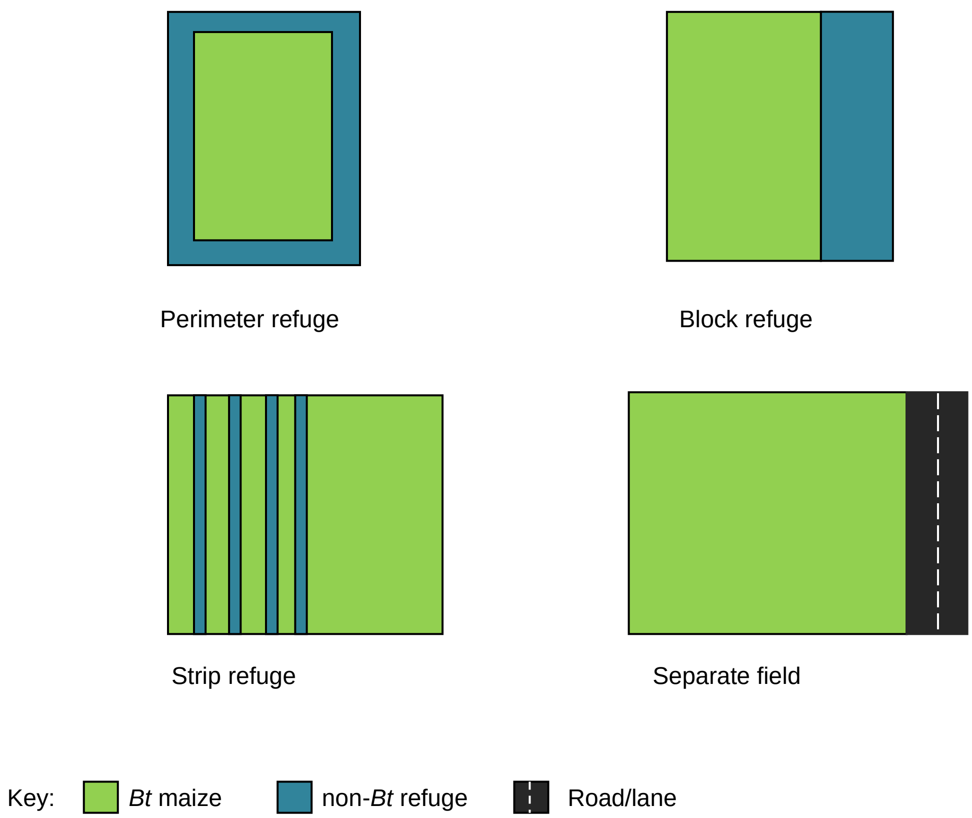


Figure 6: Refuge configurations mandated for the planting of *Bt* crops (Source: Monsanto, 2007)

Seed mixtures (also referred to as refuge-in-a-bag; Monsanto) offer an alternative method of IRM especially for smallholder farms (Andow, 2008). Although the EPA conditionally approved the use of seed mixtures in the USA in 2010 (Onstad *et al*., 2011) the introduction was met with mixed reviews especially by entomologists who criticized this method as a non-viable answer to IRM (Agi *et al*., 2001).

2.7 Emergence of insect resistance in genetically modified crops

Resistance to *Bt* crops has been described in some species of insect pests (Fabrick and Tashnik, 2012). Tang *et al.* (2001), Christou (2005) and Manyangarirwa *et al*. (2006), noted that the diamondback moth (*Plutella xylostella*) was the only insect pest to develop resistance in the field to *Bt*-based insecticides containing the Cry1Aa, Cry1Ab, Cry1Ac, Cry1F, Cry1J toxins in Hawaii, Japan, Florida and the Philippines. Tang *et al.* (2001) and Jurat-Fuentes *et al*. (2003) also reported partial *Bt* resistance in the tobacco budworm (*Heliothis virescens*) (Fabricius) (Lepidoptera: Noctuidae) whereas the pink bollworm (*Pectinophora gossypiella*) (Saunders) (Lepidoptera: Gelechiidae) showed complete *Bt* resistance with the ability to grow and reproduce in the presence of *Bt* crops (Bagla, 2010) (Table 4).

Table 4: Insect resistance noted in *Bt* crops and the countries in which resistance was observed

***Bt* resistant insect pest**	**GM host crop and GM event**	**Country and year in which resistance was observed**	**Reference**
Helicoverpa zea (Corn earworm or cotton bollworm)	*Bt* cotton (Cry1Ac; Cry2Ab)	South Eastern USA (2003)	Tabashnik *et al*., 2008; Tabashnik *et al*., 2009;
Busseola fusca (maize stem borer)	*Bt* maize (Cry1Ab)	South Africa (2007)	Van Rensburg, 2007
Pectinophora gossypiella (pink bollworm)	*Bt* cotton (Cry1Ac)	India (2008)	Bagla, 2010; Wan *et al*., 2012;
Spodoptera frugiperda (fall armyworm)	*Bt* maize (Cry1F)	Puerto Rico (2006)	Tabashnik *et al*., 2009; Storer *et al*., 2010
Diabrotica virgifera virgifera (western corn rootworm)	*Bt* maize (Cry3Bb1)	USA (2011)	Gassmann *et al*., 2011

The first reported case of resistance to Cry1F *Bt* maize was the fall armyworm (*Spodoptera frugiperda*) discovered in Puerto Rico in 2006 (Tabashnik *et al*., 2009; Storer *et al*., 2010). In a study comprising of 77 insect resistance cases conducted by Tabashnik *et al*. (2013), reduced susceptibility to *Bt* crops was observed in 5 of the 13 most important insect pest species in five continents. The authors further reported that the use of *Bt* pyramids, after resistance to one of the two-toxins was observed, decreased its effectiveness. Furthermore, Tabashnik *et al*. (2013) deduced that insect resistance to pyramids will evolve faster when single-toxin *Bt* and *Bt* pyramids are planted simultaneously. An example of insect resistance to *Bt* pyramids, caused by incorrect concurrent planting, was observed in the southeastern USA and resulted in the resistance of corn earworm (*Helicoverpa zea*) to the *Bt* cotton pyramid containing Cry1Ac and Cry2Ab (Table 4) (Tabashnik *et al*., 2008; Tabashnik *et al*., 2009; Tabashnik *et al*., 2013).

In recent studies (Kruger *et al*., 2011; Kruger *et al*., 2012), no data was available to confirm the level of compliance with respect to refuge planting and resistance management in KwaZulu-Natal, for where resistance was not previously reported. The areas in South Africa which have previously reported insect resistance by *B. fusca* growing on *Bt* maize (MON810) are Christiana (first reported in 2006) and Vaalharts (resistance noted in the 2008/2009 growing season) (Van Rensburg, 1999; Van Rensburg, 2001; Van Rensburg, 2007; Kruger *et al*., 2012). Other maize producing regions in South Africa are now recording incidents of *B. fusca* resistance to the single protein Cry1Ab-toxin (Van den Berg *et al*., 2013). Currently growers in South Africa are still planting the single protein events (Van den Berg *et al*., 2013). It has been suggested that non-compliance to refugia requirements has played a role in the evolution and spread of insect resistance in these areas (Vacher *et al*., 2005; Christou *et al*., 2006; Kunert, 2011). Kruger *et al*. (2009) stated that there could have been other reasons for tolerance of *B. fusca* to the Cry 1Ab-toxin, which includes late planting dates and different planting times of maize. This resulted in an increased number of moths as well as a constant supply of moths. Van Rensburg (1999; 2001; 2007) and Kruger *et al*. (2011) further postulated that humidity and rainfall are important factors and may affect the number of moths present. The authors suggested that moths might have a preference to irrigated maize which allowed for selection of resistant insects.

A method to measure grower compliance to GM refuge planting requirements is the use of questionnaires directed to farmers growing GM crops which must be analysed by independent third parties (Bourguet *et al*., 2005). A survey conducted by Kruger *et al*. (2012), in an attempt to measure farmer compliance in South Africa confirmed low compliance to refuge planting requirements with the mandatory 20% (sprayed) and 5% (unsprayed) stipulation by RSA DAFF and the Genetically Modified Organisms Act 15 of 1997. There are other techniques which could be used to determine grower compliance to refuge planting requirements, e.g. the use of lateral flow strips for detection of GM events, to evaluate whether

GM and non-GM zones are sufficient and this method has an additional benefit of being used on-site (Bourguet *et al*., 2005).

Chapter Three

3 Methods and materials

Full ethical clearance for the administration of the GM *Bt* farmer directed survey and field trials were obtained in January 2013 (Appendix 1).

3.1 Survey

Surveys are a useful means in determining farmer attitudes, perceptions and views regarding new technologies (Kruger *et al*., 2009; Pilcher *et al*., 2002). There are approximately 100 farmers who have previously and are currently planting GM *Bt* crops in the KZN region (as indicated by leading seed companies in KZN). However a complete account of all the communal and smallholder growers in KZN is difficult to obtain since seed companies do not have updated records of where and how GM seeds are sold and distributed in the rural communities (Gouse, 2012). According to Domholt (2005) a minimum sample size of 30 respondents is required for experimental research to be deemed viable. Therefore 100 self-administered purposive survey questionnaires were submitted to communal and commercial growers via electronic and postal mail. Purposive sampling is usually chosen in quantitative research when the researcher has specific criteria for selecting respondents or participants (Domholt, 2005). In this study, purposive sampling was chosen since the specific criteria for participation was that growers plant GM *Bt* crops. The purpose of the survey was to establish which of the refugia types were favoured by the farmers and why. The questionnaire was structured in a manner to determine which planted refuge was the most effective in terms of yield, insect damage and the cost-benefit ratio. In addition, the survey questionnaire were used to determine the level of compliance of the farmers in terms of the refuge planting as stipulated by the seed companies, DAFF and the Genetically Modified Organisms Act, 1997 (Act No.15 of 1997).

The survey questionnaire design was completed in April 2012 and disseminated in July and August 2013. The survey cover letter contained a short disclaimer stipulating the intention of the questionnaire, details on the researcher conducting the survey, contact details for feedback from the survey as well as the period of the study (Appendix 2.2). A letter of consent was attached to the questionnaire requesting permission from each respondent (Appendix 2.1). The questionnaire consisted of 21 questions, seven of which were open-ended and 14 were closed questions (Appendix 2.4). The principles used to design the survey questionnaire were initially described by Gafni *et al*. (2002) which are ease of use, objectivity and a defined measurability; in this case the type of refuge configuration implemented. The survey was directed to farmers growing GM *Bt* crops only. Van Tilburg Norland (1990) affirmed that a pilot survey is necessary to determine the reliability of a survey. In the current research study a pilot questionnaire was forwarded to a small sample (five respondents) prior to the commencement of the actual survey. The pilot survey began in May 2013 and evaluated using Genstat-Pearson chi-square test showed (Pearson chi-square value = 4.94; d.f. = 1; Probability level under null hypothesis of p = 0.026). Therefore the survey was answerable, measurable, reliable and unbiased as defined by Van Tilburg Norland (1990). No changes were made to the large-scale survey questions.

The actual survey only commenced once the results of the pilot survey were checked and analysed. The date for the return of the questionnaires was scheduled for August 2013. During the months of July and August 2013, 32 respondents answered and returned the survey questionnaires (n = 32 or 32%). The results obtained via the questionnaire were analysed between August and September 2013 using Genstat – Pearson chi-square test. The survey questionnaire was circulated and distributed by seed sales representatives in the leading seed companies in KZN via both electronic and hard-copy methods.

3.2 Field trial

A field trial was conducted over the (2012/2013) growing season. The field trial was planted in November 2012 and harvested in May 2013 after a growth period of six months. The trial site was provided by Provincial Department of Agriculture and Environmental Affairs (KZN-DAE) located in Cedara (29˚32'60" N and 30˚17'0" E), KwaZulu-Natal. The soil type was Hutton soil form. Cedara has an average temperature of between 6 to 31˚C and annual rainfall of approximately 880 mm from September to May. The trial was planted under dry-land conditions on trial site C3 range 4. The hybrid yellow grain maize seeds PAN 6Q708 BR (insect resistance-Cry1Ab and herbicide tolerance) and the control PAN 6Q508 R (HT – RR) were kindly provided by Pannar (Greytown, KwaZulu-Natal). The RR (non-*Bt*) maize seed was used for planting both the refuge areas and the controls.

3.2.1 Field trial layout

The field was planted in a randomized complete block design (RCBD) with three replicates and a total of ten treatments (Table 5) were therefore imposed. The trial was planted down the contour lengthwise to remove variation down the slope. The RCBD consisted of 30 plots in two blocks of 15 plots each (Table 6). The length of the field was 154 m with each plot length being 10 meters long and eight rows wide; each row was 0.75 m wide and the width 6 meters; each occupied 60 m^2. Plots were separated from each other by a row width (length-wise) and a meter (width-wise).

Table 5: A summary of treatments, refuge options, refuge configurations and application of insecticide in the field trial

Treatment Number	Refuge Option (%)	Refuge Configuration	Insecticide Applied
Treatment 1	Control (non-*Bt*)	Control (non-*Bt*)	No
Treatment 2	Control (non-*Bt*)	Control (non-*Bt*)	Yes
Treatment 3	5%	Block	No
Treatment 4	5%	Perimeter	No
Treatment 5	5%	Strip	No
Treatment 6	20%	Block	Yes
Treatment 7	20%	Perimeter	Yes
Treatment 8	20%	Strip	Yes
Treatment 9	5% Seed mixture[1]	Random	Yes
Treatment 10	20% Seed mixture[2]	Random	Yes

[1] Seed mixture refers to the commercial refuge in a bag option which was hand mixed for the trial; 5% consists of 5% non-*Bt* seed and 95% *Bt* seed

[2] 20% seed mixture consists of 20% non-*Bt* seed and 80% *Bt* seed

Table 6: Field trial design showing the randomized adjacent plots, replicates, plot numbers and treatments

Replicate I – Plot 1 Treatment 1	Replicate I – Plot 2 Treatment 2
Replicate I – Plot 3 Treatment 3	Replicate I – Plot 4 Treatment 7
Replicate I – Plot 5 Treatment 9	Replicate I – Plot 6 Treatment 4
Replicate I – Plot 7 Treatment 10	Replicate I – Plot 8 Treatment 6
Replicate I – Plot 9 Treatment 8	Replicate I – Plot 10 Treatment 5
Replicate II – Plot 11 Treatment 7	Replicate II – Plot 12 Treatment 1
Replicate II – Plot 13 Treatment 5	Replicate II – Plot 14 Treatment 9
Replicate II – Plot 15 Treatment 4	Replicate II – Plot 16 Treatment 6
Replicate II – Plot 17 Treatment 2	Replicate II – Plot 18 Treatment 10
Replicate II – Plot 19 Treatment 3	Replicate II – Plot 20 Treatment 8
Replicate III – Plot 21 Treatment 4	Replicate III – Plot 22 Treatment 8
Replicate III – Plot 23 Treatment 9	Replicate III – Plot 24 Treatment 10
Replicate III – Plot 25 Treatment 7	Replicate III – Plot 26 Treatment 2
Replicate III – Plot 27 Treatment 5	Replicate III – Plot 28 Treatment 3
Replicate III – Plot 29 Treatment1	Replicate III – Plot 30 Treatment 6

Yield (Kg/Ha) was measured after harvesting the mature ears and insect damage (number of damaged stems, leaves and ears) was measured at five intervals during the trials. Each configuration was planted at the same time (Figure 7) adhering to the Monsanto (2007) User Guide for the Production of YieldGard, Roundup Ready and YieldGard with Roundup Ready Maize, since planting *Bt* maize requires the grower to comply with strict protocols to minimise the emergence of insect resistance.

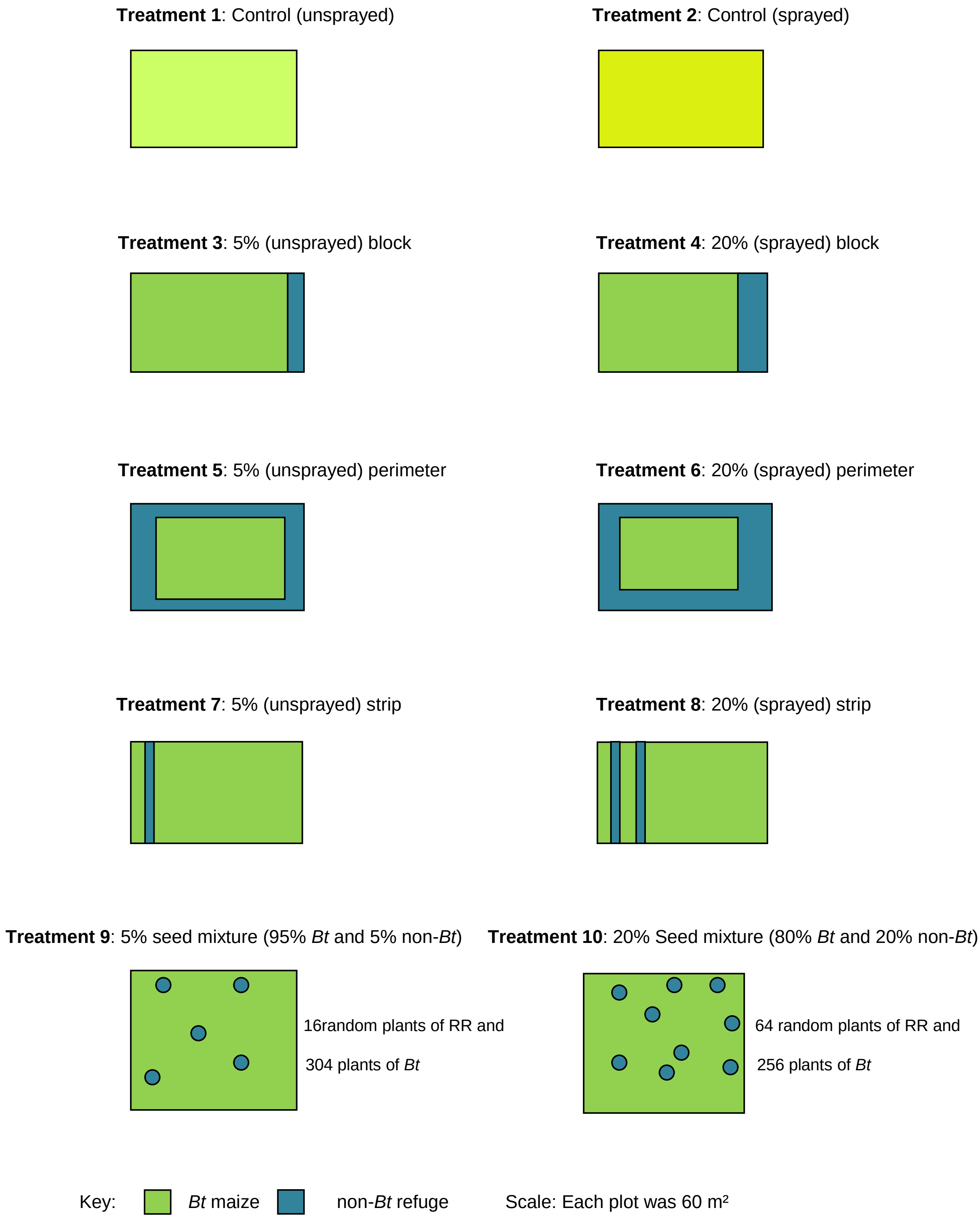


Figure 7: Field trial design based on three configurations of 5% unsprayed and 20% sprayed refuge options. Two controls of non-*Bt* maize were grown conventionally (unsprayed and sprayed). Seed mixtures (of 5% and 20%) were hand mixed and planted in two treatments. (Not drawn to scale)

3.2.2 Preparation and planting of trial site

The trial site was prepared a day before planting. The soil was tilled to remove all weeds as per normal farming practices. The trial site was chemically prepared using 160 Kg/Ha of Map (fertilizer monoammonium phosphate N:P:K-11:52:0; Kynoch), 300 ml/Ha of Calisto (herbicide active ingredient: 479.31 g/L mesotrione; Syngenta); 1.2 L/Ha of Dual (herbicide active ingredient: 958.61 g/L metolachlor; Syngenta) and 50 ml/Ha of Decis forte (insecticide active ingredient: 5.5 g/L deltamethrin; Syngenta) as per recommendation by the Cedara Research Station based on soil tests. Planting of the trial including the refuge areas began in the first week of November 2012 and was completed after two days. The trial site was hand planted using Jab seed planters (Almaco, Brazil) (Plate 1), after planting lines were created using a tractor. Two seeds were planted in each hole made by the Jab planters. A total of five people participated in planting the trial.

Plate 1: Field trial being hand planted using Jab seed planters

The plots were marked out and separated into the three configurations of the 5% and three configurations of the 20% refuge options using measuring tape and string (Plate 2). The last two treatments, T9 and T10 had seed mixtures consisting of 95% *Bt* and 5% non-*Bt* and 80% *Bt* and 20% non-*Bt* respectively. These seed mixtures were mixed by hand and were planted as follows: for the 5% seed mixture plots 16 non-*Bt* seeds were planted out of 320 planted holes; for the 20% seed mixture plots 64 non-*Bt* seeds were planted out of 320 planted holes.

Plate 2: Field trial showing marked plots, the white markers indicates the refuge areas planted with non-*Bt* maize

The two hybrids used in the field trial were PAN 6Q708 BR and PAN 6Q508 R. The remaining non-*Bt* maize was used to create border rows (Plate 3) around each plot and the entire field as per standard practice at Cedara (van Rijj, 2012)[1]. No field measurements were taken from border rows.

Plate 3: Maize trial showing younger boarders lines which were planted two weeks after the trial plots

A second application of the insecticide in this instance Karate (active ingredient: 249.24 g/L lambda-cyhalothrin; Syngenta) at a rate of 0.1 L/Ha) and herbicide Calisto (0.3 L/Ha) was administered in December 2012 (when the maize plants were between 10 cm to 11.5 cm tall). Since two seeds were planted to each hole made by the Jab planters the plots had to be thinned out by removing the less viable of the two seedlings.

[1] van Rijj, N. 2012. Personal communication. Plant scientist. KZN – DAE Cedara, Mushroom Science Division

In cases where there were missing plants, the adjacent double plants were not thinned. Crop thinning was conducted in middle December 2012, when the plants were approximately between ankle to knee height (or 5 cm to 10 cm) as per normal practice in Cedara. A top dress of 300 Kg/Ha of LAN fertilizer (containing 0.08 Kg of nitrogen per Kg; Kynoch) was required in early January 2013 to supplement the loss of nutrients due to fertilizer run off from the previous application. The insecticide Karate at a rate of 0.1 L/Ha was applied to plants using 16 L boom sprayers (Matabi, Spain) in early January 2013. However due to unexpected continuous rains the application was stopped before completion. Therefore granular insecticide (specific to stem borer insect pests; 25g/Kg Cabaryl; Kombat) was applied at a rate of 15Kg/Ha to the relevant plots. The need for additional applications of insecticide were determined by visual observations of insect damage made on the 30 plots randomly and throughout the growth of the plants and records were kept of such applications.

3.2.3 Harvest

The harvest time for conventional maize is when the plant reaches physiological and harvest maturity which is when the husk of the ears are brown and dry (Plate 4). The ears have a black mark visible at the base of each kernel and the plant moisture content is between 12 to 14 % which is predictably around six months for PAN 6Q708 BR and PAN 6Q508 R (Pannar). A moisture metre (6310 Moisture Analyser; Sinar-GrainPro, United Kingdom) was used to measure kernel moisture (Plate 5) after shelling the ear. The trial was harvested in the middle of May 2013 over a period of two days. All eight lines from 30 plots were harvested and the field masses recorded in Kg/Ha. The ears from each plot were counted and sorted to determine the number of undamaged ears and borer infested ears.

Plate 4: Physiologically mature maize ear at 25 weeks (or five months) after planting showing a brown dried husk

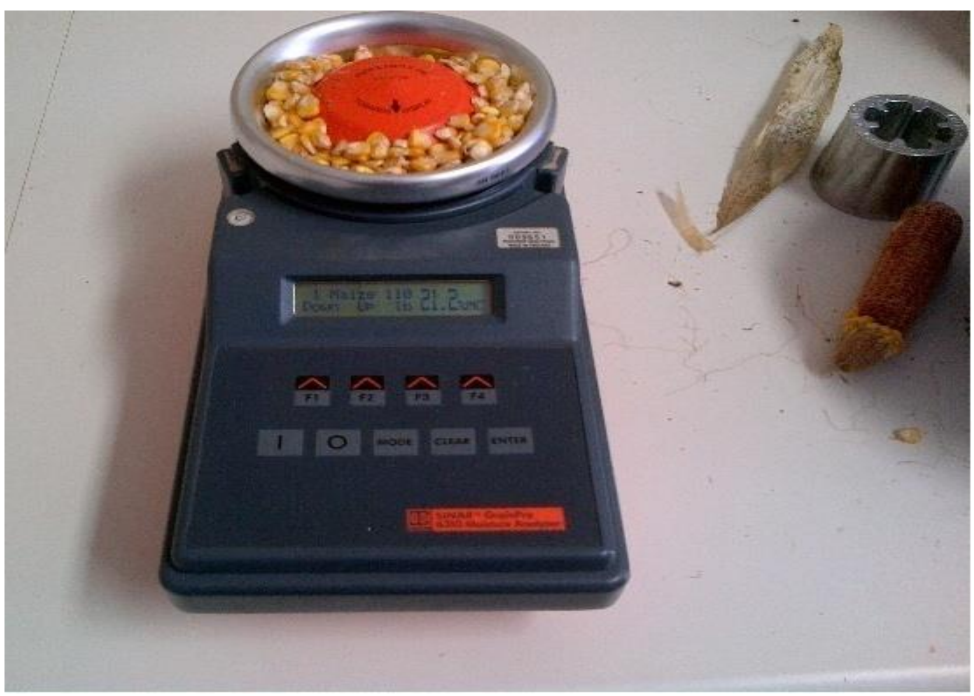


Plate 5: The moisture metre (left) used to measure the moisture content of maize and the maize shelling device (right) removes maize kernels from the ear

3.2.4 Yield parameters measured at harvest

Upon harvesting of the ears the field mass of each plot was recorded. Shelling percentages were calculated using the mass of the harvested maize kernels (grain mass) with and without the ear (Nunkumar, 2013)[1]. Representative samples of ten ears were taken from each of the 30 plots and were later shelled (Nunkumar, 2013)[1]. Moisture readings were then conducted on the samples using a moisture meter. The field mass, grain mass and moisture content readings (Appendices 10 and 11) were used to determine the final yield per plot using the following calculations:

Shelling % = grain (Kg)/grain + ear (Kg)

Moisture conversion = (100 – Moisture % /87.5)

Plot mass (Kg) = Field mass (Kg)

Grain conversion = plot mass x shelling % x moisture conversion

Tons/Ha = (grain conversion/60) x 10000/1000

Kg/Ha = Tons/Ha x 1000

Calculations were based on Nunkumar, 2013[1]

The yield of each plot was expressed in tons per hectare of harvested ears and then converted to kilograms per hectare.

[1] Nunkumar, A. 2013. Personal communication. Plant pathologist. KZN – DAE Cedara, Plant Disease Clinic Division

3.2.5 Stem borer damage

Damage caused by *B. fusca* and *Chilo partellus* was observed and recorded at five different stages of growth (vegetative, flowering, soft dough, hard dough and pre-harvest). During each of the five inspections, all 200 to 300 plants from each plot were counted and plants showing stem borer damage on the leaves and stems of each sample over the total number of plants counted (Appendices 6, 7 and 8). At each sequential inspection only 'new' borer damage was recorded. New borer damage was easy to distinguish from old borer damage because in the latter the leaves became chlorotic and necrotic and the stem borings were surrounded by a necrotic lesion. Each inspection was recorded photographically. The incidence of damaged plants was then expressed as a percentage.

Cumulative borer damage (to stems and leaves) over the five inspections was calculated by representing the percentage of borer damaged plants after six months over the total number of plants counted per plot. This was done to determine the refugia which incurred the least borer damage overall.

After harvest, the number of borer infested ears was also counted (Appendix 9). The mean percentage of borer damaged ears per plot was calculated over the total number of ears harvested in each plot.

3.2.6 Plant counts and plant lodging

The first plant count was done during the middle of December 2012 (5 weeks old). This was done to determine the number *Bt* and non-*Bt* plants that were planted per row and per plot (Appendix 3). This information was needed to later determine the cost-benefit ratio and yield per plot. The plant count changed due plant lodging (due to wind and rain damage). The first plant lodging count was done in mid-January 2013 and the second count was done in mid-February 2013

(Appendix 4). Additionally during mid-February 2013 bush pigs, were found in the trial and were responsible for extensive damage to the border lines and certain plots. This damage was counted and noted (Appendix 5). The final plant count was done in May just prior to harvesting (six months old).

3.2.7 Cost-benefit ratio

After harvest and shelling of maize ears, the grower normally sells grain to other growers or seed companies for the purposes of profit or as per land lease contracts. This seed purchased by seed companies is agreed upon and fixed per growing season and per variety. The price for varieties used in this study PAN 6Q708 BR and PAN 6Q508 R for the 2012/2013 growing season was set by the seed companies at ZAR 2000 per 1000/Kg regardless of GM trait. Costs and sales figures are shown in Table 7.

Table 7: Items purchased and grain sold for the GM *Bt* field trial

Cost item*	**South African Rand (ZAR)**
Purchase of *Bt* seed	3385.80 (per 25 Kg bag)
Purchase of non-*Bt* seeds	2964.00 (per 25 Kg bag)
Insecticide application - Karate□	25.00 (for 100ml/Ha)
Insecticide application - granules□□	72.00 (for 4 Kg/Ha)
Sale item**	**South African Rand (ZAR)**
Sale of *Bt* grain	2000.00 (per 1000 Kg)
Sale of RR grain	2000.00 (per 1000 Kg)

*Cost items were evaluated for the 2012/2013 growing season
**Sale items were determined for the 2012/2013 growing season
□□Cost per litre of Karate was ZAR 250.00 however only 100 ml/Ha was used therefore cost was ZAR 25.00
□□□Cost per 25 Kg of stalk borer granules was ZAR 450.00 however only 4Kg/Ha was used therefore the cost was ZAR 72.00

The cost price of 25 Kg *Bt* seeds (variety: PAN 6Q708 BR) for the 2012/2013 growing season was ZAR 3385.80 (inclusive of value added tax) while 25 Kg non-*Bt* (variety: PAN 6Q508R) was ZAR 2964.00 (inclusive of tax). Even though the farmer pays more for the *Bt* seed at the planting stage than the non-*Bt* seed, the seed companies will pay a standard fee for both kinds of grain after harvest (subject to the growing season).

Since there was no difference in herbicide and fertilizer usage for each of the refuge configurations, a cost-benefit ratio between the yield obtained per configuration, cost of applied additional insecticides (to all sprayed plots), labour costs and the initial cost of seed was determined. The yield was measured in Kg per hectare and equated to a value in South African Rands (ZAR) by dividing the total yield obtained in Kg/Ha by 25 Kg (which is the mass per bag of seed). This provided the number of 25 Kg bags of grain which were produced per Ha. This value was multiplied by the sale of grain (ZAR value for the sale of the grain). The resultant value was the yield obtained in ZAR. To determine the cost-benefit ratio the yield obtained in ZAR was divided by the cost of the seeds purchased. The cost-benefit calculations were performed as follows:

Number of bags per Ha = Total yield (Kg/Ha) ÷ 25 Kg (bags)
Yield obtained in ZAR/Ha = Number of bags per Ha x value of sale of seed[1](ZAR)
Cost-benefit ratio = Yield obtained (ZAR/Ha) ÷ (cost of initially purchased seeds + cost of insecticides + labour costs for insecticide application)* (ZAR)

* Labour cost for insecticide application = number of days taken to apply insecticide x number of labourers x daily rate paid to labourer by KZN-DAE
Two applications of insecticide were applied after land preparation using one labourer per day therefore: (2 x 1 x ZAR 90) = ZAR 180

[1] Value of sale of seed is what the farmer earned per 25 Kg bag of seed sold to a seed company or other grower

3.3 Statistical analyses

Statistical analysis for the survey was conducted using the statistical software package GenStat Fourteenth Edition, Release: 14.2 (GenStat Procedure Library Release PL22.2, 2011, VSN International Limited). The statistical test used for the survey was Pearson chi-square.

Analysis of variance (ANOVA) of GenStat Fourteenth Edition, Release: 14.2 (GenStat Procedure Library Release PL22.2, 2011, VSN International Limited) was also used for analysis of the field trial data and the post-hoc analysis was done using Fishers least significant difference. Statistical analysis was conducted at a 0.05% alpha level. Yield data (Appendices 10 and 11) was analysed using ANOVA and the means were separated using LSD. To accommodate for the yield losses caused by bush pigs which entered the trial site, the means were adjusted using a covariate of the pig damage. The pig damage was extensive and was correlated to the yield. Stem borer damage was analysed using ANOVA of Genstat statistical package. Cost-benefit ratio calculations were analysed using ANOVA of Genstat and adjusted with a covariate to account for damage caused by bush pigs (Appendices 12 and 13).

In order to assess if either the 5% or the 20% refuge options, regardless of the planting configuration, results in significantly improved insect damage, yield or cost-benefit ratio, the treatments were combined to formulate an overall conclusion as to the most effective refugia prior to applying statistical analyses.

Chapter Four

4 Results

4.1 Analysis of survey questionnaire responses

The survey questionnaire was designed to determine which refuge options (5 and 20%) and configurations (block, strip, perimeter and adjacent) which were employed by GM *Bt* maize growers in KwaZulu-Natal and the reasons for those choices. In addition, this study also aimed to determine the compliance of *Bt* growers with refugia planting requirements mandated by RSA DAFF.

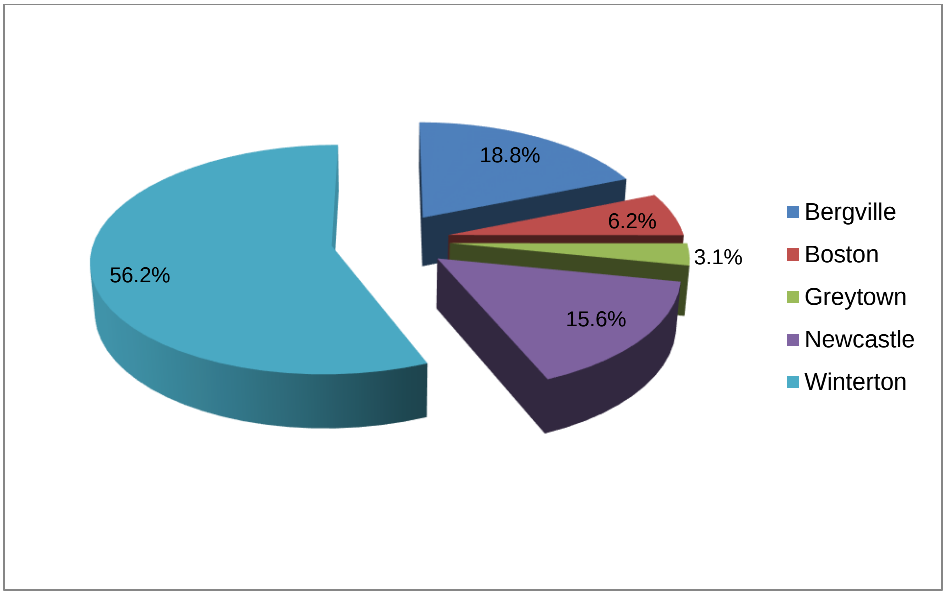
The questionnaire was divided into sections and categories which included the scale of the grower; type of GM *Bt* crop grown; which genetically modified trait(s) is used; whether or not a refuge area is planted; the refuge option employed; refuge configuration utilized; observation of insect borer damage and whether or not refuge-in-a-bag mixtures are used.

4.1.1 The scale and locality of growers planting genetically modified crops in KwaZulu-Natal

Thirty one of the 32 respondents produced commercial GM *Bt* crops (96.9%). One grower (3.1%) produced GM *Bt* crops for subsistence purposes with a total area of 300 Ha and 100 Ha planted with GM *Bt* crops. Responses received were from 5 maize producing regions in KZN (Graph 1). The mean area planted with GM *Bt* crops by the 32 growers was 5 890 Ha out of a total crop area of 16 099 Ha i.e. 36.6%. Herbicide tolerant soybeans were grown by 28 (87.5%) of respondents. No planting of GM cotton was recorded since the climate in this particular region of KZN is not suitable. The majority of growers (22 or 68.75%)

planted stacked *Bt*/HT maize and HT soybeans, the single *Bt* trait was favoured by 8 growers (or 25%) and the single HT trait was planted by 2 growers (or 6.25%).

Graph 1: The percentage of survey respondents and the 5 maize producing regions they represented in KwaZulu-Natal (n = 32)



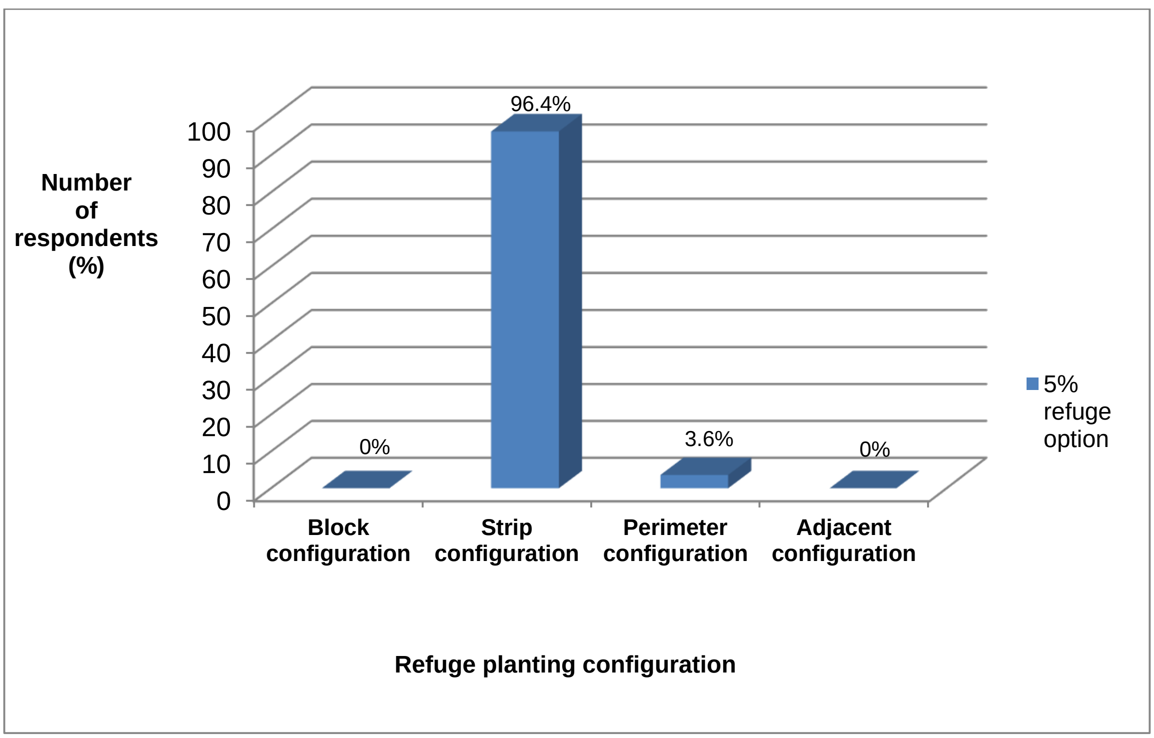
4.1.2 Planting of a refuge area

Data showed that 29 (90.6%) of the 32 growers planted a non-*Bt* refuge and three growers (9.4%) indicated they did not plant the refuge area. Respondents were further asked if they had always planted non-*Bt* refugia and 22 out of 29 growers (75.9%) indicated that they had always complied with refugia planting requirements. Seven respondents (24.1%) replied in the negative for planting refugia citing 'management crisis' (one respondent out of seven or 14.3%) 'were not told to plant a refuge area' (one respondent or 14.3%) and 57.1% (four respondents) cited 'incorrectly planted' as reasons for their non-compliance with one respondent not giving a reason.

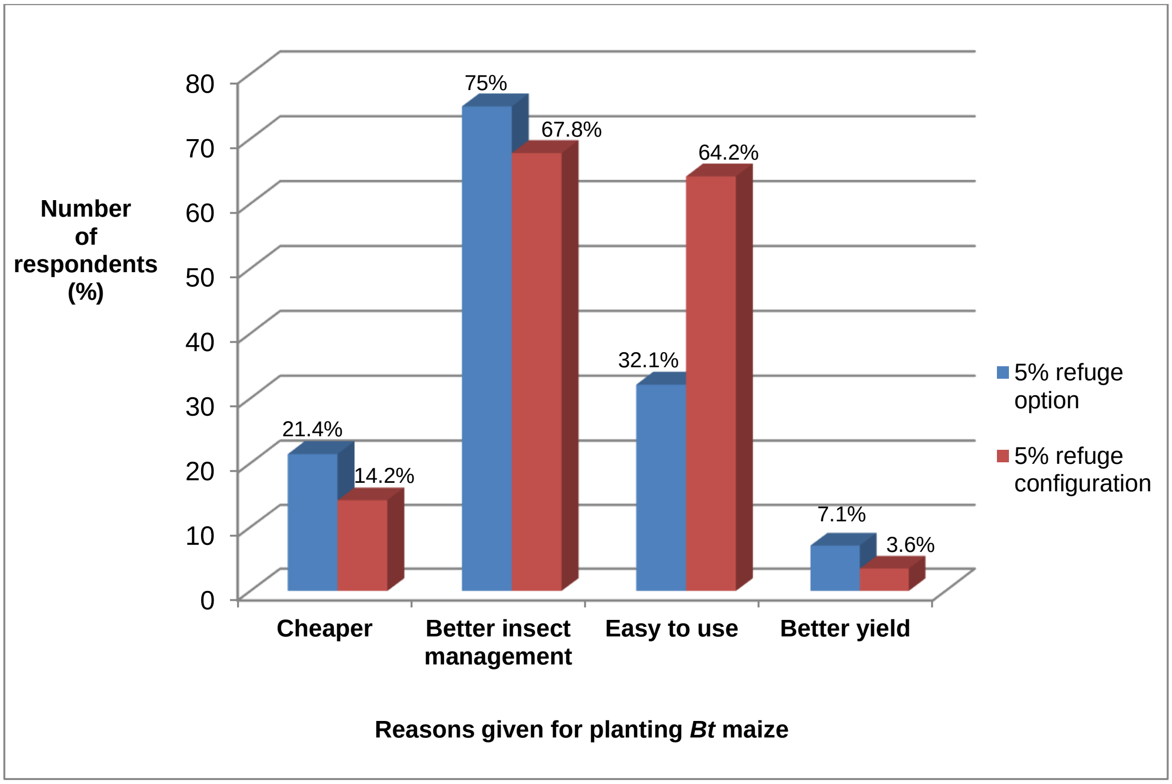
4.1.3 Refugia employed by respondents in KwaZulu-Natal and reasons for their decision

The findings of the survey showed that 29 (90.6%) respondents planted non-*Bt* refugia. Of these, 28 (96.6%) planted the 5% (unsprayed) option and one respondent (3.4%) planted the 20% (sprayed) refuge option. The configuration chosen by the majority of respondents was the 5% refugia (27 out of 28 respondents or 96.4%) using the strip configuration (Graph 3). The most prevalent reason for the choice of refuge option and configuration was 'better insect management' (Graph 4). 'Increased yield' was only chosen by two respondents (7.1%) for the choice of refuge option and by one respondent (3.6%) for refuge configuration.

Graph 2: Respondents' choice of configuration when planting the 5% refuge option (n = 28)



Graph 3: Respondents' reasons for planting the 5% refuge option and configuration (n = 28)



4.1.4 Grower observation of insect borer damage and infestation

Respondents were asked whether they observed any insect borer damage on their *Bt* crops since their planting of *Bt* maize. Seven respondents (21.9%) had observed prior damage with the first report of borer infestation in the year 2010 and 25 respondents (78.1%) did not record any borer damage (n = 32). The incidents of borer infestation were isolated in two locations i.e. Winterton and Bergville in KwaZulu-Natal. There were four instances out of seven (57.1%) reported in 2010 from the Winterton area, two (28.6%) in 2011 and one (14.3%) observed in 2012, the latter three cases were noted in the Bergville area (n = 32). In addition, four of the seven (57.1%) cases of borer damage in *Bt* crops reported by growers were from respondents that did not comply initially with refuge planting requirements.

4.1.5 Adoption of seed mixtures by respondents as an insect resistance management strategy

The growers were asked whether they would prefer to use a pre-mixed refuge-in-a-bag option in their future planting instead of the 5 and 20% refuge options which have strict planting requirements. The majority of responses replied in the negative (24 responses or 75%). Two growers (6.3%) reported ‘not sure’ which may have been due to them not being aware of seed mixture technology previously whereas 6 respondents (18.8%) replied ‘yes’.

4.2 Field trial

4.2.1 A comparison of borer damage between different refugia treatments and non-*Bt* controls

Ten different treatments consisting of 5% and 20% refuge configurations and two seed mixture refuge options were planted using Cry1Ab *Bt* and non-*Bt* maize. The purpose was to record borer damage on the stems and ears of the maize plants during the trial. Insecticide was applied three times at monthly intervals during the first three months of growth and this timing was determined by visual inspections made by scouting for insect borer activity as soon as the maize seed germinated. In general, those treatments which sustained the highest levels of infestation and damage would be the least viable option for planting as a refuge. Early symptoms of insect borer damage appeared as a series of ‘shotgun’ holes on the leaves and stems (Plate 6). Advanced borer infestation presented as necrotic lesions (Plate 7) and in severe instances resulted in whole leaf necrosis. Insect recovery for the purposes of identification was not undertaken since it is likely that damage was caused by both *B. fusca* and *C. partellus* as these insects commonly occur in the area.

Plate 6: Early maize stem borer damage resembling ‘shotgun’ holes 14 weeks after planting

Plate 7: Advanced stem borer damage of 14 week old maize leaves causing necrotic regions

Percentage insect borer damage was recorded over 5 inspection intervals. The highest percentage of insect damage was in the control (unsprayed) and this was observed in the January (7.74% damage), February (6.31% damage) and March (3.91% damage) inspections (Table 8). However, there was no significant difference in percentage damage between the sprayed and unsprayed controls over the five month period. This suggests that the insecticide application was not efficient or not frequent enough to eliminate insect activity and associated damage in the sprayed plots. In January (during the vegetative growth stage), the control (unsprayed) treatment had significantly more damage than the 5% perimeter (unsprayed) (2.90% damage), the 20% block (sprayed) refuge (3.86% damage) and both sprayed seed mixture refugia (five percent seed mixture: 3.98% damage and 20% seed mixture: 1.97% damage).

In February (at the flowering stage) there were significant differences in the levels of damage in maize plants (stems and leaves) amongst the refuge treatments (ANOVA, F pr. = 0.04). The percentage damage observed in the control (unsprayed; 6.31% damage) was significantly higher than the 5% block (unsprayed; 3.07% damage), 5% (unsprayed; 3.44% damage) and the 5% (unsprayed; 3.66% damage) treatments (Table 8) suggesting that the differences were due to the *Bt* trait. When comparing the sprayed control (4.98% damage) with the other sprayed treatments, significant differences were observed in percentage of stems and leaves damaged in the 20% block (1.86% damage) and 20% seed mixture (1.91% damage) but in the remainder of the sprayed refugia configurations (i.e. 5% seed mixture, 20% perimeter and 20% strip), there was no advantage conferred by either the planting option or the *Bt* trait.

In March (at the soft dough stage of development) the percentage borer infestation in the unsprayed control treatment (3.91% damage) was significantly higher than in the 5% block (unsprayed; 0.89% damage) and the 20% perimeter (sprayed; 1.02% damage) (Table 8).

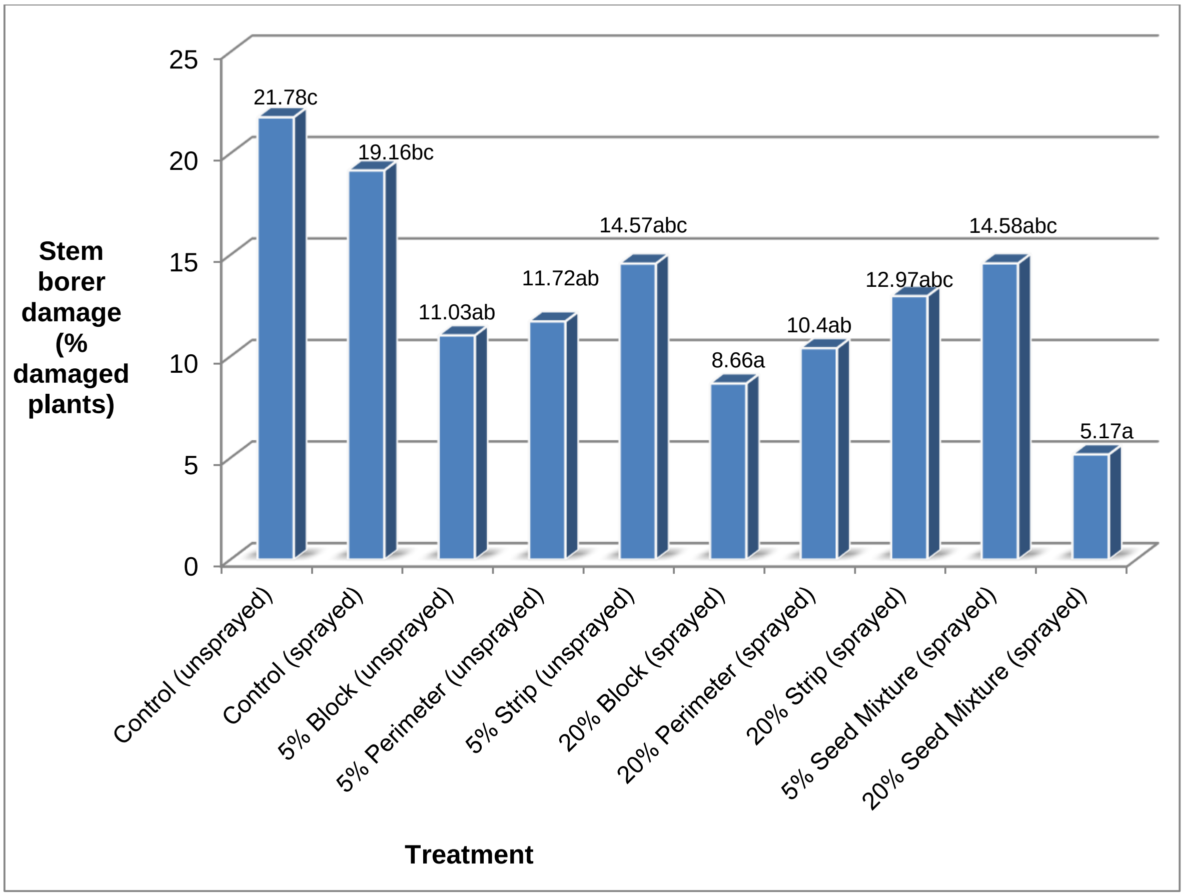
In early April (during hard dough development) the only difference between the unsprayed control (1.24% damage) and the sprayed control (3.39%) and all other treatments was the 20% seed mixture (0.22% damage) which was sprayed and had the *Bt* trait. During late April (pre-harvest stage) percentage borer damage was higher than during January, March and early April (Table 8). The percentage damage in the sprayed control treatment (2.88% damage) and the unsprayed control treatment (2.59% damage) was significantly different compared with the 20% block (0.62% damage), 20% perimeter (0.42% damage) and 20% seed mixture (0.36% damage) sprayed treatments. The trend for insect damage of those treatments that contained the *Bt* trait and had insecticide application was similar. The sprayed control treatment had more borer damage compared with the other sprayed *Bt*-containing plots, but these differences were not always significant (Table 8).

Table 8: Mean percentage of borer damage as measured by the number of damaged maize plants (stem and leaf) per plot for ten treatments over the total plant count for each inspection (n=3; results analysed by ANOVA; Post-hoc analysis by Fisher's unprotected least significant difference; significant differences are indicated by differing alphabetic letters)

Treatment	**10-January borer damage (%)**	**13-February borer damage (%)**	**19-March borer damage (%)**	**11-April borer damage (%)**	**29-April borer damage (%)**
1. Control (unsprayed)	7.74b	6.31c	3.91b	1.24ab	2.59bc
2. Control (sprayed)	5.31ab	4.98bc	2.60ab	3.39b	2.88c
3. 5% Block (unsprayed)	4.94ab	3.07ab	0.89a	1.14ab	1.00abc
4. 5% Perimeter (unsprayed)	2.90a	3.44ab	2.36ab	2.24ab	0.78ab
5. 5% Strip (unsprayed)	4.96ab	3.66ab	2.03ab	2.08ab	1.84abc
6. 20% Block (sprayed)	3.86a	1.86a	1.46ab	0.86ab	0.62a
7. 20% Perimeter (sprayed)	4.54ab	3.30ab	1.02a	1.12ab	0.42a
8. 20% Strip (sprayed)	4.36ab	3.00ab	1.89ab	1.83ab	1.90abc
9. 5% Seed Mixture (sprayed)	3.98a	4.47bc	2.53ab	1.87ab	1.74abc
10. 20% Seed Mixture (sprayed)	1.97a	1.91a	0.70a	0.22a	0.36a
Grand mean	4.46	3.60	1.94	1.60	1.41
LSD	3.49	2.48	2.82	3.15	1.97
CV%	45.6	40.2	84.9	114.7	81.1
v.r	1.71	2.65	1.05	0.69	1.87
F pr.	0.16	0.04	0.44	0.71	0.12

The cumulative percentage of borer damage over the five inspection intervals (Graph 4) showed differences between some of the treatments (ANOVA, F pr. = 0.086). The unsprayed control showed higher borer infestation of 21.8% when compared with the 20% and 5% block (sprayed and unsprayed; 8.66% and 11.03% damage, respectively), 5% perimeter (unsprayed; 11.72% damage), 20% perimeter (sprayed; 10.40% damage) and 20% seed mixture (5.17% damage). The control (unsprayed) had a higher percentage borer infestation compared with all other unsprayed treatments (5% block, perimeter and strip) due to the *Bt* trait in the five percent treatments. Planting *Bt* seed, regardless of the refuge configuration, resulted in protection against borer damage, except for the 5% seed mixture (sprayed; 14.58% damage), 5% strip (unsprayed; 14.57% damage) and 20% strip (sprayed; 12.97% damage), where damage was no different than that observed in the controls.

Graph 4: Cumulative borer damage (percentage of borer damaged plants after six months over the total number of plants) (n=3; results analysed by ANOVA; Post-hoc analysis by Fisher's unprotected least significant difference; significant differences are indicated by differing alphabetic letters; Grand mean = 13.0; LSD = 9.95; CV% = 44.60; v.r. = 2.10; F pr. = 0.086)



When the maize plants reached maturity (5 months), the borers penetrated the husks of the maize and tunneled their way into the ears (Plate 8). This caused further damage to the individual kernels (Plate 9) which impacted negatively on the maize yield obtained.

Plate 8: Borer damage visible on the husk of the mature maize ear. In this case the borer has tunneled into the ear

Plate 9: Borer damage on a mature maize ear, resulting in necrosis of severely damage areas

When comparing ear damage across the different treatments, the 5% perimeter (unsprayed) configuration showed higher damage (10.77% ear damage) than all other treatments, except the 5% block unsprayed (7.74% ear damage) (Table 9). This may have been due to the absence of insecticide in the 5% perimeter and block treatments. There were no significant differences between the controls and any of the other refugia options (Table 9). The treatments which sustained the lowest percentage of ear damage were the 5% strip (unsprayed; 2.61% ear damage), 20% perimeter (sprayed; 2.47% ear damage) and 5% seed mixture (sprayed; 3.15% ear damage), but these did not differ significantly from the controls.

Table 9: Showing the mean percentage of borer damaged ears (n=3; results analysed by ANOVA; Post-hoc analysis by Fisher's unprotected least significant difference; significant differences are indicated by differing alphabetic letters)

Treatment	**Mean percentage of borer-damaged ears (%)**
1. Control (unsprayed)	5.35ab
2. Control (sprayed)	5.58ab
3. 5% Block (unsprayed)	7.74bc
4. 5% Perimeter (unsprayed)	10.77c
5. 5% Strip (unsprayed)	2.61a
6. 20% Block (sprayed)	5.34ab
7. 20% Perimeter (sprayed)	2.47a
8. 20% Strip (sprayed)	5.25ab
9. 5% Seed Mixture (sprayed)	3.15a
10. 20% Seed Mixture (sprayed)	4.04ab
Grand mean	5.23
LSD	4.59
CV%	50.3
v.r.	0.03
F pr.	0.90

4.2.2 A comparison of *Bt* maize yields between different refugia options and configurations

Yield (Kg/Ha) of the field trial was calculated to determine the refuge option (5 and 20%) and refuge configuration (block, perimeter, strip and seed mixture) which produced the highest yield. According to the Agricultural Research Council – Grain Crops Institute (ARC – GCI) (2013), the maize cultivars PAN6Q508R and PAN6Q708BR produce a yield of between 8000 and 9000 Kg/Ha in the KZN region when planted under dry-land conditions.

No significant differences were noted in respect of yield regardless of the parameter measured (Table 10). All treatments produced a yield in the expected range except the 20% (sprayed) option in perimeter configuration which produced the lowest yield (7344.00 Kg/Ha). The 5% (unsprayed) in strip configuration produced second lowest yield (7740.2 Kg/Ha) while 20% strip (sprayed) produced yield within the yield range for these varieties and locality.

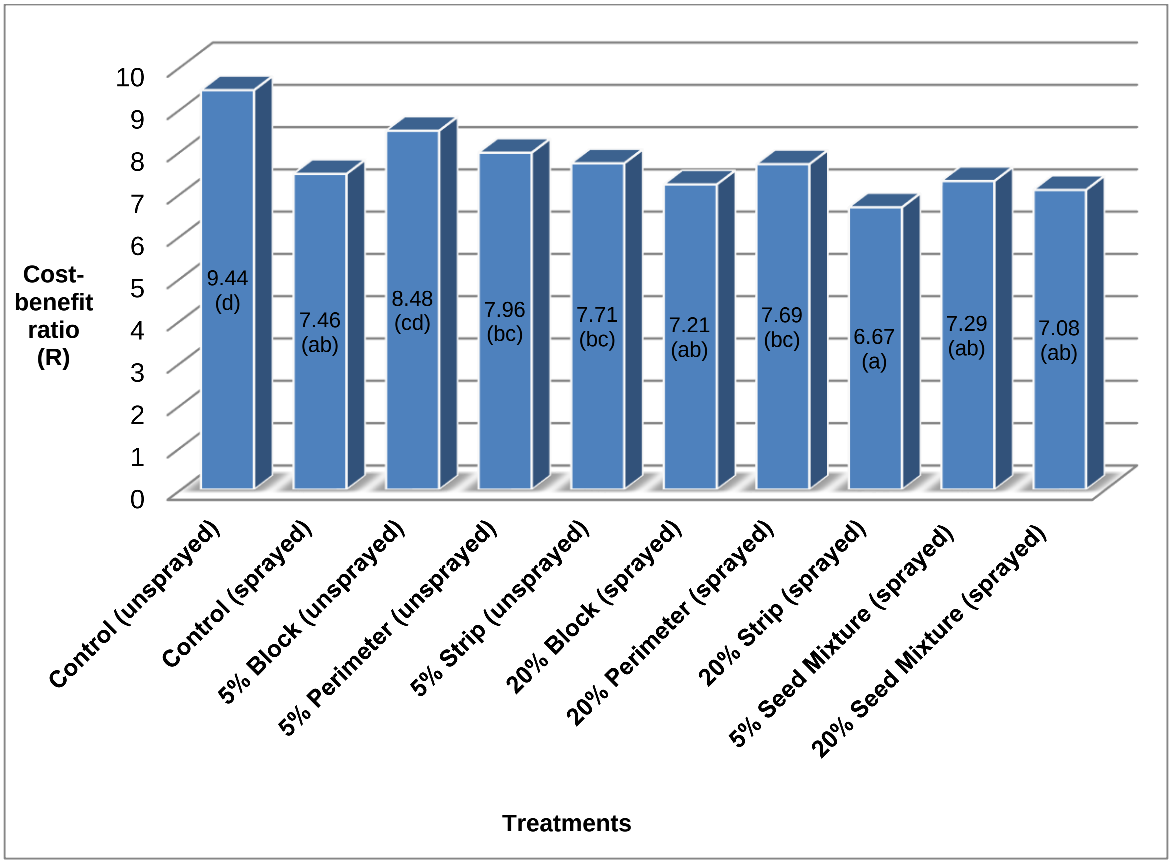
Table 10: Mean yield data for the field trial (n=3; results analysed by ANOVA; Post-hoc analysis by Fisher's unprotected least significant difference)

Treatment	Plot mass (Kg)	Shelling %	Moisture Conversion	Grain Conversion	Yield (Kg/Ha)
1. Control (unsprayed)	64.11	0.83	0.91	48.36	8060.4
2. Control (sprayed)	62.63	0.84	0.91	47.79	7964.0
3. 5% Block (unsprayed)	70.74	0.83	0.91	53.50	8916.6
4. 5% Perimeter (unsprayed)	62.72	0.84	0.91	47.63	7938.9
5. 5% Strip (unsprayed)	61.97	0.83	0.91	46.44	7740.2
6. 20% Block (sprayed)	64.91	0.84	0.91	49.76	8293.7
7. 20% Perimeter (sprayed)	57.48	0.84	0.91	44.07	7344.0
8. 20% Strip (sprayed)	63.20	0.83	0.91	48.10	8016.4
9. 5% Seed Mixture (sprayed)	67.07	0.83	0.91	50.46	8410.5
10. 20% Seed Mixture (sprayed)	66.41	0.84	0.91	50.74	8457.6
Grand mean	8110	-	-	-	-
LSD	2.01	-	-	-	-
CV%	14.5	-	-	-	-
v.r.	0.41				
F pr.	0.92				

4.2.3 Cost-benefit ratio calculation for field trial

Cost-benefit ratio calculations were conducted to determine the treatment which provided the best economic returns based on the yield of harvested grain. The highest cost-benefit value of ZAR 9.44 was obtained for the control (unsprayed) (Graph 5). This means that for every ZAR spent by the farmer a return of ZAR 9.44 is realized on the sale of grain. The lower cost-benefit ratio of ZAR 7.46 obtained in the control (sprayed) indicates that the cost of applying insecticide can reduce profit margins significantly. There were no significant differences between the cost-benefit ratios with a 5% refuge when compared with each other regardless of the planting configuration. When comparing cost-benefit ratios for the 20% refuge planting configurations, the perimeter and block configurations (ZAR 7.69 and ZAR 7.21 respectively) were better than the strip (ZAR 6.67) treatment. There were no significant differences between the remainder of the treatments.

Graph 5: Mean cost-benefit ratios determined for the different treatments (n=3; results analysed by ANOVA; Post-hoc analysis by Fisher's unprotected least significant difference; significant differences are indicated by differing alphabetic letters; Grand mean = 7.70; LSD = 0.968; CV% = 7.2; v.r. = 25.80; F pr. = 0.124)



4.2.4 Comparison of insect damage, yield and cost-benefit in the five and twenty percent refugia

There were no significant differences in the yield or total leaf and stem damage when comparing the 5 and 20% refugia. However there was a significant difference in the cost-benefit ratio between the 5 and 20% refugia (ANOVA, F pr. = 0.03) (Table 11). This difference reflects both the cost of the insecticide and the labour to apply it in the 20% option. Furthermore, there was a difference in the mean levels of damaged ears in the perimeter configuration between the 5 and 20% refugia (Table 11).

Table 11: Comparison of 5 and 20% refugia for ear damage, total plant damage, yield and cost-benefit ratio, regardless of the planting configuration (n=3; results analysed by ANOVA; Post-hoc analysis by Fisher's unprotected least significant difference)

	Ear damage (% damage)	**Total leaf and stalk damage (% damage)**	**Yield (Kg/Ha)**	**Cost-benefit ratio (R)**
Five percent (mean)	**7.11**	**10.60**	**8.20**	**7.96**
Five percent (block)	7.78	9.90	8.92	8.34
Five percent (perimeter)	10.86	9.50	7.94	7.90
Five percent (strip)	2.71	12.50	7.74	7.64
Twenty percent (mean)	**4.47**	**9.40**	**7.88**	**7.15**
Twenty percent (block)	5.42	7.80	8.29	7.11
Twenty percent (perimeter)	2.65	9.30	7.34	7.83
Twenty percent (strip)	5.35	11.10	8.02	6.52
Grand mean	5.79	10.00	8.04	7.55
LSD	4.34	10.17	0.95	1.07
CV%	38.7	59.6	-	7.10
v.r.	0.00	0.46	0.33	388.33
F pr.	0.98	-	0.58	0.03
F pr. (configuration)	0.09	0.64	0.36	0.09

Chapter Five

5 Discussion

5.1 The practices, preferences and perceptions of *Bt* maize growers in KwaZulu-Natal

The survey in this study was designed to determine grower compliance with *Bt* maize refugia plantings in KZN as well as the refuge options and configurations most widely preferred. The survey indicates that the first GM *Bt* crops planted by growers in KZN was in 1999, although *Bt* technology was first introduced into South Africa in 1997. The slow adoption of *Bt* maize in South Africa was also observed by Gouse *et al*. (2005). The authors suggested that the reason for the late adoption of *Bt* maize by South African commercial scale growers was that the yield benefit of *Bt* maize was not sufficient to make up for the technology fee charged by seed companies. The authors further reasoned that the slow acceptance of GM *Bt* technology by growers was their concern about consumers not purchasing their crops due to public misconceptions about GM food safety (Gouse *et al*., 2005).

The results of the survey indicates that the majority of growers (96.6%) plant the 5% unsprayed refugia in the strip configuration (96.4%. Graph 2), which is in accordance with the Christiana and Bothaville (South Africa) surveys conducted by Kruger *et al*. (2012) where 95% of farmers in Bothaville chose the strip configuration. The respondent who chose the 20% option cited ‘easy to use’ as the reason for their choice and the perimeter configuration was chosen for the same reason. The survey results also indicate that the majority of maize grown in KZN is by commercial scale farmers (96.9%) which are also consistent with the findings of a survey conducted by Gouse *et al*. (2009).

In the present study, farmers in KZN state that *Bt* maize is planted primarily for better insect management (75%) and ease of use (32.2%) rather than for yield benefits (Graph 3). This data is consistent with a grower survey in Ontario, Canada conducted by Powell *et al*. (1999) in which 56% of growers planted *Bt* maize to control *O. nubilalis* (European corn borer). This highlights the recurrent problem that growers have in controlling borer pests (Hutchison *et al*., 2010).

The lowest percentage of cumulated borer infestation in Graph 4 was observed in the 20% block (sprayed; 8.66% damage) and 20% seed mixture (sprayed; 5.17% damage). This indicates that growers are not well informed about the benefit of planting either seed mixtures or the 20% (sprayed) refugia options since only one respondent (3.4%) chose to plant the 20% option. The lack of knowledge pertaining to seed mixtures was highlighted again in the survey when the majority of growers chose ‘no’ (75%) and ‘not sure’ (6.3%) when asked about their preference in the planting of seed mixtures in future instead of the five percent and 20% refugia. This is not surprising since *Bt* seed mixtures are not yet available commercially in South Africa. Generally new technology is met with sceptism and adoption of such technology takes time (Kunert, 2011). This may also account for the negative response to seed mixture technology. According to a survey conducted by Alexander (2007), growers in Indiana, USA were exposed to training workshops prior to the dissemination of the survey to demonstrate the importance of planting refugia. The author concluded that the training programs were successful in both explaining the purpose of refugia and highlighting the advantages of IRM strategies (Alexander, 2007). Gouse *et al*. (2006) and Gouse (2012) reported that in South Africa *Bt* technology was first introduced to small scale and subsistence farmers by the agrochemical and seed company Monsanto (patent owner of the *Bt* trait which is licensed to other seed companies in South Africa). However the uptake of Yieldgard™ technology was slow in the rural farming communities, therefore in 2001, Monsanto Company hosted training workshops in nine rural areas in South Africa (Mpumalanga, KwaZulu-Natal, Eastern Cape and Limpopo) (Gouse *et al*., 2006; Gouse, 2012). The authors confirmed that the workshops were directed to dry land maize communal farmers and the purpose was to create *Bt* product awareness hence potential growers were provided with 250 grams of free *Bt* Yieldgard™ seed (Gouse *et al*., 2006; Gouse, 2012). Consequently, the present study highlights that prior to commercialization of seed mixtures in South Africa, extensive product workshops and training programs need to be instituted in order to get favourable responses from growers.

The survey results also indicated that three growers (9.4%) did not plant the mandatory refuge area due to the fact that they only planted HT crops which do not require the planting of a refuge area; consequently their responses regarding refugia were excluded from data analysis. Also, the current survey confirmed that there were 7 (24.1%) instances of initial incorrect planting of refugia and the absence of refugia entirely. Moreover, the reasons cited for non-compliance with refugia planting requirements were 'management crisis' (14.3%), 'were not told to plant a refuge area' (14.3%), 57.1% 'incorrectly planted' and one respondent did not cite a reason. A similar example of incorrect and missing refugia planting was reported by Kruger *et al*. (2009) and Kruger *et al*. (2012). The authors stated that 7% and 15% of farmers in Hoopstad and Reitz, respectively, did not comply with the high dose refuge requirements by incorrectly planting refugia on the corners of square fields. Kruger *et al*. (2009) and Kruger *et al*. (2012) also found that in the areas of Christiana and Hoopstad (*Bt* maize fields only), only ten and 22% of farmers, respectively, complied with the refuge requirements in the 1998 planting season. Furthermore, in the 2010 planting season 20% and 16% of farmers in Christiana and Standerton RSA respectively refrained from planting any refuge area. The reason given by the farmers for the low adherence to the refuge requirement was intensive labour costs and time restrictions (Kruger *et al*., 2009; Kruger *et al*., 2012). Andow *et al*. (2010) reported that due to time constraints, farmers in Minnesota USA did not comply with regulations and planted random patterns of refuge. According to Bourguet *et al.* (2005) complete adherence and compliance to refuge regulations have resulted in further costs to farmers since there are yield losses due to European corn borer damage in the refuge area. Estimated yield losses of 20 to 25% have been reported which consequently leads to low farmer compliance to refuge planting requirements. However, the current survey shows that *Bt* maize growers in South Africa are now 100% compliant with refuge planting requirements which was also confirmed by Kruger *et al*. (2012) where there was a steady increase in the level of compliance in the Hoopstad, Bothaville, Reitz and Delmas regions in RSA until a level of 100% was achieved in the 2009/2010 growing season for *Bt* maize.

The current survey indicated that there were 7 (21.9%) reported incidents of insect borer infestation observed by 32 *Bt* maize growers over the years. Four out of the 7 cases (57.1%) where borer damage was recorded in *Bt* maize, were from respondents that did not comply with refuge planting requirements. This incorrect refuge planting and non-compliance may have resulted in insect borer infestation in *Bt* maize similarly to what was also suggested by Vacher *et al*. (2005), Christou *et al*. (2006), Tabashnik (2008) and Kunert (2011). The cases in which borer infestation was observed were isolated and limited to Winterton and Bergville in KZN with the first occurrence of borer damage observed in 2010 and a decrease in the incidents over the years, indicating possibly *Bt* trait failure or incorrect planting of refugia in the earlier years.

One of the limitations of the survey in this study was the inclusion of GM *Bt* growers in KwaZulu-Natal province only. Possible future research could encompass more maize-growing regions within South Africa which would give a broader understanding of compliance with refugia planting requirements and insect borer resistance monitoring.

5.2 Refuge option and configuration – effect on the levels of insect damage, yield and cost-benefit ratio

<u>Insect damage</u>

The results from the single growing season using Cry1Ab *Bt* maize seed showed that there was significant insect borer activity and damage on the *Bt* plants (Table 8) especially in the 5% seed mixture (14.58% damage), 5% strip (14.57% damage) and the 20% strip (12.97% damage) refuge treatments (Graph 4). Scouting for such damage over the course of the trial necessitated the application of insecticide as is the conventional practice in commercial scenarios. There were no significant differences in percentage damage between the sprayed (19.16% damage) and unsprayed (21.78% damage) controls over the five month period. This implies that the sprayed foliar insecticide applications were not effective in controlling borer activity especially with the mature maize plants. This was also observed in the study conducted by Gunewardena and Madugalla (2011) where the efficacy of granular insecticides proved better than liquid foliar insecticides in controlling the damage caused by *C. partellus*. The authors further noted that using sprayed insecticides especially on mature maize was problematic since there was no complete coverage of the insecticide on the plants. Also, in mature maize, stem borers would have already tunneled into the stems and ears, rendering sprayed insecticides ineffective in controlling stem borers (Tabashnik *et al*., 2001; Gunewardena and Madugalla, 2011).

The field trial results indicated that the use of seed mixtures especially the 20% seed mixture (5.17% damage) proved successful in limiting the damage caused by insect borers (Graph 4). Seed mixtures involve *Bt* and non-*Bt* seeds mixed in the same bag without the need to construct defined refuge areas (Andow, 2008; Head and Greenplate, 2012). This technology allows for randomization of *Bt* and non-*Bt* seeds (in different pre-determined ratios) planted within the same field (Manjunath, 2012). Manjunath (2012) and Muralimohan and Srinivasa (2011) suggested that in countries such as the USA, Australia and South Africa refuge planting is mandated while in India farmers opt not to citing extensive yield loses for their non-compliance. According to Onstad *et al*. (2011) though, the use of seed mixtures as an IRM strategy is problematic since scouting for insect damage becomes difficult without defined refuge zones whereas using a block configuration has clearly outlined *Bt* portions and refuge areas. The authors further stated that scouting for insect damage is an important aspect in IRM and the only way to monitor whether seed mixture refugia are complying with the 5% and 20% requirements is to use on-site GM test strips, which are very expensive to purchase and are not practical to use on large cultivated areas. In addition, a field study conducted by Agi *et al*. (2001) confirmed that blended *Bt* and non-*Bt* cotton seeds proved unsuccessful in limiting the bollworm damage in their cotton fields. The authors noted extensive boll damage and yield loss and predicted that seed mixtures for controlling bollworm infestation (and other mobile insects as part of an IRM strategy) in cotton was not acceptable (Agi *et al*., 2001). However, the positive feedback from seed mixtures is that the onus of planting non-*Bt* refugia is removed from the grower and is redirected to seed companies since grower compliance is generally low and difficult to monitor (Muralimohan and Srinivasa, 2011). A survey study conducted by Alexander (2007) indicated that two thirds of Indiana-USA *Bt* maize growers preferred to plant seed mixtures instead of any of the four refuge configurations due to ease of planting. In addition, further research using seed mixtures with pyramided two-toxin *Bt* maize seeds (Agi *et al*., 2001; Gryspeirt and Grégoire, 2012) could be investigated so as to offer more information on insect pest management.

Yield

The yields in 60% of the treatments in this study were within the published yield range of 8000 to 9000 Kg/Ha obtained by ARC – GCI (2013) (Table 10). Wu (2006) and Mazzoni *et al*. (2010) observed that the infestation of maize by stem boring larvae promoted fungal infection by *Fusarium* species, a fungal pathogen, and the movement of the larvae provided a method of dispersal for the fungal spores. Dowd (2001) reported that mycotoxins produced by ear moulds are responsible for maize yield losses of hundreds of millions of USA dollars each year. Fungal rot is caused by opportunistic pathogens and are responsible for secondary infection usually entering the stem through larval borings (Dowd, 2001). It is highly likely that the maize crop in this study was exposed to secondary fungal pests which are opportunistic pathogens which may have infected already compromised (insect borer infested) plants, thereby compounding the yield reduction in some of the treatments. Although fungal pathogens also occur in non-*Bt* maize, generally more insect damage does result in higher levels of *Fusarium* and the presence of mycotoxins (Wu, 2006).

Experiments conducted by Dowd (2001) and Saladini *et al*. (2008), confirmed that by reducing the activities of maize borers using insecticides (although not economically viable), there was a reduction in the levels of *Fusarium* sp. found on harvested maize kernels. Mycotoxins, produced by a range of fungi, are carcinogenic and harmful to humans when ingested. Other fungal pathogens such as common rust (*P. sorghi*) and Northern corn leaf blight (*S. turcica*) were observed on the leaves of the maize plants during random scouting inspections conducted in this study. Yield losses of 23% were reported in Western Ethiopia and 60% in Uganda arising from the infections of common rust and Northern corn leaf blight (Fininsa and Yeun, 2001). Welz and Geiger (2000) inferred that Northern corn leaf blight can cause damage ranging from minor lesions on the maize leaves to severe foliar damage. Future work could include evaluation of the extent of secondary infection by fungal pathogens in *Bt* maize on yield.

<u>Cost-benefit ratio</u>

Cost-benefit calculations are useful as an indicator in determining whether adoption of technologies or strategies could be implemented in agriculture as was the case in the study conducted by Mudombi (2010). The author conducted an ex ante economic investigation to determine the benefit of adopting GM cassava (*Manihot esculenta*) (Crantz) (Malpighiales: Euphorbiaceae) in South Africa. The cost-benefit study concluded that planting GM cassava was not as lucrative compared with maize or potato (*Solanum tuberosum* L.) (Solanales: Solanaceae) in South Africa. Furthermore, Flannery *et al*. (2004) used cost-benefit analysis to predict the economic profitability of GM crops compared with the same non-GM types in Ireland. The cost-benefit investigation found that cultivation of GM sugar beet (*Beta vulgaris* L.) (Caryophyllales: Amaranthaceae), barley and wheat would be economically beneficial than the non-GM crops based on data from the 2002 and 2003 growing seasons in Ireland. In this study, the most cost effective treatment was the unsprayed control (ZAR 9.44 return for ZAR 1 spent by the farmer), followed by two of the unsprayed 5% configurations, namely the block (ZAR 8.48 return for ZAR 1 spent by the farmer) and perimeter (ZAR 7.96 return for ZAR 1 spent by the farmer). These findings highlighted the cost of manual operations for spraying in the cost-benefit calculations, where the most economical treatments were the ones where no insecticide was applied (Graph 5). However, as evidenced by the results of the survey, the reasons growers choose to cultivate *Bt* maize is also influenced by insect management and ease of use of the insect management option. In contrast, a study conducted by Livingston *et al*. (2004) on *Bt* cotton concluded that the sprayed refugia were more economically viable but this was dependent on yield obtained from the unsprayed refugia. These authors further stated that using their mathematical model, the yield obtained from their unsprayed refugia was less than yield for the sprayed plots (Livingston *et al*., 2004). They also suggested that a reduction in the unsprayed refuge size from 5 to 2% and a decrease in the sprayed refuge size from 20% to 16% could increase profit margins (Livingston *et al*., 2004). In addition, that investigation did not use manual labour for insecticide application which could be an additional cost. These two factors i.e. poor yield and the cost of manual application of insecticides could have impacted upon the cost-benefit ratios obtained in the present study.

Area-wide borer control in the USA (Hutchinson *et al*., 2010) is another reason why it is considered advantageous to plant *Bt* maize as target pest numbers are reduced even in non-*Bt* maize grown in surrounding areas. The researchers suggested that insect pests such as *O. nubilalis* (*Bt* maize – USA), *P. gossypiella* (*Bt* cotton – USA) and *H. armigera* (*Bt* cotton – China) populations were suppressed using *Bt* plantings and insect control was also seen in the surrounding non-*Bt* fields (Hutchinson *et al*., 2010).

<u>Five (5) versus twenty (20) percent refugia and configurations</u>

The field trial data for the combined 5 and 20% refugia for mean yield and total number of damaged plants showed no significant differences (Table 11). The cost-benefit ratio data however indicates that the 5% refuge, regardless of configuration, was significantly more economical than the 20% option. This may have been due to the 3 insecticide applications required to control stem borer in the 20% plots. The costs involved with both labour and the purchase of insecticide which resulted in the lower cost-benefit values in the 20% plots. In addition, the mean ear damage data indicates that the 20% (2.65% mean ear damage) refuge planted in the perimeter configuration was better than the 5% perimeter (10.86% mean ear damage) approach (Table 11). This may have been due to the absence of insecticide application in the 5% perimeter plots.

5.3 Managing borer resistance

Based on studies conducted in RSA by Van Rensburg (1999; 2001; 2007), Kruger *et al*. (2011), Campagne *et al*. (2013) and Van den Berg *et al*. (2013), *B. fusca* has developed resistance to Cry1Ab *Bt* maize and the single-toxin *Bt* maize trait is no longer able to control this stem borer in certain areas (Van den Berg *et al*., 2013). Insect resistance management using a single Cry gene may be less effective compared with two-toxin or pyramided *Bt* seed due to the way in which insects evolve resistance to either insecticides or *Bt* toxins (Roush, 1998).

Manyangarirwa *et al*. (2006) and Andow (2008) have suggested the use of gene stacking to control stalk borers since each of the Cry genes in the GM plant is independently toxic to the insect. The idea behind gene stacking is that the different Cry genes will provide a greater protection against more insect pests (Head and Greenplate, 2012). Additionally the target pest is less likely to develop a single mechanism of resistance that will allow for tolerance to two proteins at the same time therefore the proteins selected for use in gene stacking need to have different modes of action against the insect pest (Roush, 1998; Jurat-Fuentes *et al*., 2003; Head and Greenplate, 2012; Gryspeirt and Grégoire, 2012). Gryspeirt and Grégoire (2012) also suggested that the use of pyramided *Bt* crops allow for a reduction in refuge size compared with non-pyramided *Bt* crops and in order to maintain efficacy of the single-toxin *Bt* crops the dose needs to be high whereas the two-toxin approach can have one toxin at a lower dose. Furthermore, Gryspeirt and Grégoire (2012) and Van den Berg *et al*. (2013) stated that internationally the trend of using two-toxins is being accepted by multinational developers because of the decreased risk and the delayed rate of development of resistance compared with the single gene approach.

In order to determine whether *B. fusca* and *C. partellus* developed field-evolved resistance to single-toxin *Bt* maize, many successive growing seasons need to be planted and this could be investigated in a possible future study. Recurring susceptibility and ultimately the development of resistance of insect pests is measured using laboratory bioassays that involves the exposure of the insect larvae to food sources containing *Bt* toxins (Tabashnik *et al*., 2008). Bates *et al*. (2005) suggested that accidental insect resistance may also be possible due to the pollen transfer from *Bt* crops to non-*Bt* crops creating a low *Bt* toxin presence in the second generation of non-*Bt* crops. This low dose *Bt* toxin is not sufficient to kill otherwise HDR single-toxin *Bt* susceptible insect types (Bates *et al*., 2005).

Van den Berg *et al*. (2013) confirmed that the presence of *Bt* resistant *B. fusca* observed over the last three years in the Vaalharts region of RSA highlights the miscalculation in the early mathematical models designed to predict the emergence of *Bt* insect resistance. The authors further noted that seed companies, research institutes and the government need to work together to develop resistance monitoring and HDR compliance protocols in order to delay further development of resistance and to report on possible *Bt* crop failure. This concept was reported before by Tabashnik *et al*. (2009), in which the large-scale failure and retraction of *Bt* maize event Cry1F susceptible to *S. frugiperda* occurred and was subsequently the quickest resistance reported in the field and became the first instance of a *Bt* event to be retracted from commercial sales. However Gassmann *et al*. (2009) suggested that the reason insect pests like *H. zea* have not caused more *Bt* (Cry1Ac) cotton crop failure throughout the world is due to the widespread control of this pest using stacked gene (Cry2Ab and Cry1Ac) *Bt* cotton and continued insecticide spraying. Additionally, retraction of a GM event may not always be warranted since the particular trait might still be useful against other insect pests (Van den Berg *et al*., 2013).

De Villiers and Hoisington (2011) and Bates *et al*. (2005) found that the emergence of field resistance in GM technology has been lower than expected. They further postulated that the slow semblance of insect resistance may be due to three factors namely: (a) the fitness costs to non-susceptible types, in particular the individuals developed in the laboratory, which do not survive in the wild; (b) the limited number of resistant alleles and; (c) the mating of resistant individuals with susceptible types which creates more heterozygous resistant alleles and the high dosage of *Bt* toxin released by *Bt* crops. The authors also suggested that insect resistance development was further delayed by the advent of refuge strategy on the larger scale farms and by the proper use of non-*Bt* host plants in small holder farms.

In an effort to increase farmer compliance to GM refuge planting requirements, DAFF and seed companies in South Africa established that prior to planting of *Bt* crops, it is mandatory to sign an agreement between the seed company and the potential grower (Bates *et al*., 2005; Bourguet *et al*., 2005). The agreement specifies the allowed configuration types and refuge requirements (Bourguet *et al*., 2005). Bourguet *et al.* (2005) further suggested that there should be a continuance of grower education programs designed to inform new and current GM growers. The authors also emphasized the importance of investigation by authorities and departments such as the US EPA and RSA DAFF of suspected non-compliance cases, the identification of non-conforming growers and possible repercussions.

Kruger *et al*. (2009) and Kunert (2011) further projected that in order to delay future potential insect resistance, there needs to exist a greater compliance to planting requirements and refuge strategies. This could be achieved by increasing communication among extension officers, farmers and seed companies. Current resistance levels also need to be closely monitored and reported accordingly. It was proposed that instead of the current highlighting of spatial distances in refugia, spatial configurations of *Bt* and non-*Bt* crops be contemplated as a means of insect resistance management (IRM) (Bourguet *et al*., 2005; Kruger *et al*., 2009). Another consideration in IRM is the implementation of different modes of action of *Bt* traits (Kruger *et al*., 2009).

Chapter Six

6 Conclusions

It can be concluded from the field trial results obtained in the current study that mean yield benefits and mean insect damage on *Bt* plants were no different for either the 5 or 20% refugia in any of the three configurations namely strip, block and perimeter. However, from the cost-benefit comparisons, the 5% refugia seemed more cost effective than the 20% configurations due to the use of insecticide in the latter refuge treatment. This economic advantage offered by the 5% refugia was also reflected in the survey results where 96.6% of KZN *Bt* maize growers were shown to have planted the 5% option. The 20% perimeter option incurred less ear damage compared with the 5% perimeter option although ease of planting may prevent adoption of such a planting configuration.

The 20% seed mixture refuge showed less damage than the sprayed control and may be a viable refuge option due to ease of planting. However, if ease of planting by growers is to be considered as a motivating factor for adopting this option prior to commercialization in South Africa, the advantages and limitations of this technology need to be highlighted and communicated to growers by seed companies.

Survey results obtained in this study suggested that incorrectly planted and missing refugia in the initial stages of *Bt* maize adoption may have contributed to incidence of insect borer damage. Therefore stricter compliance monitoring methods by RSA DAFF and seed companies need to be established in order to limit further insect damage and resistance. Continuous monitoring, coupled with feedback communication, is likely to compel growers to comply with refugia planting requirements. The suggestion from this study is that RSA DAFF needs to establish a panel consisting of representatives from seed companies, entomologists, agricultural scientists and officials from RSA DAFF that conduct random inspections aimed at determining grower compliance and the monitoring of insect borer resistance. Insect borer surveillance, growing of *Bt* maize field trials (by the panel) and grower directed surveys will provide the salient data that could trace the evolution of insect resistance within the maize regions in South Africa and predict future patterns of resistance. This information can be filtered to growers who can implement the suggested strategies within the specified time allocation. The success of such a panel is highly dependent on open communication between all the involved stakeholders and the willingness of growers to adopt the measures stipulated by the panel.

6.1 Possible future research on planting refugia

<u>GM *Bt* maize farmer directed surveys in the future could encompass the following parameters</u>:

- The current study showed that in the particular survey area, the total area planted with GM *Bt* crops in KZN is 36.6%. Future surveys could show whether this figure changes over time. Since the evolution of pest resistance to *Bt* maize may negatively impact on grower preference for use of *Bt* technology especially due to the fact that the majority of growers in KZN stated that planting *Bt* maize was to control borer damage.

- The present survey indicates that growers favoured the *Bt*/HT stacked gene varieties (68.75%) however it would be interesting to determine whether growers who opt for two-toxin pyramided genes crops record a beneficial rate of insect control.

<u>Future *Bt* maize field trials could include</u>:

- The inclusion of stem borer population counts so as to fully ascribe crop damage observed in the field to stem borer infestation. Stem borer population counts were not conducted in the present study since it involved the use of destructive sampling but will be considered for future work.
- Using seeds with pyramided insecticidal traits.
- Establishing the yield losses due to fungal colonization.
- The use of pyramided seed mixtures and trials over several seasons to monitor insect borer resistance and management thereof.

Chapter Seven

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Appendices

1 Ethical clearance for survey and field trial research

UNISA university of south africa

2013-01-23

Ref. Nr.: 2012/CAES/031

To:
Student: Ms O Moodley **Student nr: 48109711**
Supervisor: Prof D Modise
Department of Agriculture and Animal Health
College of Agriculture and Environmental Sciences

Dear Prof Modise and Ms Moodley

Request for Ethical approval for the following research project:

An investigation of refugia configurations implemented in insect resistant genetically modified maize (Zea mays) by means of a farmer survey and field trial analyses

The application for ethical clearance in respect of the above mentioned research has been reviewed by the Research Ethics Review Committee of the College of Agriculture and Environmental Sciences, Unisa. Ethics clearance for the above mentioned project (Ref. Nr.: 2012/CAES/031) **is granted** after careful consideration of all documentation and submitted to the CAES Ethics committee. The committee suggests the use of pseudo names instead of company names to protect the identify and anonymity of the companies involved. A project number was issued from the Department of Forestry and Fisheries to indicate the approval of the project.

Please be advised that the committee needs to be informed should any part of the research methodology as outlined in the Ethics application (Ref. Nr.: 2012/CAES/031), change in any way. In this instance a memo should be submitted to the Ethics Committee in which the changes are identified and fully explained.

We trust that sampling, data gathering and processing of the relevant data will be undertaken in a manner that is respectful of the rights and integrity of all participants, as stipulated in the UNISA Research Ethics Policy.

The Ethics Committee wishes you all the best with this research undertaking.

Kind regards,

Prof E Kempen,
CAES Ethics Review Committee Chair

University of South Africa
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2 Survey questionnaire

2.1 Survey participation consent form

To whom it may concern

I ....the undersigned (the owner/manager of the below farm/plot), hereby grant Miss Odeshnee Moodley permission to carry out trials on the above mentioned farm/plot using a questionnaire for the purpose of her Master of Science in Agriculture studies with the University of South Africa. Miss Moodley has undertaken not to reveal your name, your farm name or the name(s) of your employee(s) in her dissertation as well as to protect your **privacy and confidentiality**. I understand that my participation is **voluntary** and that I may withdraw from the research at any time, in writing, without giving a reason. I also understand that there are no risks involved in the participation of this study and I agree to participate.

Disclaimer: This research questionnaire has been designed in accordance with UNISA's policy on Research Ethics.

Name of farm/plot: ....

Name of farm owner: ....

Signature: ....

Date: ....

Name of witness: ....

Signature of witness: ....

Date: ....

2.2 Survey cover letter

Dear colleague

I am a part-time MSc. Student with the Department of Agriculture and Animal Health, University of South Africa (UNISA). My field of interest is **genetically modified (GM) crops and refugia configurations** used in insect resistant genetically modified maize (*Zea* mays). South Africa is one of few countries planting GM crops in which insect resistant (*Busseola fusca*-maize stem borer) has been found in the field. One reason for this is thought to be non-compliance with refuge requirements. The enclosed questionnaire aims to evaluate refuge planting practices by growers of GM maize. This survey will also be used to determine which refuge configuration: perimeter, block, split planter /strip or separate field/adjacent patterns, is most frequently used by farmers and why. It will be appreciated if you could spend ten minutes to answer the survey questionnaire as honestly as possible keeping in mind that the answers will be kept strictly confidential. Please forward all completed survey questionnaires either via electronic or postal mail using the details below before the end of August 2013. The respondents wishing for feedback from the survey and the field trial study where different refuge options will be planted should apply at the end of the study directly in writing to the researcher using the details listed below. Please note that study is proposed to conclude in October 2013 and applications should be made accordingly. Results of the study will be made available to the respondents upon request in November and December 2013 in writing.

Researcher: Odeshnee Moodley

Postal Address: 20 Orleans Place

Reservoir Hills

4091

E-mail: odeshnee.moodley@gmail.com

2.3 Question guide

Please note that this questionnaire is intended for farmers/growers planting **GM crops only**. Please indicate your choice of response by ticking the relevant box(es). Should you wish to further elaborate your response please do so in the space below the answer box. If your response for question 9.2 is “no” then please proceed directly to question 10.1.

2.4 Questionnaire:

<u>Title: Refugia: grower preference/response and compliance to GM maize planting</u>

1. What is your farm/plot name or number? If more than one please list all.

____

____

____

2. Where is your farm(s) located? Please indicate the city and/or province of all.

____

____

____

3. Do you grow GM crops? Please tick the relevant box.

☐ Yes

☐ No

4. In what year/growing season did you first start planting GM seeds?

____

____

5. What is the estimated size of your farm? Please answer in hectares (Ha).

____

____

6. How much of your farm is planted to GM maize? Please answer in hectares (Ha).

____

____

7. Which of the following scales/sizes best applies to your farm?

☐ Communal scale

☐ Commercial scale

8. Which GM crops do you grow? Please tick the relevant box(es).

☐ *Glycine max* (soybean)

☐ *Zea mays* (maize)

☐ *Gossypium hirsutum* (cotton)

9.1 What type of GM technology have you been using? Please tick the box(es).

- ☐ Insect resistance (*Bt*)
- ☐ Herbicide tolerance (HT)

9.2 Do you plant a non-GM refuge? Please tick the relevant box.

- ☐ Yes
- ☐ No

9.3 If no, why? Please proceed to Question 10.1

____

____

____

9.1 Have you always planted a non-GM refuge? Please tick the relevant box.

- ☐ Yes
- ☐ No

9.2 If yes, in which year/growing season did you first plant the non-GM refuge?

____

____

9.3 If no, why?

____

____

9.4 Which refuge option do you plant? Please tick the relevant box(es).

- ☐ 5% unsprayed non GM refuge
- ☐ 20% sprayed non GM refuge

9.5 Why did you choose the above option? Please tick the relevant box(es).

- ☐ Cheaper option
- ☐ Better insect management
- ☐ Easy to use
- ☐ Better yield

9.6 If you use the 5% unsprayed option, which of the four refuge configurations do you plant? Please tick the relevant box(es).

- ☐ Perimeter
- ☐ Block
- ☐ Split planter/strip
- ☐ Separate field/adjacent

9.7 What is your reason for planting this configuration? Please tick the relevant box(es).

- ☐ Cheaper option
- ☐ Better insect management
- ☐ Easy to use
- ☐ Better yield

9.8 If you use the 20% sprayed option, which of the four refuge configurations do you plant? Please tick the relevant box(es).

- ☐ Perimeter
- ☐ Block
- ☐ Split planter/strip
- ☐ Separate field/adjacent

9.9 What is your reason for planting this configuration? Please tick the relevant box(es).

- ☐ Cheaper option
- ☐ Better insect management
- ☐ Easy to use
- ☐ Better yield

10.1 Have you observed any insect or borer damage on your GM maize? Please tick the relevant box.

☐ Yes

☐ No

10.2 If so, in which year/growing season did you first observe this damage?

____

____

____

11. Would you prefer to use refuge in a bag/seed mixtures (a mixture of *Bt* and non-*Bt* maize) in the future, instead of the 5% and 20% refuge options?

☐ Yes

☐ No

☐ Not sure

Thank you for your participation

3 Initial and final plant counts for *Bt* maize field trial

Repetition number	Plot number	Treatment	RR plant count	*Bt* plant count	Final plant count
1	1	Control (unsprayed)	313	0	254
1	2	Control (sprayed)	331	0	172
1	3	5% Block (unsprayed)	15	320	253
1	4	20% Perimeter (sprayed)	61	273	168
1	5	5% Seed mixture (sprayed)	16	316	237
1	6	5% Perimeter (unsprayed)	17	319	132
1	7	20% Seed mixture (sprayed)	61	269	222
1	8	20% Block (sprayed)	67	269	215
1	9	20% Strip (sprayed)	58	286	236
1	10	5% Strip (unsprayed)	17	328	228
2	11	20% Perimeter (sprayed)	69	278	230
2	12	Control (unsprayed)	335	0	190
2	13	5% Strip (unsprayed)	18	310	241
2	14	5% Seed mixture (sprayed)	19	314	277
2	15	5% Perimeter (unsprayed)	16	315	289
2	16	20% Block (sprayed)	67	260	247
2	17	Control (sprayed)	371	0	335
2	18	20% Seed mixture (sprayed)	56	277	309
2	19	5% Block (unsprayed)	15	304	303
2	20	20% Strip (sprayed)	67	267	282
3	21	5% Perimeter (unsprayed)	18	307	303
3	22	20% Strip (sprayed)	65	256	284
3	23	5% Seed mixture (sprayed)	13	306	287
3	24	20% Seed mixture (sprayed)	64	248	277
3	25	20% Perimeter (sprayed)	66	267	285
3	26	Control (sprayed)	346	0	280
3	27	5% Strip (unsprayed)	17	259	221
3	28	5% Block (unsprayed)	17	319	288
3	29	Control (unsprayed)	329	0	276
3	30	20% Block (sprayed)	69	259	283

4 Accumulative plant lodging counts over five inspection intervals

Repetition number	Plot number	Treatment	10 January	13 February	19 March	11 April	29 April
1	1	Control (unsprayed)	15	22	0	0	0
1	2	Control (sprayed)	29	35	0	0	0
1	3	5% Block (unsprayed)	18	16	0	0	0
1	4	20% Perimeter (sprayed)	11	21	0	0	0
1	5	5% Seed mixture (sprayed)	8	15	0	0	0
1	6	5% Perimeter (unsprayed)	28	19	0	0	0
1	7	20% Seed mixture (sprayed)	1	3	0	0	0
1	8	20% Block (sprayed)	6	9	0	0	0
1	9	20% Strip (sprayed)	3	4	0	0	0
1	10	5% Strip (unsprayed)	4	8	0	0	0
2	11	20% Perimeter (sprayed)	6	5	0	0	0
2	12	Control (unsprayed)	8	6	0	0	0
2	13	5% Strip (unsprayed)	7	0	0	0	0
2	14	5% Seed mixture (sprayed)	3	9	0	0	0
2	15	5% Perimeter (unsprayed)	2	1	0	0	0
2	16	20% Block (sprayed)	1	1	0	0	0
2	17	Control (sprayed)	2	3	0	0	0
2	18	20% Seed mixture (sprayed)	6	4	0	0	0
2	19	5% Block (unsprayed)	3	0	0	0	0
2	20	20% Strip (sprayed)	9	7	0	0	0
3	21	5% Perimeter (unsprayed)	3	2	0	0	0
3	22	20% Strip (sprayed)	5	2	0	0	0
3	23	5% Seed mixture (sprayed)	0	0	0	0	0
3	24	20% Seed mixture (sprayed)	1	5	0	0	0
3	25	20% Perimeter (sprayed)	1	1	0	0	0
3	26	Control (sprayed)	9	2	0	0	0
3	27	5% Strip (unsprayed)	2	0	0	0	0
3	28	5% Block (unsprayed)	4	3	0	0	0
3	29	Control (unsprayed)	2	2	0	0	0
3	30	20% Block (sprayed)	1	2	0	0	0

5 Accrued pig damage counts

Repetition number	Plot number	Treatment	13 February	19 March	11 April	29 April
1	1	Control (unsprayed)	0	7	12	26
1	2	Control (sprayed)	2	7	82	88
1	3	5% Block (unsprayed)	1	2	26	36
1	4	20% Perimeter (sprayed)	11	8	93	123
1	5	5% Seed mixture (sprayed)	0	36	45	67
1	6	5% Perimeter (unsprayed)	8	73	94	114
1	7	20% Seed mixture (sprayed)	7	48	65	84
1	8	20% Block (sprayed)	1	49	67	79
1	9	20% Strip (sprayed)	1	55	74	65
1	10	5% Strip (unsprayed)	1	46	43	63
2	11	20% Perimeter (sprayed)	1	37	64	80
2	12	Control (unsprayed)	5	68	75	110
2	13	5% Strip (unsprayed)	5	27	35	45
2	14	5% Seed mixture (sprayed)	3	16	23	45
2	15	5% Perimeter (unsprayed)	1	19	17	32
2	16	20% Block (sprayed)	1	15	16	34
2	17	Control (sprayed)	0	4	6	14
2	18	20% Seed mixture (sprayed)	0	7	5	12
2	19	5% Block (unsprayed)	1	9	9	38
2	20	20% Strip (sprayed)	0	16	28	23
3	21	5% Perimeter (unsprayed)	0	7	7	16
3	22	20% Strip (sprayed)	1	8	21	33
3	23	5% Seed mixture (sprayed)	3	22	34	40
3	24	20% Seed mixture (sprayed)	0	21	26	27
3	25	20% Perimeter (sprayed)	8	33	30	45
3	26	Control (sprayed)	15	31	27	30
3	27	5% Strip (unsprayed)	7	35	48	50
3	28	5% Block (unsprayed)	21	36	40	53
3	29	Control (unsprayed)	7	37	36	48
3	30	20% Block (sprayed)	4	19	25	31

6 Total borer damage counts of leaves and stems for 30 plots over the five inspection intervals

Repetition number	Plot number	Treatment	10 January	13 February	19 March	11 April	29 April
1	1	Control (unsprayed)	30	20	15	6	9
1	2	Control (sprayed)	16	17	4	14	9
1	3	5% Block (unsprayed)	16	18	7	4	7
1	4	20% Perimeter (sprayed)	22	7	6	2	1
1	5	5% Seed mixture (sprayed)	20	21	9	13	10
1	6	5% Perimeter (unsprayed)	9	10	0	1	0
1	7	20% Seed mixture (sprayed)	7	7	0	0	1
1	8	20% Block (sprayed)	21	8	4	0	2
1	9	20% Strip (sprayed)	20	10	3	0	4
1	10	5% Strip (unsprayed)	27	21	7	9	10
2	11	20% Perimeter (sprayed)	14	21	3	2	0
2	12	Control (unsprayed)	23	21	5	1	5
2	13	5% Strip (unsprayed)	13	10	7	9	5
2	14	5% Seed mixture (sprayed)	19	16	10	2	3
2	15	5% Perimeter (unsprayed)	17	19	14	14	5
2	16	20% Block (sprayed)	14	8	9	8	2
2	17	Control (sprayed)	35	17	22	8	9
2	18	20% Seed mixture (sprayed)	4	4	0	1	1
2	19	5% Block (unsprayed)	28	7	1	6	1
2	20	20% Strip (sprayed)	11	11	8	8	3
3	21	5% Perimeter (unsprayed)	2	3	8	5	2
3	22	20% Strip (sprayed)	12	8	6	8	9
3	23	5% Seed mixture (sprayed)	0	5	3	0	0
3	24	20% Seed mixture (sprayed)	8	7	6	1	1
3	25	20% Perimeter (sprayed)	9	4	0	5	2
3	26	Control (sprayed)	3	12	0	1	3
3	27	5% Strip (unsprayed)	8	4	3	0	0
3	28	5% Block (unsprayed)	3	3	0	0	0
3	29	Control (unsprayed)	20	16	12	3	5
3	30	20% Block (sprayed)	3	2	0	0	1

7 Total plant counts for borer damaged leaves and stems – calculations for 30 plots over five inspection intervals

Repetition number	Plot number	Treatment	10 January	13 February	19 March	11 April	29 April
1	1	Control (unsprayed)	298	276	269	264	250
1	2	Control (sprayed)	302	265	260	185	179
1	3	5% Block (unsprayed)	317	300	299	275	265
1	4	20% Perimeter (sprayed)	323	291	294	209	179
1	5	5% Seed mixture (sprayed)	324	309	273	264	242
1	6	5% Perimeter (unsprayed)	308	281	185	164	144
1	7	20% Seed mixture (sprayed)	329	319	278	261	242
1	8	20% Block (sprayed)	330	320	272	254	242
1	9	20% Strip (sprayed)	340	336	282	263	272
1	10	5% Strip (unsprayed)	341	332	287	290	270
2	11	20% Perimeter (sprayed)	341	335	299	272	256
2	12	Control (unsprayed)	327	316	253	246	211
2	13	5% Strip (unsprayed)	321	316	294	286	276
2	14	5% Seed mixture (sprayed)	330	318	305	298	276
2	15	5% Perimeter (unsprayed)	329	327	309	311	296
2	16	20% Block (sprayed)	326	324	310	309	291
2	17	Control (sprayed)	359	356	352	350	342
2	18	20% Seed mixture (sprayed)	327	323	316	318	311
2	19	5% Block (unsprayed)	316	315	307	307	278
2	20	20% Strip (sprayed)	325	318	302	290	295
3	21	5% Perimeter (unsprayed)	322	320	313	313	304
3	22	20% Strip (sprayed)	316	313	306	293	281
3	23	5% Seed mixture (sprayed)	319	316	297	285	279
3	24	20% Seed mixture (sprayed)	311	306	285	280	279
3	25	20% Perimeter (sprayed)	332	323	298	301	286
3	26	Control (sprayed)	337	320	304	308	305
3	27	5% Strip (unsprayed)	274	267	239	226	224
3	28	5% Block (unsprayed)	332	308	293	289	276
3	29	Control (unsprayed)	327	318	288	289	277
3	30	20% Block (sprayed)	327	321	306	300	294

8 Percentage borer damaged plants for 30 plots over five inspection intervals (vegetative, flowering, soft dough, hard dough and pre-harvest)

Repetition number	Plot number	Treatment	10 January borer damage (%)	13 February borer damage (%)	19 March borer damage (%)	11 April borer damage (%)	29 April borer damage (%)
1	1	Control (unsprayed)	10.07	7.25	5.58	2.27	3.6
1	2	Control (sprayed)	5.30	6.42	1.54	7.57	5.03
1	3	5% Block (unsprayed)	5.05	6.00	2.34	1.45	2.64
1	4	20% Perimeter (sprayed)	6.81	2.41	2.04	0.96	0.56
1	5	5% Seed mixture (sprayed)	6.17	6.80	3.30	4.92	4.13
1	6	5% Perimeter (unsprayed)	2.92	3.56	0	0.61	0
1	7	20% Seed mixture (sprayed)	2.13	2.19	0	0	0.41
1	8	20% Block (sprayed)	6.36	2.5	1.47	0	0.83
1	9	20% Strip (sprayed)	5.88	2.98	1.06	0	1.47
1	10	5% Strip (unsprayed)	7.92	6.33	2.44	3.10	3.70
2	11	20% Perimeter (sprayed)	4.10	6.27	1.00	0.74	0
2	12	Control (unsprayed)	7.03	6.65	1.98	0.41	2.37
2	13	5% Strip (unsprayed)	4.05	3.16	2.38	3.15	1.81
2	14	5% Seed mixture (sprayed)	5.76	5.03	3.28	0.67	1.09
2	15	5% Perimeter (unsprayed)	5.17	5.81	4.53	4.50	1.69
2	16	20% Block (sprayed)	4.29	2.47	2.90	2.59	0.69
2	17	Control (sprayed)	9.75	4.78	6.25	2.29	2.63
2	18	20% Seed mixture (sprayed)	1.22	1.24	0	0.31	0.32
2	19	5% Block (unsprayed)	8.86	2.22	0.33	1.95	0.36
2	20	20% Strip (sprayed)	3.39	3.46	2.65	2.76	1.02
3	21	5% Perimeter (unsprayed)	0.62	0.94	2.56	1.60	0.66
3	22	20% Strip (sprayed)	3.80	2.56	1.96	2.73	3.20
3	23	5% Seed mixture (sprayed)	0	1.58	1.01	0	0
3	24	20% Seed mixture (sprayed)	2.57	2.29	2.11	0.36	0.36
3	25	20% Perimeter (sprayed)	2.71	1.24	0	1.66	0.70
3	26	Control (sprayed)	0.89	3.75	0	0.32	0.98
3	27	5% Strip (unsprayed)	2.92	1.50	1.26	0	0
3	28	5% Block (unsprayed)	0.90	0.98	0	0	0
3	29	Control (unsprayed)	6.12	5.03	4.17	1.04	1.81
3	30	20% Block (sprayed)	0.92	0.62	0	0	0.34

9 Healthy and borer damaged ears recorded at harvest

Repetition number	Plot number	Treatment	Healthy ears	Borer damaged ears	Total number of ears	Percentage ear damage (%)
1	1	Control (unsprayed)	324	5	329	1.52
1	2	Control (sprayed)	210	25	235	10.64
1	3	5% Block (unsprayed)	362	29	391	7.42
1	4	20% Perimeter (sprayed)	225	2	227	0.88
1	5	5% Seed mixture (sprayed)	310	14	324	4.32
1	6	5% Perimeter (unsprayed)	169	30	199	15.08
1	7	20% Seed mixture (sprayed)	288	10	298	3.36
1	8	20% Block (sprayed)	278	9	287	3.13
1	9	20% Strip (sprayed)	284	21	305	6.89
1	10	5% Strip (unsprayed)	323	6	329	1.82
2	11	20% Perimeter (sprayed)	300	25	325	7.69
2	12	Control (unsprayed)	258	17	275	6.18
2	13	5% Strip (unsprayed)	365	19	384	4.95
2	14	5% Seed mixture (sprayed)	369	4	373	1.07
2	15	5% Perimeter (unsprayed)	343	44	387	11.37
2	16	20% Block (sprayed)	385	32	417	7.67
2	17	Control (sprayed)	421	19	440	4.32
2	18	20% Seed mixture (sprayed)	376	23	399	5.76
2	19	5% Block (unsprayed)	342	31	373	8.31
2	20	20% Strip (sprayed)	337	19	356	5.34
3	21	5% Perimeter (unsprayed)	400	25	425	5.88
3	22	20% Strip (sprayed)	377	15	392	3.83
3	23	5% Seed mixture (sprayed)	360	12	372	3.23
3	24	20% Seed mixture (sprayed)	372	7	379	1.85
3	25	20% Perimeter (sprayed)	379	10	389	2.57
3	26	Control (sprayed)	357	6	363	1.65
3	27	5% Strip (unsprayed)	324	5	329	1.52
3	28	5% Block (unsprayed)	382	21	403	5.21
3	29	Control (unsprayed)	349	33	382	8.64
3	30	20% Block (sprayed)	385	19	404	4.70

10 Field mass calculations taken after harvest for 30 plots and used to determine yield

Repetition number	Plot number	Treatment	Mass bag one (Kg)	Mass bag two (Kg)	Mass bag three (Kg)	Mass bag four (Kg)	Total mass or plot mass (Kg)
1	1	Control (unsprayed)	22.25	15.41	15.97	21.15	74.78
1	2	Control (sprayed)	11.93	38.12	N/A	N/A	50.05
1	3	5% Block (unsprayed)	28.26	30.73	19.85	N/A	78.84
1	4	20% Perimeter (sprayed)	32.53	16.59	N/A	N/A	49.12
1	5	5% Seed mixture (sprayed)	17.46	23.30	28.79	N/A	69.55
1	6	5% Perimeter (unsprayed)	19.07	23.51	N/A	N/A	42.58
1	7	20% Seed mixture (sprayed)	18.49	16.49	27.95	N/A	62.93
1	8	20% Block (sprayed)	29.36	25.31	N/A	N/A	54.67
1	9	20% Strip (sprayed)	18.92	21.84	22.21	N/A	62.97
1	10	5% Strip (unsprayed)	30.52	30.92	N/A	N/A	61.44
2	11	20% Perimeter (sprayed)	14.60	17.96	26.67	N/A	59.23
2	12	Control (unsprayed)	30.28	19.01	N/A	N/A	49.29
2	13	5% Strip (unsprayed)	19.96	25.92	20.51	N/A	66.39
2	14	5% Seed mixture (sprayed)	31.00	30.32	N/A	N/A	61.32
2	15	5% Perimeter (unsprayed)	25.58	19.44	24.16	N/A	69.18
2	16	20% Block (sprayed)	29.41	28.05	11.39	N/A	68.85
2	17	Control (sprayed)	25.68	21.26	28.18	N/A	75.12
2	18	20% Seed mixture (sprayed)	44.65	26.78	N/A	N/A	71.43
2	19	5% Block (unsprayed)	25.72	22.42	19.05	N/A	67.19
2	20	20% Strip (sprayed)	28.32	33.17	N/A	N/A	61.49
3	21	5% Perimeter (unsprayed)	22.38	25.16	28.87	N/A	76.41
3	22	20% Strip (sprayed)	37.45	27.70	N/A	N/A	65.15
3	23	5% Seed mixture (sprayed)	20.59	24.31	25.43	N/A	70.33
3	24	20% Seed mixture (sprayed)	29.16	35.72	N/A	N/A	64.88
3	25	20% Perimeter (sprayed)	16.49	19.87	27.74	N/A	64.10
3	26	Control (sprayed)	35.18	27.53	N/A	N/A	62.71
3	27	5% Strip (unsprayed)	32.85	25.22	N/A	N/A	58.07
3	28	5% Block (unsprayed)	22.42	23.21	20.55	N/A	66.18
3	29	Control (unsprayed)	37.19	31.06	N/A	N/A	68.25
3	30	20% Block (sprayed)	30.97	40.25	N/A	N/A	71.22

11 Maize shelling percent, moisture percent and moisture conversion used to calculate yield

Repetition number	Plot number	Treatment	Grain (Kg)	Grain + cob (Kg)	Shelling %	Moisture %	Moisture conversion
1	1	Control (unsprayed)	1.9	2.29	0.83	20.9	0.9
1	2	Control (sprayed)	1.84	2.18	0.84	19.8	0.92
1	3	5% Block (unsprayed)	2.12	2.53	0.84	19.8	0.92
1	4	20% Perimeter (sprayed)	1.77	2.1	0.84	19.8	0.92
1	5	5% Seed mixture (sprayed)	2.2	2.65	0.83	21.2	0.9
1	6	5% Perimeter (unsprayed)	1.95	2.29	0.85	20.7	0.91
1	7	20% Seed mixture (sprayed)	2.17	2.59	0.84	19.7	0.92
1	8	20% Block (sprayed)	1.75	2.07	0.85	20.3	0.91
1	9	20% Strip (sprayed)	1.98	2.35	0.84	20.5	0.91
1	10	5% Strip (unsprayed)	1.86	2.23	0.83	20.3	0.91
2	11	20% Perimeter (sprayed)	1.61	1.92	0.84	19.2	0.92
2	12	Control (unsprayed)	1.97	2.35	0.84	20.2	0.91
2	13	5% Strip (unsprayed)	1.91	2.32	0.82	20.4	0.91
2	14	5% Seed mixture (sprayed)	1.87	2.25	0.83	20.7	0.91
2	15	5% Perimeter (unsprayed)	1.97	2.37	0.83	20.6	0.91
2	16	20% Block (sprayed)	1.78	2.12	0.84	19.9	0.92
2	17	Control (sprayed)	1.74	2.09	0.83	20.3	0.91
2	18	20% Seed mixture (sprayed)	1.95	2.32	0.84	20.2	0.91
2	19	5% Block (unsprayed)	1.68	2.04	0.82	20.9	0.9
2	20	20% Strip (sprayed)	1.85	2.23	0.83	19.8	0.92
3	21	5% Perimeter (unsprayed)	1.95	2.35	0.83	20.4	0.91
3	22	20% Strip (sprayed)	1.78	2.15	0.83	20.8	0.91
3	23	5% Seed mixture (sprayed)	1.87	2.26	0.83	20.8	0.91
3	24	20% Seed mixture (sprayed)	1.86	2.25	0.83	20.2	0.91
3	25	20% Perimeter (sprayed)	1.88	2.25	0.84	20.9	0.9
3	26	Control (sprayed)	1.96	2.33	0.84	20.4	0.91
3	27	5% Strip (unsprayed)	1.81	2.17	0.83	21	0.9
3	28	5% Block (unsprayed)	1.72	2.07	0.83	20.5	0.91
3	29	Control (unsprayed)	1.95	2.35	0.83	20.7	0.91
3	30	20% Block (sprayed)	1.88	2.26	0.83	20.5	0.91

12 Data used to calculate the cost-benefit ratio

Repetition number	Plot number	Treatment	Yield (Tons/ Ha)	Yield in R/Ha	Input cost (R)	Cost-benefit ratio (R)
1	1	Control (unsprayed)	9.31	18620.22	1778	10.47
1	2	Control (sprayed)	6.45	12892.88	2055	6.28
1	3	5% Block (unsprayed)	10.15	20309.18	2018.35	10.06
1	4	20% Perimeter (sprayed)	6.33	12653.31	2257.4	5.61
1	5	5% Seed mixture (sprayed)	8.66	17317.95	2295.35	7.54
1	6	5% Perimeter (unsprayed)	5.49	10978.54	2018.35	5.44
1	7	20% Seed mixture (sprayed)	8.11	16210.77	2257.4	7.18
1	8	20% Block (sprayed)	7.05	14095.75	2257.4	6.24
1	9	20% Strip (sprayed)	8.02	16044.76	2257.4	7.11
1	10	5% Strip (unsprayed)	7.73	15468.54	2018.35	7.66
2	11	20% Perimeter (sprayed)	7.63	15257.65	2257.4	6.76
2	12	Control (unsprayed)	6.28	12559.09	1778	7.06
2	13	5% Strip (unsprayed)	8.26	16513.41	2018.35	8.18
2	14	5% Seed mixture (sprayed)	7.72	15438.33	2295.35	6.73
2	15	5% Perimeter (unsprayed)	8.71	17417.22	2018.35	8.63
2	16	20% Block (sprayed)	8.87	17735.76	2257.4	7.86
2	17	Control (sprayed)	9.46	18912.71	2055	9.20
2	18	20% Seed mixture (sprayed)	9.10	18200.36	2257.4	8.06
2	19	5% Block (unsprayed)	8.26	16528.74	2018.35	8.19
2	20	20% Strip (sprayed)	7.83	15651.25	2257.4	6.93
3	21	5% Perimeter (unsprayed)	9.62	19237.49	2018.35	9.53
3	22	20% Strip (sprayed)	8.20	16402.60	2257.4	7.26
3	23	5% Seed mixture (sprayed)	8.85	17706.75	2295.35	7.71
3	24	20% Seed mixture (sprayed)	8.17	16334.62	2257.4	7.24
3	25	20% Perimeter (sprayed)	8.08	16153.20	2257.4	7.16
3	26	Control (sprayed)	7.99	15978.51	2055	7.78
3	27	5% Strip (unsprayed)	7.23	14459.43	2018.35	7.16
3	28	5% Block (unsprayed)	8.33	16661.92	2018.35	8.26
3	29	Control (unsprayed)	8.59	17183.08	1778	9.66
3	30	20% Block (sprayed)	8.97	17930.82	2257.4	7.94

13 ANOVA input data to determine cost-benefit ratio

Repetition number	**Plot number**	**Treatment**	**Karate**	**Sprayed**	***Bt***	**RR**
1	1	Control (unsprayed)	0	0	0	1
1	2	Control (sprayed)	1	1	0	1
1	3	5% Block (unsprayed)	0	0	0.95	0.05
1	4	20% Perimeter (sprayed)	1	1	0.8	0.2
1	5	5% Seed mixture (sprayed)	1	1	0.95	0.05
1	6	5% Perimeter (unsprayed)	0	0	0.95	0.05
1	7	20% Seed mixture (sprayed)	1	1	0.8	0.2
1	8	20% Block (sprayed)	1	1	0.8	0.2
1	9	20% Strip (sprayed)	1	1	0.8	0.2
1	10	5% Strip (unsprayed)	0	0	0.95	0.05
2	11	20% Perimeter (sprayed)	1	1	0.8	0.2
2	12	Control (unsprayed)	0	0	0	1
2	13	5% Strip (unsprayed)	0	0	0.95	0.05
2	14	5% Seed mixture (sprayed)	1	1	0.95	0.05
2	15	5% Perimeter (unsprayed)	0	0	0.95	0.05
2	16	20% Block (sprayed)	1	1	0.8	0.2
2	17	Control (sprayed)	1	1	0	1
2	18	20% Seed mixture (sprayed)	1	1	0.8	0.2
2	19	5% Block (unsprayed)	0	0	0.95	0.05
2	20	20% Strip (sprayed)	1	1	0.8	0.2
3	21	5% Perimeter (unsprayed)	0	0	0.95	0.05
3	22	20% Strip (sprayed)	1	1	0.8	0.2
3	23	5% Seed mixture (sprayed)	1	1	0.95	0.05
3	24	20% Seed mixture (sprayed)	1	1	0.8	0.2
3	25	20% Perimeter (sprayed)	1	1	0.8	0.2
3	26	Control (sprayed)	1	1	0	1
3	27	5% Strip (unsprayed)	0	0	0.95	0.05
3	28	5% Block (unsprayed)	0	0	0.95	0.05
3	29	Control (unsprayed)	0	0	0	1
3	30	20% Block (sprayed)	1	1	0.8	0.2