

An investigation into the development and status of resistance of *Busseola fusca* (Lepidoptera: Noctuidae) to Bt maize

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Thesis submitted in fulfilment of the requirements for the award of the
degree Doctor of Environmental Science at the North-West University
(Potchefstroom Campus)

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November 2010

Potchefstroom, South Africa

Acknowledgments

A special word of thanks to my Creator who granted me the opportunity to study His creation and nature. I thank Him that I could learn so much about nature. I also want to praise Him for the privilege of being able to share this knowledge with other people by means of this thesis.

There are several other people without whom this thesis and the work it describes would not have been possible at all. I would like to thank those people who have contributed towards the successful completion of this work.

Without the direction and advice given by my supervisor Prof. Johnnie van den Berg this project would not have been completed. I would like to thank him for all the time and energy he spent making this thesis a success. I am sincerely thankful for the opportunity of travelling through our country, exploring new places I would have never been able to visit if it was not for his unfailing support.

Prof. Koos van Rensburg thank you for your time, input, dedication and thoroughness.

The technical assistance of Mrs. Pierrie Els, Anton Swanepoel, Riaan Rossouw and Hendrik Kruger from Monsanto in collection of diapause larvae is highly appreciated.

Warm thanks to all the farmers who gave me the opportunity to do surveys on their fields, without them this study would not be possible. Mrs. Andre Bezuidenhout, Pierrie Els, Sam Kramer, Attie Meintjes, Schalk Meintjes, Nelius Möll, Mynhardt Noeth, Joe Olevano and Riaan Rossouw, Jaco Vermeulen thank you for your time and assistance with this survey.

Rozelle Keulder and Marijke Coetsee whom without I wouldn't have been able to do the laboratory studies. Thank you for your time and all that you have done. You were always there to help and were great co-workers and friends. You never despaired even

when some of my techniques were time consuming. To all the other Plant Protection students who gave a helping hand during my surveys, thank you so much for that. You have been great.

Moses Phetoe, thank you for all the technical assistance during my study and especially for your dedication in planting maize to make sure I have enough material to complete my experiments.

Dr. Suria Ellis for your assistance with the statistical analysis, it is greatly appreciated.

Laura Quinn and Annemie Erasmus, thank you for your contributions and friendship.

To Annatjie and Buks my parents, Lydia, Chrisjan and Johan thank you for your love, understanding and support.

The financial assistance of the Maize Trust Fund and National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF.

This work forms part of the Environmental Biosafety Cooperation Project between South Africa and Norway coordinated by the South African National Biodiversity Institute and we accordingly give due acknowledgment.

Abstract

Based on surface area, South Africa is currently ranked 8th in planting genetically modified (GM) crops in the world. The stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is of economic importance throughout sub-Saharan Africa. Bt maize (MON810) has been grown to control lepidopterous stem borers in South Africa since its first release in 1998. The first report of resistance to Bt maize was made in the Christiana area of South Africa in 2007. The objectives of this study were to evaluate the status of resistance of populations of *B. fusca* to Bt maize; to evaluate farmers' perceptions of the regulatory aspects guiding the planting of Bt maize and refugia and how the field situation developed between 1998 and 2010; to compare the fitness of the fertility, fecundity and longevity of Bt-resistant and susceptible *B. fusca* populations and to determine if there are fitness costs associated with resistance of *B. fusca* to Bt maize. Questionnaire surveys were conducted amongst 185 farmers in seven districts throughout the maize production region. The questionnaire addressed signing of contracts upon purchasing GM seed, refuge compliance, pest management practices, perceived benefits and risks relating to Bt maize. In order to study fitness and fitness costs that may be associated with resistance development, the life history parameters of known Bt-resistant and susceptible populations were compared in the laboratory using a diapause-as well as second-generation populations collected in maize fields. The following parameters were compared between different stem borers populations and treatments: pupal mass, moth longevity, fecundity, fertility, larval mass and survival, and sex ratio. This study confirmed resistance of *B. fusca* to the Cry1Ab toxin (MON810) and that larvae collected from refugia at Vaalharts were resistant and survived on Bt maize. Compliance to refugia requirements was low especially during the initial 5 - 7 years after release. An alarmingly high number of farmers applied insecticides as preventative sprays on Bt maize and refugia. Except for moth longevity and LT50-values, no other fitness costs were observed to be associated with the resistance trait in the highly resistant *B. fusca* population used in this study. The LT50 may indicate some degree of fitness cost but does not translate into observable costs in terms of fecundity, larval mass and survival. The absence of fitness costs may promote the use of alternative Bt-resistance management strategies, such as the introduction of a multi-

gene strategy. The introduction of a stacked event such as MON89034 which produces more than one protein with activity active against the resistant target pest, together with compliance to the refuge strategy, is most likely the only solution to managing Bt-resistant stem borer populations in South Africa.

Keywords: Bt maize; *Busseola fusca*; fitness costs; life history parameters; resistance development; refuge compliance.

Uittreksel

Op grond van die area onder verbouing van geneties-gemodifiseerde (GM) gewasse, is Suid-Afrika die 8^{ste}-grootste in die wêreld. Die mieliestamboorder, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), is van ekonomiese belang in die hele sub-Sahara Afrika. In Suid-Afrika word Bt-mielies (MON810) sedert 1998 vir die beheer van mieliestamboorders aangeplant. Die eerste weerstand teen Bt-mielies in Suid-Afrika is in 2007 gerapporteer. Die doelwitte van hierdie studie was om die status van weerstand van *B. fusca* teen Bt-mielies te evalueer; om boere se persepsies te bepaal ten opsigte van wetlike aspekte rakende die plant van Bt-mielies, toevlugsoorde en stamboorderweerstandontwikkeling tussen 1998 en 2010 te evalueer; om fekunditeit en fertiliteit asook lewensduur van Bt-weerstandbiedende en vatbare *B. fusca* populasies te bepaal. Daar is ook bepaal of daar geskiktheidskoste verbonde is aan die teenwoordigheid van weerstand teen Bt-mielies. Vraelys-opnames is onder 185 boere in sewe distrikte regoor die mielieproduksiegebied onderneem. Die vraelys het die volgende aspekte aangepreek: onderteken van tegnologie-ooreenkomste met die aankoop van GM saad, nakoming van toevlugsoordvereistes, plaagbestuur asook voordele en risiko geassosieer met Bt-mielies. In die geskiktheid- en geskiktheidskostestudies is die lewensgeskiedenis-parameters van bekende Bt-weerstandbiedende stamboorderpopulasies vergelyk met die van vatbare populasies onder laboratoriumtoestande. Diapouse- sowel as tweede-generasiepopulasies wat in die veld versamel is, is in hierdie studies gebruik. Die volgende lewensgeskiedenis-parameters is tussen weerstandbiedende en vatbare populasies vergelyk: papiemassa, lewensduur van motte, fekunditeit, fertiliteit, larwale massa, oorlewing en geslagsverhouding. Hierdie studie het weerstand van *B. fusca* teen die Cry1Ab-toksien (MON810) bevestig en getoon dat larwes wat in nie-Bt-mielie toevlugsoorde by Vaalharts voorkom, ook weerstandbiedend is teen die Bt-toksien. Gedurende die eerste 5 - 7 jaar na vrystelling van Bt-mielies was die nakoming van toevlugsoord-vereistes laag. 'n Groot aantal boere het insekdoder voorkomend toe op Bt-mielies en toevlugsoorde toegedien. Behalwe vir lewensduur van motte en LT50-waardes is geen ander geskiktheidskoste waargeneem in die boorderpopulasie met 'n hoë vlak van weerstand nie. Die hoër LT50-waarde mag 'n graad van geskiktheidskoste aandui maar

dit word nie sigbaar uitgedruk of hou nie verband met geskiktheidskoste in terme van fekunditeit, larwale massa of oorlewing nie. Die afwesigheid van geskiktheidskoste in Bt-weerstandbiedende populasies kan die gebruik van alternatiewe Bt-weerstandbestuurstrategieë soos 'n multigeenstrategie, bevorder. Die vrystelling van 'n stapelgeen soos MON89034 wat meer as een proteïen produseer wat aktief is teen die teikenplaag, tesame met nakoming van die vlugoordstrategie, is moontlik die enigste oplossing vir die bestuur van Bt-weerstandbiedende stamboorderpopulasies in Suid-Afrika.

Sleutelwoorde: Bt-mielies; *Busseola fusca*; geskiktheidskoste; lewensgeskiedenis-parameters; stamboorderweerstandsonwikkeling; toevlugsoordvereistes.

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Chapter 1

Introduction and literature review

1.1 History of genetically modified maize in South Africa

Large-scale planting of Bt crops in the world commenced during 1996 with South Africa quickly adopting Bt maize and cotton for control of lepidopteran pests. Based on surface area, South Africa is currently ranked 8th in planting genetically modified (GM) crops in the world (Fig. 1.1) (James 2008, 2009).

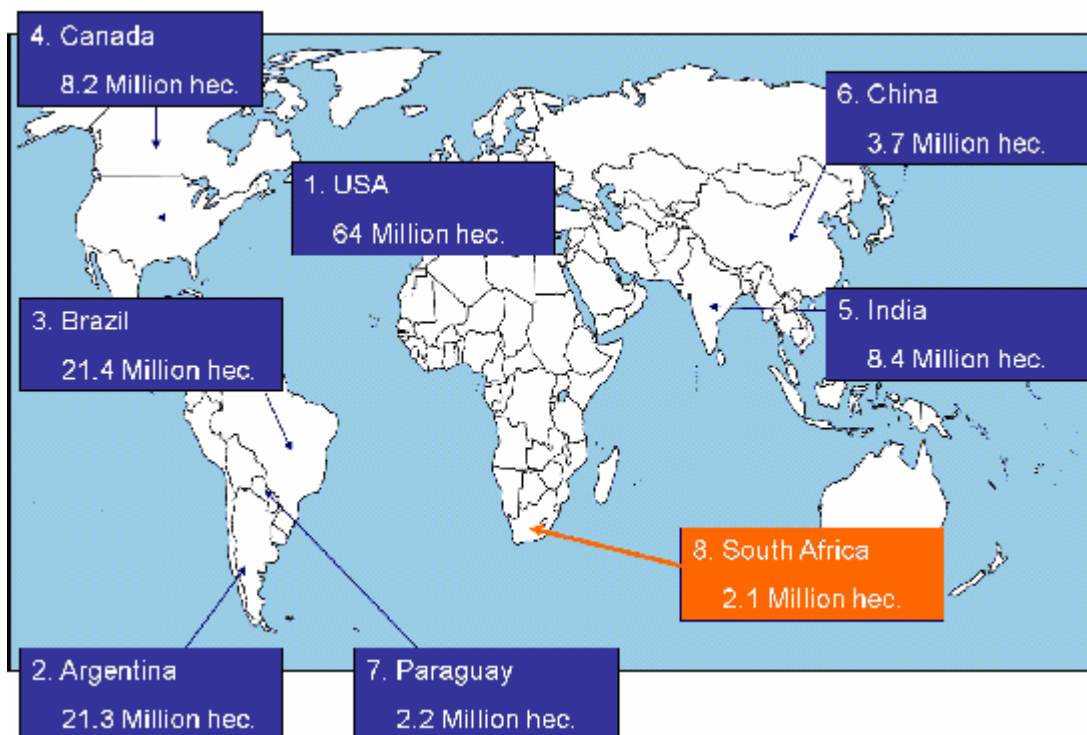


Fig. 1.1 Based on surface area, South Africa is currently ranked 8th in planting genetically modified (GM) crops in the world (Figure adapted from James (2009)).

The stem borers, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) (Fig. 1.2), *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) (Fig. 1.3) and *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) (Fig. 1.4) are of economical importance in South Africa. Especially *B. fusca* and *C. partellus* cause serious yield losses in maize (Van den Berg, 1997).



Fig. 1.2 *Busseola fusca* larva inside a maize stem.



Fig. 1.3 *Chilo partellus* larvae damage to maize stem.



Fig. 1.4 *Sesamia calamistis* second-instar damage symptoms on the inside surface of a leaf sheath.

An application to the South African Department of Agriculture during 1989 to perform field trials with genetically modified cotton, initiated the South African biosafety process and the first trials with transgenic crops on the African continent (Gouse, 2005). The first evaluation of various Bt maize events for control of the maize stem borer, *B. fusca*, in South Africa was conducted during the 1994/95 growing season (Van Rensburg, 1999). This was done by means of field and greenhouse evaluations of Bt maize under artificial infestation with the target pest. Results concluded that various events of the *Cry1Ab* gene were not equally effective for control of *B. fusca*. MON810 and MON802 seemed to be superior to other events, both in inbred material and in hybrid combinations.

In 1997 South Africa became the first country in Africa to commercially produce transgenic crops (Gouse *et al.*, 2005). To date the commercial release of insect-resistant (Bt) cotton and maize as well as herbicide-tolerant (RR) soybeans, cotton and maize have been approved. Farmers started adopting Bt cotton varieties during the 1997/98 season and Bt yellow maize during the 1998/99 season. Bt white maize

was introduced during the 2001/02 season and 2002/03 saw the first season of large-scale Bt white maize production (Gouse, 2005). The area planted to Bt-maize increased to approximately 1 million ha in 2007.

1.2 Transgenic maize and Bt toxin

The introduction of transgenic hybrid maize varieties began in 1996 in the United States of America (Glaser & Matten, 2003). Apart from the use of *Bacillus thuringiensis* (Bt) in spray formulations, genes from this bacteria encoding for crystalline proteins, that are toxic to Lepidopteran larvae, have also been used in the genetic modification of maize. Transgenic maize plants produce Bt toxin at a high level throughout the growing season. The Bt gene encodes for a crystalline protein, known as a Cry protein. The promoter of the Bt gene inserted into the maize genome turns on the production of an inactive form of the toxin in maize cells (Van Rie *et al.*, 1989). Once ingested by a target insect, crystalline inclusions dissolve in the larval midgut releasing Cry proteins (Van Rie *et al.*, 1989). Cry proteins are protoxins (the inactive toxin) that are proteolytically converted into smaller toxic polypeptides inside the insect midgut (Höfte & Whiteley, 1989). The toxin binds to specific receptors on the epithelial cells of the larval gut and ruptures cell walls, leading to subsequent paralysis of the gut and eventual death of the insect (Kumar *et al.*, 1996).

1.3 Potential advantages of transgenic crops

From an environmental and human-health perspective, the use of genetically modified crops promises benefits. While many broad-spectrum insecticides reduce the impact of biological control agents that help to control insect and mite pests, studies also indicated that Bt maize is compatible with biological control and has little effect on natural enemies of pests (Bessin, 2010).

Control of lepidopteran pests with Bt endotoxins provides four advantages from the grower's perspective. Firstly, control is no longer affected by the weather. The crop is protected even if the field conditions do not allow spray equipment to enter into fields or if conditions are unsuitable for aerial application (Meeusen & Warren, 1989). A second and related advantage is the protection of plant parts that are difficult to reach with insecticide spray or the protection of new growth that emerges after spray applications, such as tillers and ears of maize (Meeusen & Warren, 1989).

Thirdly, the crop is protected continuously in the field and scouting may no longer be needed. The problem of realizing the presence of lepidopteran pests too late is consequently eliminated (Bessin, 2010). The importance of scouting should however, not be underestimated and could play an important role in detection of resistance. Chemical control of stem borers is complicated because of cryptic feeding deep inside plant whorls where larvae do not easily come into contact with insecticides. A further complication in the chemical control of *C. partellus* is the overlapping of generations owing to staggered pupation and the recurrence of infestation of the same planting at later crop growth stages (Van Rensburg & Van den Berg, 1992). No special application equipment is necessary for insecticide application in post-flowering period when a Bt crop is planted. Bt crops do not require any specialized equipment and could therefore be effective on farms of all sizes (Bessin, 2010).

Finally, and most likely the most important advantage is the reduction in insecticide applications. A reduction in pesticide application also reduces the potential pesticide drift onto other crops or environmentally sensitive areas (Meeusen & Warren, 1989). Because the active Bt toxin material is produced directly in the crop tissue, concerns such as spray drift and groundwater contamination are obviated (Meeusen & Warren, 1989). The use of transgenic crops reduces the use of insecticides and minimizes the impact of these chemicals on non-target organisms and has positive health consequences for farm workers (Barton & Dracup, 2000).

The benefits of GM crops is also evident from an example from the Makhathini Flats region of KwaZuluNatal, South Africa, where 95% of smallholder (1-3 hectares) cotton producers grew rainfed Bt cotton during the 1999/2000 growing season. Farmers that adopted Bt cotton reported higher yields, reduced insecticide use and a reduction in labor inputs (Ismael *et al.*, 2001). A typical farmer, often a woman, was spared 12 days of arduous spraying, saves more than a 1000 liters of water used for spraying, and walks 100 km less per year (Conway, 2004). A study during the first three seasons of Bt white maize production by small-scale farmers in South Africa showed significant yield increases. During the first season yield increases between 21 and 62 % with an average of 32 % was reported with Bt maize above the

conventional isoline (Gouse, 2005). Despite a lower than normal rainfall and stem borer pressure in 2002/03, small-scale farmers in KwaZulu-Natal enjoyed a statistically significant yield increase of 16 % due to better stem borer control with Bt maize. Bt maize adopting-farmers were better off than farmers who planted conventional hybrids, despite the additional technology fee in terms of seed costs (Gouse, 2005).

1.4 Potential disadvantages and uncertainties about transgenic crops

GM crops will and have become a major component of insect control strategies. A proper perspective of their potential demands a close look at the limitations and uncertainties, which may reduce its impact on agriculture.

Bt maize seed is more expensive than comparable non-Bt seed. Bt maize is only an advantage when a specific insect pest is present and there is no advantage to planting seed with the Bt gene if the specific pest is not present. Stem borer populations can vary in abundance from year to year and their pest status is not predictable. This is evident from research done by Van Rensburg *et al.* (1985) on *B. fusca*. The seasonal abundance of *B. fusca* moths at five localities in the maize production area of South Africa was monitored by means of Robinson light traps during the 1970's and 1980's. Geographical variation in the flight patterns was shown to exist between localities from east to west. Both the onset and magnitude of the three seasonal moth flights seem to be governed by climatic factors (Van Rensburg *et al.*, 1985). In some production areas and during certain growing seasons infestation may be severe or very late.

Maize is wind pollinated and can be cross-pollinated with maize pollen from fields within several hundred meters. This may present a problem for producers of food grade maize or non-Bt maize when it is important to keep the grain free of Bt maize. Care should be taken to reduce Bt contamination through cross pollination (Bessin, 2010). Luna *et al.* (2001) conducted experiments to investigate the duration of pollen viability and the effectiveness of isolation distance for controlling gene flow. In this experiment the theoretical, maximum distance that viable pollen could move was 32 km if pollen was transported linearly at the maximum average afternoon wind speeds for the specific location, viability was maintained for 2 h, and pollen settling rate was

ignored. Cross pollinations that occurred at a maximum distance of 200 m from the source planting was observed (Luna *et al.*, 2001).

Another potential disadvantage is that biotechnology is being pursued to repair the problems caused by previous agrochemical technologies. Based on the fact that more than 500 species of pests have already evolved resistance to conventional insecticides, surely pests can also evolve resistance to Bt toxin in GM crops (Altieri, 2004). This was confirmed by Van Rensburg (2007) who reported resistance of *B. fusca* to Bt maize.

1.5 Resistance evolution of target pests to Bt- sprays and crops

Since the first deployment of Bt crops there has been concern with regard to evolution of resistance of target pests (Tabashnik, 1994; Gould, 1998). The large scale use of Bt crops have put considerable selection pressure on target species (Tabashnik, 1994).

Resistant strains of pests to Bt-sprays are often reported to show disadvantages in life-history characteristics (Roush & McKenzie, 1987). To assess these disadvantages that result from resistance, various components of fitness, such as mating, fertility, fecundity and development time must be measured (Roush & McKenzie, 1987). The potential of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) to develop Bt resistance has led studies involving long-term selection with the Cry1Ab and Cry1Ac toxins with Dipel ES (Bt-spray) in the laboratory (Huang *et al.*, 1997). The study conducted by Huang *et al.* (1997), showed a rapid loss of susceptibility to Dipel. The rapid change in *B. thuringiensis* susceptibility in the five colonies suggested there was an adaptation or an enhancement of an existing defence system in *O. nubilalis* population which is already widespread.

The diamond back moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), has evolved high levels of field resistance to formulated Bt insecticide (Tabashnik, 1994, Tabashnik *et al.*, 1994). The ability of Lepidoptera to evolve resistance to Bt-toxins has been noted by Tabashnik (1994) who indicated that species in the Noctuidae, Pyralidae and Plutellidae can develop resistance when exposed to Bt in selection experiments under laboratory conditions and in the field.

Various events of the Bt genes are available, exhibiting high levels of toxicity to *O. nubilalis* and southwestern corn borer, *Diatraea grandiosella* (Dyer) (Lepidoptera: Pyralidae) in the USA (Sharma *et al.*, 2000).

The resistance strains of *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) showed increased ability to survive and develop on Bt cotton and on Cry1Ac-treated diet, compared to their performance on non-Bt cotton or untreated diet. Resistant individuals reared on Bt cotton or diet with a high concentration of Cry1Ac had reduced survival, slower development, reduced pupal weight and lower fecundity (Liu *et al.*, 2001). This was also observed for *O. nubilalis* (Bourguet *et al.*, 2000) indicating that the efficacy of the refuge strategy may be decreased, since moths derived from Bt-maize have less probability of mating with susceptible counterparts from non-Bt fields, as was shown with *O. nubilalis* (Bourguet *et al.*, 2000).

Chaufaux *et al.* (2001) reported that all selected strains of *O. nubilalis* developed significantly increased tolerance to Cry1Ab after chronic exposure to this protein in laboratory studies. In this study a significantly decreased susceptibility of *O. nubilalis* population was observed after exposure to *B. thuringiensis* toxin for multiple generations and further indicated that the chronic exposure to the Cry1Ab toxin throughout development did select for increased *B. thuringiensis* tolerance (Chaufaux *et al.*, 2001). These results suggest that low levels of resistance are common among widely distributed *O. nubilalis* populations in Nebraska (Chaufaux *et al.*, 2001). This could therefore also be the case with indigenous stem borers in Africa but no studies have been conducted yet.

To date field evolution of resistance to Bt-toxins in the world has been rare and only detected in *B. fusca* in South Africa (Van Rensburg, 2007), *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in the south-eastern United States (Tabashnik, 2008; Tabashnik *et al.*, 2008a) and *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) in Puerto Rico (Matten *et al.*, 2008). Resistance to Bt cotton has also recently been reported in the Pink bollworm (*P. gossypiella*) in India (Monsanto, 2010).

1.6 The high dose/refuge strategy for resistance management

Prior to the introduction of Bt crops in 1995, insect resistance management was rarely implemented before field resistance to insecticides occurred and generally another insecticide was available to replace the old one (Glaser & Matten, 2003).

The main environmental threat of Bt crops is that their widespread cultivation could lead to insect resistance development to Bt toxins and the subsequent reversal to the use of broad spectrum insecticides. High dose/refuge resistance management strategies may make theoretical sense, but the practical situation is still uncertain because there are huge gaps in knowledge about pest genetics and about pest ecology in cropping systems (Renner, 1999).

In 1988 four basic strategies were outlined that could be used to delay insect adaptation to transgenic insecticidal cultivars (Gould, 1998). Firstly, mixtures of toxic and non-toxic cultivars (refuge approach); secondly, gene stacking two or more toxins in each transgenic insecticidal cultivar; thirdly, low doses of toxins that act in concert with natural enemies to decrease pest populations and fourthly, tissue-, time- or signal-dependent expression of toxins (Gould, 1998). Absent from the list was the high-dose approach, which was not even considered until 1991 when Monsanto (St. Louis, MO) scientists demonstrated that they could produce plants with toxin titers that were much higher than that needed to kill 100 % of the susceptible genotype of target insects (Perlak *et al.*, 1991, cited by Gould, 1998).

Analysis of more than a decade of resistance monitoring data for six Lepidoptera species targeted by Bt maize and cotton suggests that the principles of the refuge strategy may apply in the field (Tabashnik *et al.*, 2008b).

The high dose/refuge strategy, employed to limit resistance development, comprises a combination of Bt maize plants producing high doses of toxin and non-Bt plants in close proximity to one another (Fig. 1.5). The purpose of the high dose of toxin is to kill as many individuals of the target pest as possible, whereas the purpose of the refuge is to sustain pest individuals that survive on that particular crop (Renner, 1999; Gould, 2000). The goal is to ensure that those rare, Bt resistant individuals that survive on the Bt crop do not produce completely resistant offspring by mating

with other toxin-resistant individuals. Instead, susceptible individuals from the refuge can mate with toxin-resistant individuals that survive on the engineered plants. Offspring from the combination of a susceptible and resistant individual are expected to have only a low to moderate level of toxin resistance. These individuals should not be able to survive on plants with high Bt toxin levels (Renner, 1999; Gould, 2000), lowering the chance of resistant populations developing in a given area.

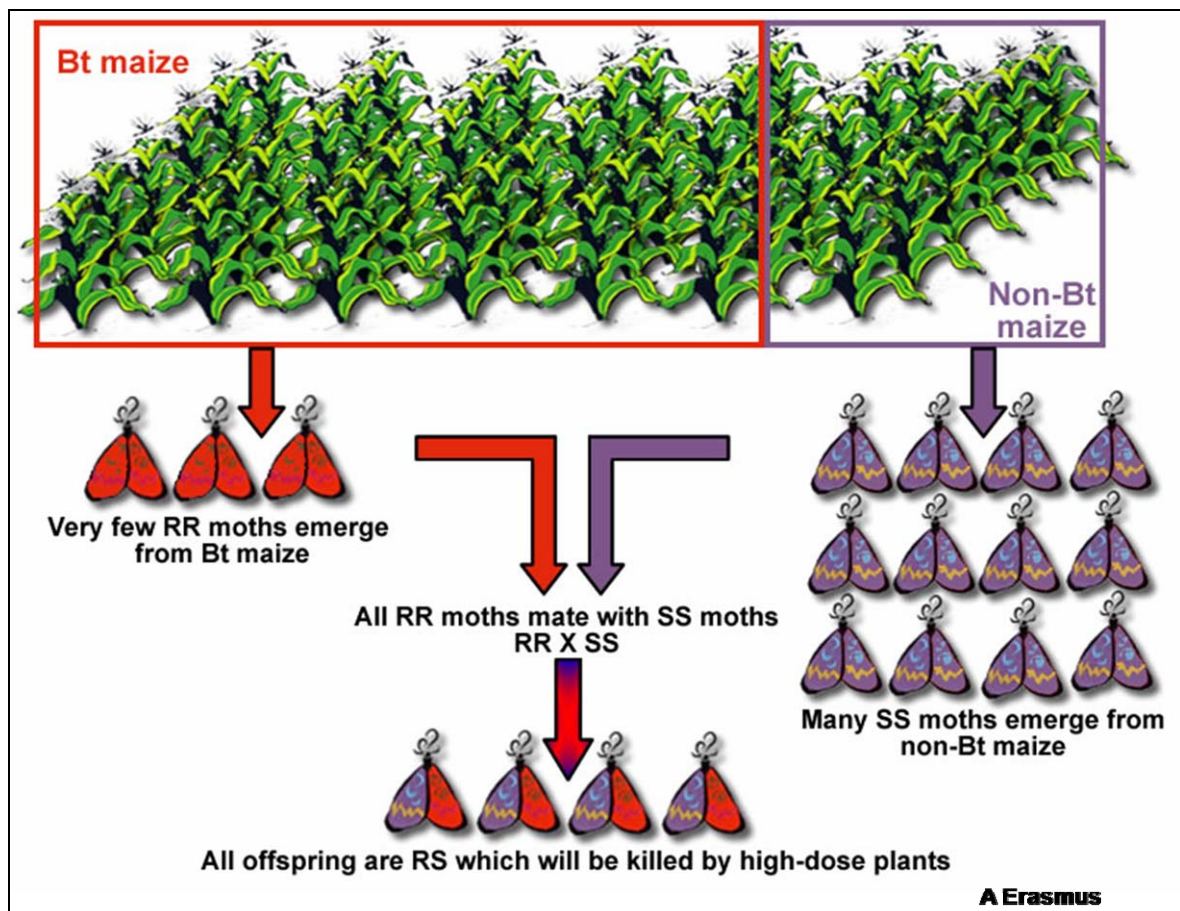


Fig. 1.5 Illustration of the high dose/refuge strategy for resistance management.

The use of the high-dose refuge strategy is limited by a number of practical considerations. Delivery of the high-dose and separation of toxic and non-toxic refuge plants in space may be compromised by the expected contamination of Bt seed with non-expressing 'off-types' that may comprise up to 3 % of the crop (Gould, 1998) and create, in effect, a seed mixture (Bates *et al.*, 2005). Models indicate that even a low frequency of non-Bt plants in a Bt field could result in a marked acceleration of resistance development. In addition Bates *et al.* (2005)

suggested that pollen flow from Bt plants to non-Bt plants may result in exposure of pests to low levels of Bt in developing seed tissue in at least some areas of the refuge (Bates *et al.*, 2005).

Generally, the high-dose/structured refuge strategy has received the most attention as an optimal insect resistance management strategy (Glaser & Matten, 2003). This strategy assumes that resistance is recessive and is conferred by a single locus with two alleles in three insect genotypes (RR, SS and RS), and that resistance alleles are initially rare and that there will be random mating between resistant and susceptible adults (Roush, 1994; Gould, 1998; USEPA, 1998, 2001). The basic requirements for the high-dose toxin strategy include:

- resistance genes must be recessive
- heterozygote survival is less than 5 % of RR
- resistance genes are rare
- non-transgenic refuge will sustain susceptible pest population
- refuge proximity is sufficient to ensure nearly random mating
- crop will express 25 times the toxin required to kill 99 % of the susceptible pests (Glaser & Matten, 2003).

1.7 Refuge strategy and requirements

The basis of integrated resistance management (IRM) is that growers must plant sufficient hectareage of non-Bt crops to serve as refuges for pests. This decreases the selection pressure for the development of Bt resistant insects and ensures that Bt susceptible pests will be available as mates for Bt resistant insects, should they develop (Thomson, 2002). In Africa it should be possible to use this system on commercial farms but it remains to be seen how effectively it can be managed in small-scale farming systems. The authorities in charge of regulating the use of GM crops in these countries will have to pay particular attention to this (Thomson, 2002).

For every particular Bt crop in any country however, it is necessary to understand the resistance risk and what sort of IRM options may be feasible. This could be done by collecting information on the agricultural system, the biology of the target pest and the behaviour of growers and generating local information on product performance

(Head, 2010). This helps to define the precise IRM tactics that can and should be used. The following factors should be considered:

- The cropping system – Is the crop grown in monoculture or polyculture? What is the approximate farm size? What pest control methods are used for the target pests? What level of product adoption is expected? Do transgenic products already exist in this crop or in other crops in the same agricultural system? (Head, 2010).
- Biology of target pest – For each target pest, how many generations occur per year and how many of these are on the crop of interest? Does the insect use wild or cultivated hosts other than the crop of interest as hosts? How much do the adult insects move before and after mating? Where do the insects mate? Do they have a history of developing resistance to insecticides and Bt's? Are transgenic products used to control this species in this crop or other crops in the same agricultural system? (Head, 2010).
- Grower behaviour and attitudes – Will growers be willing to practice IPM and IRM? Do they have a history of doing so? What is their knowledge level? Have growers been sensitized to the concept of resistance through the loss of other pest control tools? (Head, 2010).
- Product performance – For each target pest species, what is the level of control (% mortality) of the Bt crop for each important life stage infesting the crop? Will the use of insecticides be part of the overall IPM system in the Bt crop? If so, will the insecticide applications still be needed on the Bt crop for this target pest? (Head, 2010).

Because of local differences in cropping systems and farmer behaviour, IRM plans may vary with respect to: the nature of the refuge strategy (whether a structured refuge is needed and, if so, its size and placement); and the means by which farmers are educated on IRM (the medium used, the focal points for education, and the

educational messages used). As an illustration, consider the following logic for defining an appropriate refuge strategy (Head, 2010).

Although the planting of refugia is compulsory to limit resistance development (Monsanto, 2007 & 2009), the level of compliance by farmers in South Africa is not known. The current refuge requirements are either a 20 % refuge planted to conventional maize which may be sprayed with insecticides, or a 5 % refuge area that may not be sprayed (Fig. 1.6).



Fig. 1.6 Bt maize field with its adjacent non-Bt refuge. The non-Bt maize planting (left) is lighter in colour due to later flowering than Bt maize plants.

The basic theory underlying the refuge strategy was to reduce the heritability of resistance by providing susceptible adults to mate with rare resistant adults surviving on Bt crops. More over the value of refuges is enhanced if the concentration of toxin in the Bt crop is high enough to kill the progeny from mating between resistant and susceptible insects. Recognition of the prevalence and impact of fitness costs adds a new dimension to the refuge strategy in that refuges can select against resistance (Gould, 1998; Bourguet *et al.*, 2000; Gassmann *et al.*, 2009).

Uncertainties associated with refuge implementation may affect the success of the high-dose/refuge strategy. If the compliance rate to refuge adoption can be increased, then insect resistance management for Bt maize would have a higher probability of success (Bourguet, 2004). No information exists on annual adoption rates of Bt maize or compliance to regulatory requirements regarding planting of Bt maize in South Africa.

1.8 Resistance development in South Africa

At harvest of the 1999 growing season, crop damage to the lower stems of maize caused by *B. fusca* was noticed on a considerable scale at a number of localities, involving various Bt maize hybrids (Van Rensburg, 2001). Van Wyk *et al.* (2007) also reported increased incidence of *B. fusca* larvae on Bt-maize during the post-flowering period in the Highveld region of South Africa. Since no leaf feeding damage occurred during the vegetative growth stages of these plants, this damage seemed to indicate increased survival of larvae resulting from late oviposition, presumably during the period from tasseling to grain filling. No yield losses could be attributed to these infestations, but the observation caused concern due to the possibility that similar infestations may in future result in significant damage to ears, particularly in years when late spring rains necessitate the use of relatively late planting dates (Van Rensburg, 2001). It could also contribute towards the development of Bt-tolerant stem borer populations. Since YieldGard technology claims control of only the first two larval instars, it seemed probable that increased survival of neonate and second instar larvae on Bt-maize may have resulted from feeding on some less toxic plant parts followed by stem tunneling at late plant growth stages (Van Rensburg, 2001).

The first official report of resistance of *B. fusca* to Bt maize was made by Van Rensburg (2007) in South Africa. Studies conducted in Christiana in the North-West province indicated that the larval population on Bt maize at certain localities has attained a level of resistance where some larvae were able to survive in the presence of the Bt-toxin but not without some detrimental effect on larval growth rate (Van Rensburg, 2007).

1.9 Fitness costs

Fitness costs occur when the fitness of individual's bearing resistant (R) alleles is less than that of homozygous susceptible (SS) individuals in the absence of toxin. Because resistance alleles are rare in populations not previously exposed to insecticides (Bentur *et al.*, 2000), fitness of heterozygous individuals (RS) impacts strongly on the early dynamics of resistance evolution. If fitness costs are not recessive, RS are less fit than SS individuals in refuges. Yet with functionally recessive resistance, RS and SS individuals are equally fit in transgenic fields. This suggests that the spread of recessive R alleles with no recessive fitness costs could be prevented with an appropriate refuge/high dose strategy (Carrière & Tabashnik, 2001). Understanding the fitness of resistant insects is important in development of effective resistance management strategies (Wu *et al.*, 2009). Bt resistance have been studied in 14 species of moths (Table 1; Gassmann *et al.*, 2009) indicating detection of costs by different experimental methods.

Rapid adaptation of insect pests could shorten the success of crops that are genetically modified to produce toxin from *B. thuringiensis* (Tabashnik, 1994). As in the case with insecticides (Dipel), resistance alleles may have pleiotropic effects that could compromise normal functions and impose a fitness cost in the absence of Bt toxin. This can also be implied for the *B. fusca* resistance to Bt maize in South Africa. Fitness costs may therefore contribute to the delay of resistance evolution to Bt toxins when refugia are present (Carrière & Tabashnik, 2001).

Oppert *et al.* (2000) investigated fitness costs in a laboratory study with Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). The fitness parameters (development time and survival) of Bt-susceptible and Bt-resistant Indian meal moth colonies were examined on untreated or Bt-treated diets. Oppert *et al.* (2000) concluded that there were modest costs associated with resistance to Bt in some *P. interpunctella* colonies but not in others. In another study Groeters *et al.* (1994) observed fitness trade-offs associated with evolution of resistance in the diamondback moth, *P. xylostella*, with lower fecundity and fertility as well as reduced survival.

Carrière *et al.* (2001a) conducted a study to estimate development time of resistant and susceptible pink bollworm strains (*P. gossypiella*) on cotton. Except for a slight (3 %) difference in development time detected, no evidence was found for fitness cost affecting development time. Results further showed that resistance to Bt cotton in pink bollworm had little or no effect on development rate on non-Bt cotton (Carrière *et al.*, 2001a).

Table 1. Fitness costs of Bt resistance have been studied in 14 species of lepidoptera (Gassmann *et al.*, 2009).

Species	Number of studies	References*
Gelichiidae		
<i>Pectinophora gossypiella</i> (Pink bollworm)	10	(Carrière <i>et al.</i> , 2005; Carrière <i>et al.</i> , 2004; Carrière <i>et al.</i> , 2006a; Carrière <i>et al.</i> , 2007; Carrière <i>et al.</i> , 2001a; Carrière <i>et al.</i> , 2001b; Carrière <i>et al.</i> , 2006b; Gassmann <i>et al.</i> , 2006; Gassmann <i>et al.</i> , 2008; Higginson <i>et al.</i> , 2005)
Noctuidae		
<i>Busseola fusca</i> (Maize stalk borer)	1	(Van Rensburg, 2007)
<i>Helicoverpa armigera</i> (Cotton bollworm)	7	(Akhurst <i>et al.</i> , 2003; Bird & Akhurst, 2004; Bird & Akhurst, 2005; Bird & Akhurst, 2007; Liang <i>et al.</i> , 2007; Liang <i>et al.</i> , 2008; Zhao <i>et al.</i> , 2008a)
<i>Helicoverpa zea</i> (Corn earworm)	3	(Burd <i>et al.</i> , 2003; Jackson <i>et al.</i> , 2004; Jackson <i>et al.</i> , 2006)
<i>Helicoverpa virescens</i> (Tobacco budworm)	6	(Gahan <i>et al.</i> , 2005; Gould & Anderson, 1991; Jackson <i>et al.</i> , 2007; Johnson <i>et al.</i> , 1997; Sims & Stone, 1991; Stone <i>et al.</i> , 1989)
<i>Spodoptera exigua</i> (Beet armyworm)	2	(Dingha <i>et al.</i> , 2004; Moar <i>et al.</i> , 1995)
<i>Spodoptera littoralis</i> (Cotton leafworm)	1	(Müller-Cohn <i>et al.</i> , 1996)
<i>Trichoplusia ni</i> (Cabbage looper)	4	(Janmaat & Myers, 2003; Janmaat & Myers, 2005; Janmaat & Myers, 2006; Tamez-Guerra <i>et al.</i> , 2006)
Plutellidae		
<i>Plutella xylostella</i> (Diamondback moth)	25	Baur <i>et al.</i> , 1998; Cerda <i>et al.</i> , 2003; Groeters <i>et al.</i> , 1993; Groeters <i>et al.</i> , 1994; Hama <i>et al.</i> , 1992; Imai & Mori, 1999; Liu <i>et al.</i> , 2001; Liu <i>et al.</i> , 1996; Perez <i>et al.</i> , 1995; Ramachandran <i>et al.</i> , 1998; Raymond <i>et al.</i> , 2007a; Raymond <i>et al.</i> , 2005; Raymond <i>et al.</i> , 2006; Raymond <i>et al.</i> , 2007b; Sayyed <i>et al.</i> , 2000a; Sayyed <i>et al.</i> , 2000b; Sayyed <i>et al.</i> , 2004; Sayyed & Wright, 2001; Shirai <i>et al.</i> , 1998; Tabashnik <i>et al.</i> , 1994; Tabashnik <i>et al.</i> , 1991; Tabashnik <i>et al.</i> , 1995; Tang <i>et al.</i> , 1997; Wright <i>et al.</i> , 1997; Zhao <i>et al.</i> , 2000b)
Pyralidae		
<i>Cadra cautella</i> (Almond moth)	1	(McGaughey, 1985)
<i>Chilo suppressalis</i> (Asiatic rice borer)	1	(Alinia <i>et al.</i> , 2000)
<i>Homoeosoma electellum</i> (Sunflower moth)	1	(Brewer, 1991)
<i>Ostrinia nubilalis</i> (European corn borer)	5	(Bolin <i>et al.</i> , 1999; Huang <i>et al.</i> , 2005; Huang <i>et al.</i> , 2002; Huang <i>et al.</i> , 1999; Li <i>et al.</i> , 2007)
<i>Plodia interpunctella</i> (Indian meal moth)	4	(Johnson & McGaughey, 1996; McGaughey, 1985; McGaughey & Beeman, 1988; Oppert <i>et al.</i> , 2000)

*References cited by Gassmann *et al.* (2009).

1.10 Farmers' surveys and perceptions

When assessing farmers' perceptions of innovations such as transgenic crops, surveys can be useful tools (Grieshop *et al.*, 1988). Surveys can generally determine the success or failure of an innovation after the technology has been introduced and used by farmers (Pilcher & Rice, 1998). A unique opportunity exists in this case with Bt maize to gain insight from farmers on the use of Bt maize for stem borer control. A survey of farmers' perceptions and knowledge will be beneficial to extension specialists, crop consultants and industry in developing educational information for farmers (Pilcher & Rice, 1998) and to determine levels of compliance to regulatory requirements such as planting of refugia.

Sanvido *et al.* (2009) also suggested that farmer questionnaires be used as part of general surveillance to record unusual pest outbreaks that could possibly be associated with changes in functional biodiversity. In Sweden, surveys amongst GM crop farmers showed a negative attitude towards the technology (Lehrman & Johnson, 2008), while South African farmers were very positive towards the technology, mainly ascribing it to ease of management.

Yellow maize is grown in large quantities and is primarily used as animal feed and as an input in the food industry in South Africa (Gouse *et al.*, 2005). The initial spread of Bt yellow maize was quite slow in 2000/01, with farmers planting less than 3 % of the total maize area under Bt maize. Possible reasons for this were that the Bt hybrids were not well adapted to the local production conditions and that many farmers did not foresee a significant productivity increase from the use of Bt seed. Many farmers believed that if they managed to plant at the recommended time, in order to escape periods of peak stem borer moth flights, their crop would suffer limited damage whether they plant Bt maize or not. The third reason was the farmers' concern that they might not be able to sell their harvest because of consumer concerns about genetically modified food (Gouse, 2005). Gouse *et al.* (2005), however, found that commercial maize farmers benefited economically from the use of Bt maize. Despite paying more for seed, farmers who adopted Bt maize enjoyed increased income from Bt maize compared to conventional maize through savings on pesticides and increased yield due to better pest control.

1.11 Conclusions

This first report of resistance development of a target pest to Bt maize was done in South Africa (Van Rensburg, 2007). Over a period of one year, the presence of another possible resistant population has been observed by farmers in another area close to the first report at the Vaalharts irrigation scheme Northern Cape province, South Africa.

No investigation has so far been made to quantify the status of resistance, attempt an explanation for resistance development or develop strategies to manage it. Currently no effective management strategy exists to combat the spread of resistant populations, or to delay resistance development in this area. It seems that the prescribed high dose/refuge strategy may not be very effective and the level of compliance is unknown in the main maize production areas of the country.

The possibility exists that non-compliance to refuge requirements in the Highveld region can play a role in evolution of resistance or the possible spread of resistance in these areas. A unique opportunity exists after 12 years of cultivation of Bt maize in South Africa to gain insight from farmers on the use of Bt maize as well as regulatory aspects pertaining to the planting thereof. Furthermore it will also determine levels of compliance to regulatory requirements such as planting of refugia and highlight possible areas where resistance development may be a risk.

1.12 Objectives of the study

1.12.1 General objective

The general objective was to study the status of resistance of different populations of *B. fusca* to Bt maize and to compare fitness of Bt resistant and susceptible populations of stem borers on maize. Surveys were also done to determine farmers' perceptions on the use of Bt maize and compliance to refugia requirements.

1.12.2 Specific objectives

- to evaluate the status of resistance of different populations of *B. fusca* to Bt maize and the level of resistance of borer populations occurring on the refuge-plantings of maize at the localities where resistance was reported.
- to evaluate farmers' perceptions of the regulatory aspects guiding the planting of Bt maize and refugia, its benefits and disadvantages as well as their perceptions of the pest status of *B. fusca* in the Vaalharts irrigation scheme and Highveld region of South Africa.
- to compare the fitness of Bt-resistant and susceptible *B. fusca* populations.
- to determine if there are fitness costs associated with resistance of *B. fusca* to Bt maize.

Results of this study will be presented in the form of chapters with the following titles:

- Status of Bt resistance in a Vaalharts population of the maize stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae).
- Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa.
- Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa.
- Development and reproductive biology of Bt-resistant and susceptible larvae of the maize stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae).
- Fitness costs associated with resistance of *Busseola fusca* to Bt maize.

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Chapter 2

Resistance to Bt maize in *Busseola fusca* (Lepidoptera: Noctuidae) from Vaalharts, South Africa

Abstract

The first report of resistance of the maize stem borer (*Busseola fusca*) to Bt maize (MON810) was made in the Christiana area of South Africa during 2007. The objective of this study was to evaluate the status of resistance of other populations of *B. fusca* to Bt maize. One greenhouse and two laboratory studies were conducted. *Busseola fusca* populations were collected on Bt maize as well as the adjacent refugia (conventional maize/non-Bt maize) in the Vaalharts area, 50 km from the Christiana site. Control populations were collected from sites where Bt maize was not planted. In the greenhouse study 720 potted plants were each artificially infested with 10 neonate larvae of the F1-generation after the field collected populations were reared through to adults. Numbers of live larvae and larval mass per plant were determined at regular intervals over a 35-day period. Larvae of the Christiana conventional population (Bt-susceptible) on Bt-maize (CHR08Con-Bt) and Bethal conventional population (Bt-susceptible) on Bt-maize (BET08Con-Bt) did not survive on Bt-maize for longer than 12 days. The populations collected from both Bt (VAA08Bt-Bt) maize and refuges (VAA08Ref-Bt) at Vaalharts were resistant and the subsequent generation of larvae completed their life cycle on Bt-maize. Similar results were observed in the laboratory experiments. This study confirmed resistance of *B. fusca* to the Cry1Ab toxin (MON810). The geographical distribution of resistance was shown to include at least the Vaalharts area, in addition to the original report for the Christiana area. These observations that larvae collected from refugia at Vaalharts were resistant, show that the efficacy of the refuge strategy is compromised in this area because the contribution of refugia did not produce large enough numbers of susceptible individuals to mate with moths of which larvae survived inside Bt maize fields.

Accepted for publication as: **Kruger, M., Van Rensburg, J.B.J. and Van den Berg, J.** 2011. Resistance to Bt maize in *Busseola fusca* (Lepidoptera: Noctuidae) from Vaalharts, South Africa. Environmental Entomology 40.

2.1 Introduction

Large-scale planting of Bt crops in the world began during 1996 with South Africa quickly adopting Bt maize and cotton for the control of lepidopteran pests. Based on surface area planted to genetically modified (GM) crops, South Africa is currently ranked 8th in planting these crops in the world (James, 2009).

Since the first deployment of Bt crops there has been concern with regard to evolution of resistance of target pests (Gould, 1998). The ability of Lepidoptera to evolve resistance to Bt-toxins have been noted by Tabashnik (1994) who indicated that species in the Noctuidae, Pyralidae and Plutellidae can develop resistance when exposed to Bt in selection experiments under laboratory conditions and in the field. The large scale use of Bt crops have put considerable selection pressure on target species. To date field evolution of resistance has been rare and only detected in *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in South Africa (Van Rensburg, 2007), *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in the south-eastern United States (Tabashnik, 2008, Tabashnik *et al.*, 2008a) and *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in Puerto Rico (Matten *et al.*, 2008; Storer *et al.* 2010). Resistance to Bt cotton has also recently been reported in the Pink bollworm (*Pectinophora gossypiella*) (Saunders) (Lepidoptera: Gelechiidae) in India (Monsanto, 2010).

The importance of refugia in the delay of resistance evolution has been pointed out by several authors (Bourguet, 2004; Tabashnik *et al.*, 2003; Tabashnik, 2008). Refuges are defined as habitats in which the target pest is not under selection pressure due to the toxin and it therefore provides a sustainable habitat for pest development. The principle underlying the high dose/refuge strategy is that any resistant insects emerging from the Bt crop are more likely to mate with one of the much larger number of susceptible pest insects emerging from refugia than with each other, thereby decreasing the selection of Bt resistance alleles (Bourguet, 2004). Analysis of more than a decade of resistance monitoring data for six Lepidoptera species targeted by Bt maize and cotton suggests that the principles of the refuge strategy may apply in the field (Tabashnik *et al.*, 2008b).

The first evaluation of various Bt maize events for control of the maize stem borer, *B. fusca* in South Africa was conducted between the 1994/95 and 1996/97 growing seasons (Van Rensburg, 1999). This was done by means of field and greenhouse evaluations of Bt maize under artificial infestation with the target pest. Results concluded that various events of the *Cry1Ab* gene were not equally effective for control of *B. fusca*. MON810 and MON802 was reported to be superior to other events, both in inbred material and in hybrid combinations. While Bt11 has been released in South Africa the majority of hybrids contain event MON810. Bt maize was released in South Africa, during the 1998/99 growing season when approximately 50 000 ha were planted. The area planted to Bt-maize increased to approximately 1 million ha in 2007. At harvest of the 1999 growing season, crop damage to the lower stems caused by *B. fusca* was noticed on a considerable scale at a number of localities, involving various Bt maize hybrids (Van Rensburg, 2001). Van Wyk *et al.* (2007) also reported increased incidence of *B. fusca* larvae on Bt-maize during the post-flowering period on the Highveld region of South Africa. Since no leaf feeding damage occurred during the vegetative growth stages of these plants, this damage seemed to indicate increased survival of larvae resulting from late oviposition, presumably during the period from tasseling to grain filling. No yield losses could be attributed to these infestations, but the observation caused concern due to the possibility that similar infestations may in future result in significant damage to ears, particularly in years when late spring rains necessitate the use of relatively late planting dates (Van Rensburg, 2001). It could also contribute towards the evolution of Bt-tolerant stem borer populations. Since YieldGard technology claims control of only the first two larval instars, it seems probable that increased survival of neonate and second instar larvae on Bt maize may result from feeding on some less toxic plant parts followed by stem tunneling at late plant growth stages (Van Rensburg, 2001).

The first report of field resistance of *B. fusca* to Bt-maize was subsequently made in the Christiana area by Van Rensburg (2007) who showed that the larvae on Bt maize at certain locations attained a level of resistance where some larvae were able to survive in the presence of the Bt-toxin but not without some detrimental effect on larval growth rate.

Within one year of the first report of resistance of *B. fusca* another reportedly resistant population was observed by farmers at the Vaalharts irrigation scheme, approximately 50 km from the initial site.

The objective of this study was to evaluate the status of resistance of different populations of *B. fusca* to Bt maize and to evaluate the level of resistance of borer populations occurring on the refuge-plantings of maize at the locations where resistance was reported.

2.2 Material and methods

One greenhouse and two laboratory studies were conducted during 2008 and 2009, respectively.

2.2.1 Greenhouse study (January - February 2008)

2.2.1.1 Collection sites

Four *B. fusca* populations were collected during December 2007 at three farms in Vaalharts (Fig. 2.1) from infested maize plants that were between four and seven weeks old. Larvae were collected by dissecting between 100 and 200 plants inside the respective Bt and conventional/non-Bt fields described below (Fig. 2.2). Approximately 250 4th-instar larvae were collected from each of the Bt and conventional maize fields at each locality, reared through to adults on Bt and conventional maize respectively and the F1-generation used in the experiment.

After farmers reported another possible resistant population in the Vaalharts irrigation scheme, larvae were collected from Bt maize (designated VAA08Bt), as well as the refuge of conventional maize planted adjacently to the Bt maize field (designated VAA08Ref). These fields were planted as prescribed in the user guide for the production of YieldGard maize (Monsanto, 2007).

A susceptible population (designated CHR08Con) was collected on conventional maize planted on a farm near Christiana where Van Rensburg (2007) reported resistance, 50 km from the Vaalharts site (Fig. 2.1). Another susceptible population of stem borers outside of the Vaalharts region was collected from conventional maize in the Bethal area (Mpumalanga province) approximately 450 km away from

the above mentioned sites (designated BET08Con). Planting of Bt maize is less common in the Mpumalanga Province where dryland maize production is the norm. The larvae were reared to adult stage and the F1 used in the larval survival study which was done under greenhouse conditions.

2.2.1.2 Larval survival study

The experiment comprised of eight treatments which consisted of four stem borer populations evaluated on two maize hybrids. These stem borer populations were designated the VAA08Bt, VAA08Ref, CHR08Con, BET08Con populations which were tested on each of a Bt- and conventional hybrid. The following two hybrids were used: DKC 78-15B (transgenic, MON810), CRN 3505 (non-Bt iso-hybrid for DKC 78-15B).

The study was conducted in a greenhouse using potted maize plants (Fig. 2.3). There were 90 potted plants for each treatment. Plants were infested by placing 10 neonate larvae into the whorl of each plant by means of a camel-hair brush, 20 days after emergence. Nine plants of each treatment were dissected 2, 4, 6, 8, 12, 16, 20, 25, 30 and 35 days after inoculation. Dissection of plants inoculated with larvae from the BET08Con population was terminated on day 25 due to a limited number of available F1 neonate larvae and infested plants.

The numbers and mass of live larvae per plant were determined at each sampling date. The experiment was terminated on day 35 when the first pupae started to form. For this reason larval mass determined on day 30 was used in analyses to avoid the effect that the change to pupae could have on mass. Mean larval mass was calculated for each sampling date and survival was expressed as a percentage.

The percentage and number of dead and live larvae of the different borer populations on Bt and conventional maize, 30 days after inoculation was also calculated. Lethal time (LT50), indicating the time (number of days) until 50 % mortality was observed was calculated for each population.

2.2.2 Laboratory study (February – March 2009)

2.2.2.1 Collection sites

A total of approximately 1200 fourth-instar stem borer larvae (200 per farm) were collected from Bt maize on six farms at the Vaalharts irrigation scheme during December 2008, after reports of larvae surviving on Bt maize were received from farmers. Each population was reared on Bt maize for one generation to confirm resistance (reported below), after which populations were pooled (designated VAA09Bt). One population was also collected from the refugia (200 larvae) consisting of conventional maize on one of the six farms (designated VAA09Ref). A susceptible control population of 150 larvae was also collected from conventional maize at Viljoenskroon (designated Vil09Con) in the Free State province, 220 km away from the Vaalharts irrigation scheme. At this locality maize is planted under dryland conditions and Bt maize is less common. The F1-neonate larvae originating from populations collected from the Bt-, refuge- and conventional fields were used in the laboratory experiment.

2.2.2.2 Larval survival and mass

The experiment consisted of six treatments viz. three stem borer populations (VAA09Bt, VAA09Ref and Vil09Con) on each a Bt- and conventional hybrid. The following two hybrids were used: DKC 78-15B (transgenic, MON810), CRN 3505 (non-Bt iso-hybrid for DKC 78-15B).

Larvae were reared in glass test tubes (200 x 25 mm) containing a cut maize stem with the base of the whorl still intact (Fig. 2.4). Stems were cut from 4 – 6 week old potted plants grown in a greenhouse. Ten neonate larvae were used per test tube and were placed into the whorl of the cut stem. For the first 15 days the same whorl tissue was provided since the stem was cut approximately 10 cm below the whorl of the plant and 5 cm of the base of the whorl was left intact. From 18 days onwards, when larvae reached late 3rd and 4th instars, only a 15 cm long cut stem was provided as food and every three day a new stem was provided. All tubes were kept at room temperature which ranged between 20 – 25 °C.

To ensure representative samples of each stem borer population, each test tube contained larvae originating from a different female moth. Due to the shortage of female moths, there were nine replicates for the Vil09Con, while the VAA09Ref and VAA09Bt consisted of 21 and 29 replications (offspring per female) respectively. Due to variation in age of larvae collected in the field, asynchrony in development and availability of male and female moths, the number of replications differed between populations.

The number and mass of live larvae per plant were determined at 3-day intervals up to 35 days after inoculation. The experiment was terminated on day 35 when the first pre-pupae were observed. Survival was recorded and expressed as percentages. Data on larval mass determined on day 30 was used in analysis to avoid the effect that the change to pupae could have on mass. The percentage and number of dead and live larvae 30 days after inoculation was calculated. Lethal time (LT50) was calculated for each population.

2.2.3 Statistical analysis

Mean mass per larva was calculated for each of the different populations at day 30 after inoculation and compared between populations by means of ANOVA (StatSoft Inc., 2009) after which means were separated using the Tukey test for significant differences. Lethal time (LT50), indicating the time (number of days) until 50 % mortality was observed for each population was calculated using logistic regressions of larval survival over time (StatSoft Inc., 2009). Fiducial limits were calculated for each population.

2.3 Results

2.3.1 Greenhouse study (January - February 2008; F1)

2.3.1.1 Larval survival

The lowest levels of survival on Bt maize were observed for the populations collected at Christiana (designated CHR08Con-Bt) and the Bethal population (BET08Con-Bt). Survival of these two populations decreased rapidly until 100% mortality was observed within 12 days (Fig. 2.5). The highest levels of survival were observed for the VAA08Ref-NBt and the CHR08Con-NBt populations on conventional maize. The F1-generation of the VAA08Bt-Bt population collected from Bt maize and the

adjacent refuge planting (VAA08Ref-Bt) survived on Bt maize plants until the pupal stage was reached with 15.5 % and 7.7 % survival respectively (Fig. 2.5). Survival of the VAA08Ref and VAA08Bt maize-collected populations was similar on Bt- and conventional maize, respectively. The VAA08Ref-Bt population had the lowest LT50 of 3.41 days and BET08Con-NBt population on conventional maize the highest LT50 of 4.8 days (Table 2.1). There were no significant differences observed between LT50-values of the VAA08Bt-Bt and VAA08Ref-Bt populations tested on Bt maize (Table 2.1).

2.3.1.2 Larval mass

Mean mass increased steadily and did not differ significantly between any of the treatments up to 25 days after inoculation (Fig. 2.6). Due to shortage of inoculated plants for the BET08Con population only one dissection was done 25 days after inoculation. Larvae of this population had the greatest mass on day 25.

There were significant differences in mean larval mass between different populations collected from VAA08Ref, VAA08Bt and CHR08Con maize on day 30 (Table 2.3) with larvae from the CHR08Con-NBt population on conventional maize being the largest. There were no significant differences in larval mass between the VAA08Bt-Grh populations collected from Bt- and conventional maize. However, larvae from the CHR08Con population collected at Christiana were significantly heavier than any of the other populations from Vaalharts.

2.3.2 Laboratory study (February – March 2009; F2)

2.3.2.1 Larval survival

Hundred percent mortality was observed for larvae of the Vil09Con-Bt population, six days after inoculation onto Bt maize. The highest levels of survival were observed for larvae of the VAA09Bt-Bt population on Bt maize and those collected from the VAA09Ref-Bt population feeding on Bt maize (Fig. 2.7). The decline in larval survival of the Vil09Con-NBt population from Viljoenskroon on conventional maize was similar to that of the VAA09Bt-Bt population on Bt maize (Fig. 2.7). In this study the VAA09Bt-NBt population on conventional maize had the highest LT50 (18.02 days) of the different *B. fusca* populations, while the Vil09Con-Bt population on Bt maize had the lowest LT50 and died within three days after inoculation (Table 2.2).

2.3.2.2 Larval mass

The mean mass per larva increased steadily up to day 18 after which a rapid increase was observed for all treatments (Fig. 2.8). Mass of larvae of the VAA09Bt and VAA09Ref populations were significantly greater on conventional than on Bt maize compared to those in the greenhouse study. Mass of larvae of the Vil09Con-NBt population on conventional maize was greatest throughout the study and was significantly different from all the other populations 30 days after inoculation (Table 2.4).

2.4 Discussion

Evolution of resistance is defined as a heritable decrease in a population's susceptibility to a toxin (Gassmann *et al.*, 2009; Tabashnik, 1994; Tabashnik *et al.*, 2008b). Results from these studies confirmed resistance of *B. fusca* at Vaalharts to Bt maize since larvae that were collected from the field, were reared to the adult stage on Bt after which the F₁-generation also survived on Bt maize. In the greenhouse study the observed tendencies on larval survival showed three groupings of lines (Fig. 2.4), indicating resistance levels that seemingly varied from susceptible to resistant. The borer population with expected resistance to Bt maize (VAA08Bt) as well as its population (VAA08Ref) successfully completed their life cycles on Bt maize, with no significant difference observed in either the level of survival (Table 2.3) or mean larval mass (Fig. 2.6) compared with controls on non-Bt maize. This result indicated that the larvae that occurred in the refuge were also resistant to Bt maize. The question arises whether the use of refugia could still serve its purpose in the Vaalharts irrigation scheme where resistance now seems to be wide-spread. If a large enough susceptible pest population is not maintained, alternative strategies will have to be investigated to manage the further spread of resistance.

Larvae of the Vaalharts population used in the greenhouse study were reared successfully for another three generations on Bt-maize after completion of the experiment. Similarly, larvae from the laboratory experiment were successfully reared for another generation after completion of the experiment.

In the greenhouse study, larvae of both the Christiana- (CHR08Con population) and Bethal populations (BET08Con) died on Bt-maize within 12 days, indicating that these populations were highly susceptible to Bt-maize. The latter's survival levels were similar to those observed for Bt-susceptible *B. fusca* populations during initial resistance evaluations by Van Rensburg (2001) and another noctuid species, *Sesamia calamistis* (Van den Berg & Van Wyk, 2007; Van Wyk *et al.*, 2009) on Bt maize. The survival of larvae collected from non-Bt maize at Christiana indicates that the specific population that was collected was susceptible to Bt maize and that resistance was not wide spread in the area where the first report of resistance to Bt maize was made by Van Rensburg (2007).

The decrease in survival over time observed in all treatments can also partly be ascribed to density dependent larval migration. In this study a significant number of larvae disappeared due to emigration from plants and only the larvae present on plants were counted. The reduced numbers of larvae inside plants was ascribed to immigration and not mortality of larvae. Since stem borer larvae have cryptic feeding habits in whorls and stems of plants, the remains of dead or dying larvae are present and can easily be observed. Previous studies by Van Rensburg & Van Rensburg (1987) on conventional maize under field conditions reported a gradual decline in *B. fusca* survival over time until 16 days after infestation. A marked decrease occurs around 20 days after infestation and, under field conditions, larvae leave the whorl and migrate to adjacent plants in a search of suitable feeding and pupating sites (Van Rensburg & Van Rensburg, 1987).

An effective high dose/refuge strategy requires three main components. First, the increase in fitness conferred by resistance alleles must be recessive so that individuals heterozygous for a resistance allele are killed by the toxin produced by plant tissues. Second, resistance alleles must be rare so that few homozygotes survive on the Bt crop. Thirdly, one of the assumptions of the high dose/refuge strategy is that resistant insects selected on Bt crops mate randomly, or preferentially with susceptible insects preserved on non-Bt crops (Bourguet, 2004). Results from this study indicate that the high dose/refuge strategy may be compromised in effectiveness in this geographical area since larvae that occur on non-Bt maize plants inside refugia also showed resistance to Bt maize.

Although the evolution of resistance of *B. fusca* can probably be ascribed to several factors, the low initial levels of compliance to refuge requirements most likely played an important role. Although the planting of refugia is compulsory to limit evolution of resistance (Monsanto, 2007), the level of compliance between 1998 and 2006 was shown to be low in the region where resistance was reported in South Africa (Kruger *et al.*, 2009, Chapter 3). Furthermore, Van Rensburg & Van Rensburg (1987) indicated that rainfall and humidity are important environmental factors affecting the abundance of *B. fusca* moths. Moths possibly prefer irrigated maize, which could have contributed to increased selection pressure towards the evolution of resistance to the Bt toxin (Van Rensburg, 2007). Van Wyk *et al.* (2008) also indicated that the strong linkage of stem borers to the maize ecosystem in irrigated areas and especially the planting of Bt-maize in these systems results in strong selection pressure for evolution of resistance.

This study confirmed resistance by *B. fusca* to the *Cry1Ab* toxin, indicating the geographical distribution of resistant populations to include at least the Vaalharts area, in addition to the original report by Van Rensburg (2007) for the Christiana area. Further research is needed on possible fitness costs associated with resistance evolution as well as insect resistance management and the high/dose refuge strategy in order to limit the evolution and spread of Bt-resistant populations to other maize production regions in the country.

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Table 2.1 LT 50 (number of days) of different *Busseola fusca* populations on Bt and conventional maize in a greenhouse study.

Population	LT50 (days)	95% Fiducial limits
BET08Con-Bt	2.22	1.82 – 2.60
CHR08Con-Bt	2.41	2.08 – 2.72
VAA08Bt-NBt	3.21	2.47 – 3.95
VAA08Bt-Bt	3.27	2.47 – 4.05
VAA08Ref-Bt	3.41	2.70 – 4.11
BET08Con-NBt	4.80	4.27 – 5.32
VAA08Ref-NBt	7.21	6.10 – 8.33
CHR08Con-NBt	8.71	7.49 – 9.95

VAA08Ref-Bt = Refuge population on Bt maize; VAA08Ref-NBt = Refuge population on conventional maize; VAA08Bt-Bt = Vaalharts Bt population on Bt maize; VAA08Bt-NBt = Vaalharts Bt population on conventional maize; CHR08Con-Bt = Christiana conventional population on Bt maize; CHR08Con-NBt = Christiana conventional population on conventional maize; BET08Con-Bt = Bethal population on Bt maize; BET08Con-NBt = Bethal population on conventional maize.

Table 2.2 LT 50 (number of days) of different *Busseola fusca* populations on Bt and conventional maize in a laboratory experiment.

Population	LT50 (days)	95% Fiducial limits
Vil09Con-Bt	3.02	–
VAA09Ref-Bt	6.84	5.77 – 7.83
Vil09Con-NBt	7.57	6.41 – 8.61
VAA09Bt-Bt	8.96	8.19 – 9.69
VAA09Ref-NBt	12.77	11.79 – 13.71
VAA09Bt-NBt	18.02	17.23 – 18.80

VAA09Ref-Bt = Refuge on Bt maize; VAA09Ref-NBt = Refuge population on conventional maize; VAA09Bt-Bt = Vaalharts Bt population on Bt maize; VAA09Bt-NBt = Vaalharts Bt population on conventional maize; Vil09Con-Bt = Viljoenskroon conventional population on Bt maize; Vil09Con-NBt = Viljoenskroon conventional population on conventional maize.

Table 2.3 Mean larval mass and percentage survival of *Busseola fusca* populations feeding on Bt and conventional maize under greenhouse conditions. (SE = standard error).

<i>Busseola fusca</i> population	Mean larval mass (mg) on day 30 ± SE*	Percentage survival on day 30 ± SE**	Percentage survival on day 35 ± SE***
VAA08Ref-Bt	0.8 a ± 0.001	2.2 a ± 1.9	7.7 a ± 2.77
VAA08Bt-Bt	2.4 a ± 0.001	1.1 a ± 0.90	3.3 a ± 1.66
VAA08Bt-NBt	5.0 a ± 0.001	2.2 a ± 1.46	5.5 a ± 1.76
VAA08Ref-NBt	15.7 a ± 0.04	8.8 ab ± 5.04	8.8 a ± 3.80
CHR08Con-NBt	69.5 b ± 0.02	17.7 b ± 5.47	12.2 a ± 3.23

* Means within columns followed by the same letter do not differ significantly according to Tukey test-HSD (F = 10.25; df = 40; P = 0.0008).

** Means within columns followed by the same letter do not differ significantly according to Tukey test-HSD (F = 3.84; df = 40; P = 0.009).

*** Means within columns followed by the same letter do not differ significantly according to Tukey test-HSD (F = 1.68; df = 40; P = 0.171).

VAA08Ref-Bt = Refuge population on Bt maize; VAA08Ref-NBt = Refuge population on conventional maize; VAA08Bt-Bt = Vaalharts Bt population on Bt maize; VAA08Bt-NBt = Vaalharts Bt population on conventional maize; CHR08Con-NBt = Christiana conventional population on conventional maize.

Table 2.4 Mean larval mass and survival of *Busseola fusca* populations feeding on Bt and conventional maize under laboratory conditions. (SE = standard error).

<i>Busseola fusca</i> population	Mean mass (mg) on day 30 ± SE*	Percentage survival on day 30 ± SE**	Percentage survival on day 33 ± SE***
VAA09Ref-Bt	186.5 a ± 0.26	12.0 a ± 2.45	10.3 ab ± 2.01
VAA09Bt-Bt	201.8 a ± 0.21	14.1 a ± 2.19	12.1 a ± 2.26
VAA09Ref-NBt	261.9 a ± 0.30	17.9 a ± 3.23	15.1 a ± 2.96
VAA09Bt-NBt	278.4 b ± 0.26	30.6 c ± 2.62	24.8 c ± 2.51
VIL09Con-NBt	289.9 b ± 0.16	2.4 b ± 0.94	2.4 b ± 0.94

*Means within columns followed by the same letter do not differ significantly according to the Tukey-HSD (F = 10.39; df = 140; P = 0.0411).

** Means within columns followed by the same letter do not differ significantly according to Tukey test-HSD (F = 18.14; df = 140; P = 0.0001).

*** Means within columns followed by the same letter do not differ significantly according to Tukey test-HSD (F = 13.00; df = 140; P = 0.0001).

VAA09Ref-Bt = Refuge on Bt maize; VAA09Ref-NBt = Refuge population on conventional maize; VAA09Bt-Bt = Vaalharts Bt population on Bt maize; VAA09Bt-NBt = Vaalharts Bt population on conventional maize; Vil09Con-NBt = Viljoenskroon conventional population on conventional maize.

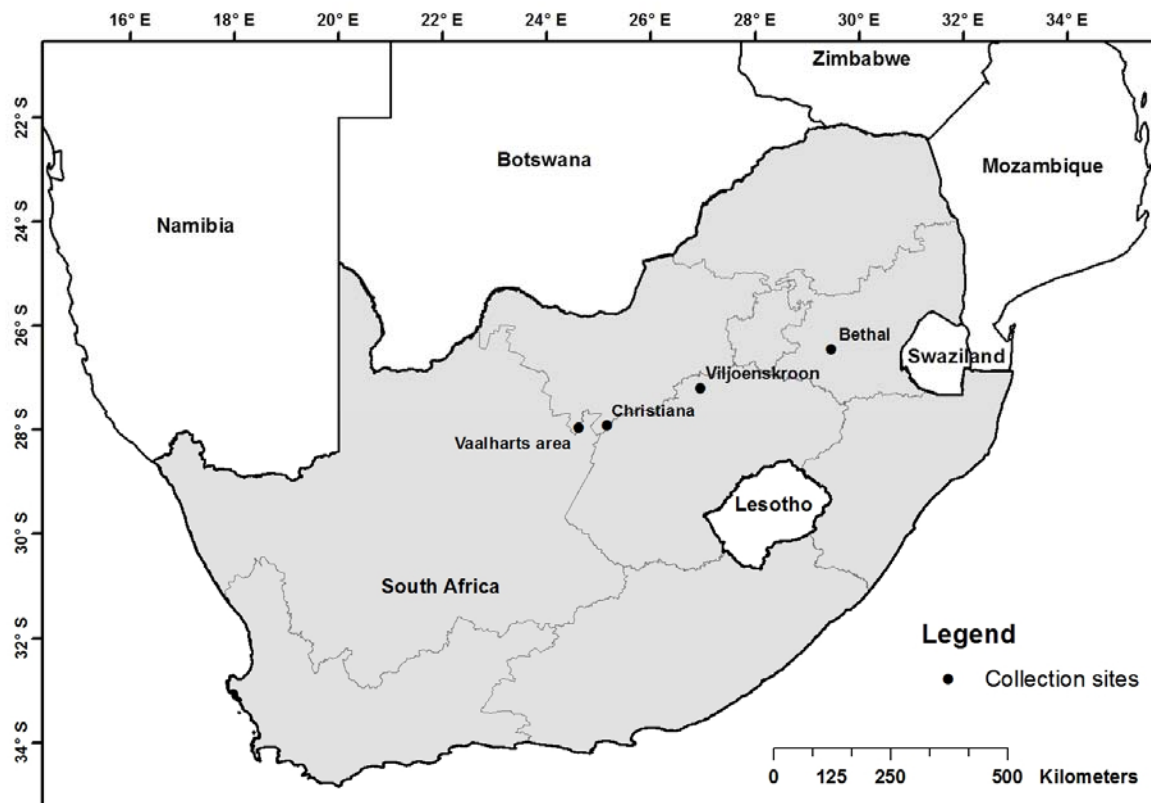


Fig.2.1 Collection sites of different *Busseola fusca* populations in South Africa.



Fig. 2.2 Collection of stem borer infested maize plants.



Fig. 2.3 Greenhouse study potted maize plants.



Fig. 2.4 Test tubes used to rear stem borer larvae in the laboratory.

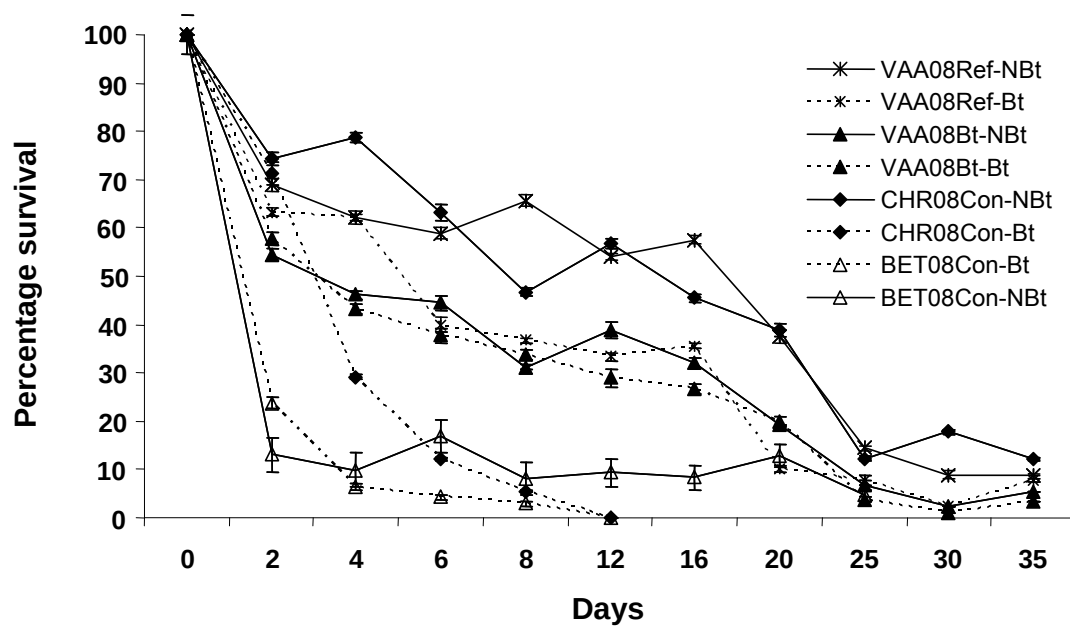


Fig. 2.5 Larval survival of different *Busseola fusca* populations on Bt and conventional maize under greenhouse conditions (Bars indicate Standard errors). Solid lines represent conventional maize and dotted lines represent Bt maize. Refuge and Bt populations were collected at Vaalharts, the conventional populations were from Christiana and Bethal.

VAA08Ref-Bt = Refuge population on Bt maize; VAA08Ref-NBt = Refuge population on conventional maize; VAA08Bt-Bt = Vaalharts Bt population on Bt maize; VAA08Bt-NBt = Vaalharts Bt population on conventional maize; CHR08Con-Bt = Christiana conventional population on Bt maize; CHR08Con-NBt = Christiana conventional population on conventional maize ; BET08Con-Bt = Bethal population on Bt maize; BET08Con-NBt = Bethal conventional population on conventional maize.

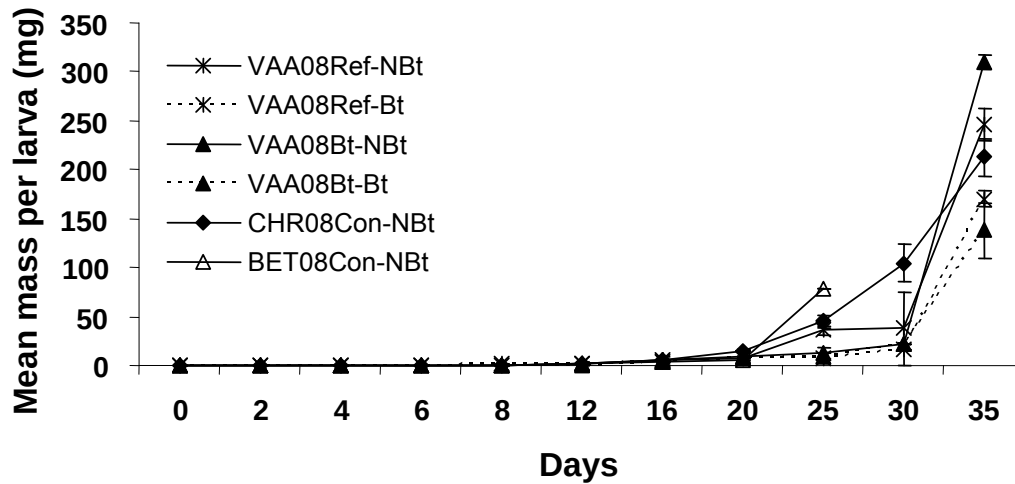


Fig. 2.6 Mean larval mass of different *Busseola fusca* populations on Bt and conventional maize under greenhouse conditions (Bars indicate Standard errors). Solid lines represent conventional maize and dotted lines represent Bt maize. Refuge and Bt populations were collected at Vaalharts, the conventional populations were Christiana and Bethal.

VAA08Ref-Bt = Refuge population on Bt maize; VAA08Ref-NBt = Refuge population on conventional maize; VAA08Bt-Bt = Vaalharts Bt population on Bt maize; VAA08Bt-NBt = Vaalharts Bt population on conventional maize; CHR08Con-NBt = Christiana conventional population on conventional maize; BET08Con-NBt = Bethal conventional population on conventional maize.

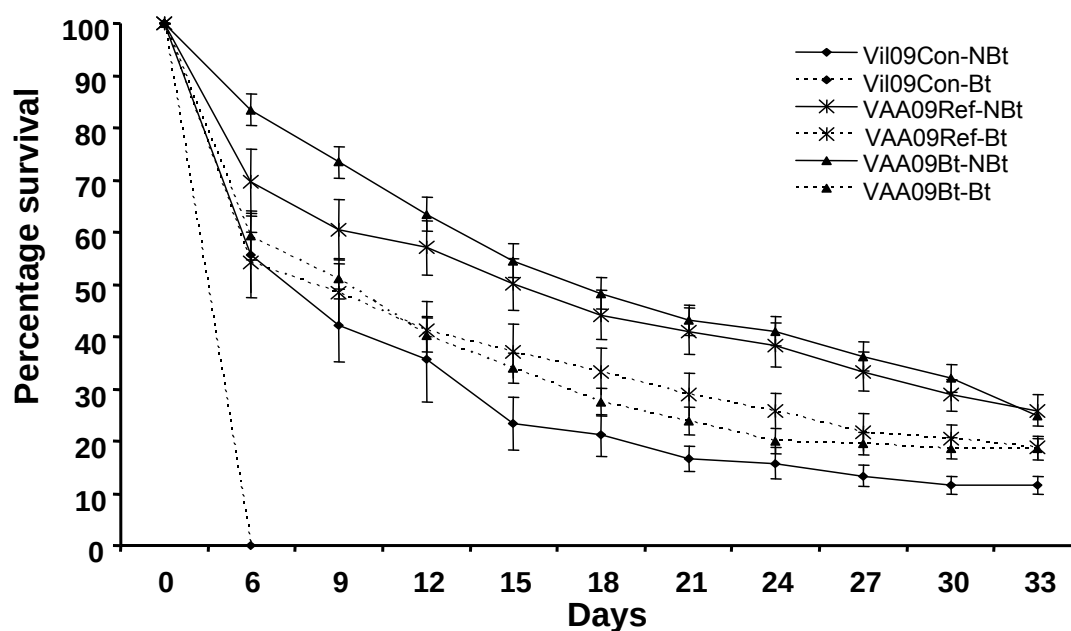


Fig. 2.7 Larval survival of different *Busseola fusca* populations on Bt and conventional maize under laboratory conditions (Bars indicate Standard errors). Solid lines represent conventional maize and dotted lines represent Bt maize. The conventional population was collected at Viljoenskroon while Refuge and Bt populations were collected at Vaalharts.

VAA09Ref-Bt = Refuge population on Bt maize; VAA09Ref-NBt = Refuge population on conventional maize; VAA09Bt-Bt = Vaalharts Bt population on Bt maize; VAA09Bt-NBt = Vaalharts Bt population on conventional maize; Vil09Con-Bt = Viljoenskroon conventional population on Bt maize; Vil09Con-NBt = Viljoenskroon conventional population on conventional maize.

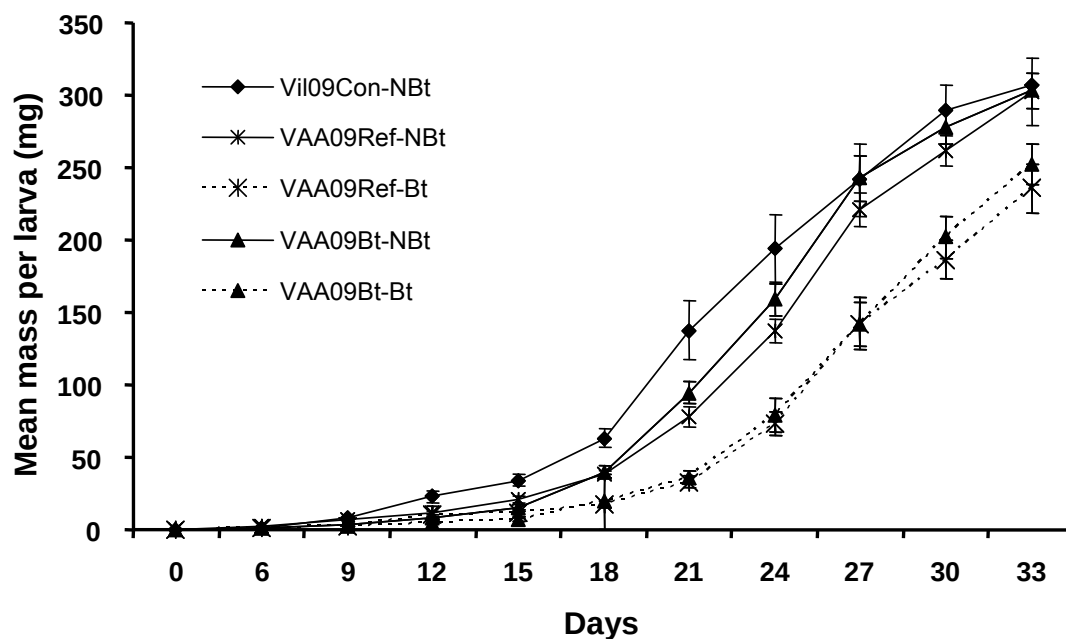


Fig. 2.8 Mean larval mass of different *Busseola fusca* populations on Bt and conventional maize under laboratory conditions (Bars indicate Standard errors). Solid lines represent conventional maize and dotted lines represent Bt maize. The conventional population was collected at Viljoenskroon while the refuge and Bt populations were collected at Vaalharts.

VAA09Ref-Bt = Refuge population on Bt maize; VAA09Ref-NBt = Refuge population on conventional maize; VAA09Bt-Bt = Vaalharts Bt population on Bt maize; VAA09Bt-NBt = Vaalharts Bt population on conventional maize; Vil09Con-NBt = Viljoenskroon conventional population on conventional maize.

Chapter 3

Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa

Abstract

Bt maize has been grown at the Vaalharts irrigation scheme in South Africa since its first release during 1998. Interest in Bt maize refuge compliance, pest incidence and production practices at Vaalharts was recently stimulated by the first report of field resistance of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) to Bt maize. Objectives of this study were to evaluate farmer's perceptions of the regulatory aspects guiding the planting of Bt maize and refugia and how the field situation developed between 1998 and 2008. A survey, using a self-administered questionnaire, was conducted amongst 80 farmers at the irrigation scheme. The questionnaire addressed signing of contracts upon purchasing genetically modified (GM) seed, refuge compliance, refuge design and general farming practices. Farmers were also questioned on the perceived benefits and disadvantages of Bt maize and their perceptions of the pest status of *B. fusca*. The two greatest advantages associated with Bt maize were shown to be convenient management (88 %) and increased productivity (61.3 %) while 42.5 % of respondents indicated that they perceived Bt technology to be environmentally friendly. Initial levels of refuge compliance was low, and even though farmers were obligated to plant a refuge area for each Bt maize field, only 7.7 % of farmers planted refuges during 1998. This number increased to 100 % during 2008. Eight percent of farmers, however, indicated that they did not plant a refuge field for each Bt maize field which was justified on the basis of small farm sizes (25 ha). Nearly all farmers (99.8 %) allow no spatial separation between the Bt maize field and adjacent refuge area. Farmers preferred to plant the refuge option where 5 % of the field area is planted to conventional maize which is not sprayed with insecticide instead of the 20 % refuge area on which insecticide application against the target pest is allowed. In South Africa stewardship programs instituted during the 2008/2009 growing season, involve grower education

programs as well as the compulsory signing of contracts between companies and farmers that contractually bind them to comply with refuge requirements accompanied by on-farm inspections. It appears that stem borer resistance to Bt maize in the Vaalharts area resulted from a combination of a late general planting date with consequent increased levels of infestation and variance in time of planting providing a continuous supply of moths.

Published as: **Kruger, M., Van Rensburg, J.B.J. and Van den Berg, J.** 2009. Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa. *Crop Protection* 28: 684-689.

3.1 Introduction

In South Africa Bt maize has been planted since 1998 and the country is the 8th biggest producer of Bt maize in the world (James, 2009). Bt maize has been grown at the Vaalharts irrigation scheme since its first release in South Africa during 1998. The target pests of Bt maize in South Africa are the stem borers *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). Interest in Bt maize production practices and refuge compliance at Vaalharts was recently stimulated by the first report of field resistance of a target pest (*B. fusca*) to Bt maize (Van Rensburg, 2007). Within one year of the first report of resistance another resistant population was recorded in the Vaalharts irrigation scheme, approximately 60 km from the site of the initial report (Kruger *et al.*, accepted, Chapter 2). No effective management strategy currently exists to limit the spread and further development of resistant populations. Although the planting of refugia to limit resistance development is compulsory (Monsanto, 2007), the level of compliance is not known. The current refuge requirements are either a 20 % refuge planted to conventional maize which may be sprayed with insecticides, or a 5 % refuge area that should not be sprayed. The reasons for development of resistance by *B. fusca* to Bt maize are not known. It is, however, possible that non-compliance to refuge requirements played a role.

The high dose/refuge strategy, employed to limit resistance development, comprises a combination of Bt maize plants producing high doses of toxin and non-Bt plants in close proximity to one another. The purpose of the high dose of toxin is to kill as many individuals of the target pest as possible, whereas the purpose of the refuge is to produce pest individuals that survive on that particular crop (Renner, 1999; Gould, 2000). The goal is to ensure that those rare, Bt resistant individuals that survive on the Bt crop do not produce completely resistant offspring by mating with other toxin-resistant individuals. Instead, susceptible individuals from the refuge can mate with toxin-resistant individuals that survive on the engineered plants. Offspring from the combination of a susceptible and resistant individual are expected to have only a low to moderate level of toxin resistance. These individuals should not be able to survive on

plants with high Bt toxin levels (Renner, 1999; Gould, 2000), lowering the chance of resistant populations developing in a given area.

When assessing farmers' perceptions of innovations such as transgenic crops, surveys can be useful tools (Grieshop *et al.*, 1988). Surveys can generally determine the success or failure of an innovation after the technology has been introduced and used by farmers (Pilcher & Rice, 1998). A unique opportunity exists in this case of Bt maize to gain insight from farmers on the use of Bt maize for stem borer control. A survey of farmers' perceptions and knowledge will be beneficial to extension specialists, crop consultants and industry in developing educational information for farmers (Pilcher & Rice, 1998) and to determine levels of compliance to regulatory requirements such as planting of refugia.

The objectives of this study were to evaluate farmers' perceptions of the regulatory aspects guiding the planting of Bt maize and refugia, its benefits and disadvantages as well as their perceptions of the pest status of *B. fusca* in the Vaalharts irrigation scheme.

3.2 Material and methods

3.2.1 Study area

The Vaalharts irrigation scheme (S24°48'693 E27°38'330) is situated in the semi-arid Northern Cape Province of South Africa, between 1050 and 1150 m above sea level. The irrigation scheme comprises an area approximately 80 km from North to South and 30 km from East to West. In total there is currently 30 000 ha of crop production under irrigation (Bornman, 1988; De Jager, 1994) all divided into 25 ha plots (Fourie, 2006). The main crops planted in the area are maize, wheat, barley, lucerne and groundnuts with a myriad of less significant crops also being grown (Vermeulen, J., Agronomist; Senwes-Coop., personal communication, 2008). Maize has only been recognised as one of the main crops since 1998, with an annual average of 9000 ha being grown under flood- or centre-pivot irrigation. This shift in favour of maize can be attributed to the higher yield provided by centre-pivot irrigation in conjunction with its lower labour

costs compared to flood irrigation. This trend was also influenced by the development of ultra-short growing season maize cultivars which allow farmers to plant an additional crop per year which was not previously possible. The large scale advent of centre-pivot irrigation in the area, five years ago, also allowed farmers to start planting earlier in the season.

3.2.2 Farmer survey

A survey was conducted amongst farmers in the Vaalharts irrigation scheme during May 2008. The survey was designed as a self-administered questionnaire. Questionnaires were pre-tested with three farmers and modifications were subsequently made. The survey sample of 80 farmers (from an approximate number of 400 maize farmers) was randomly selected from a list of producers in the irrigation scheme and any farmer cultivating maize was given an equal probability of being selected.

The questionnaire addressed basic questions on maize farming and the importance of genetically modified (GM) maize as a crop in their specific farming systems. The questionnaire was divided into five major categories that addressed the history of maize production in the area, farmer's perceptions of Bt maize, regulatory aspects and compliance to refuge requirements. Other questions addressed farming- and pest management practices.

3.2.3 Data analysis

Data were summarized in Excel and expressed as percentages that were calculated based on the total number of farmers who responded to a particular question. Farmers who did not respond to a particular question were excluded from the calculation of percentage values for that question. When a farmer selected more than one answer to a question, percentages were calculated using all answers given.

3.3 Results and discussion

A summary of the survey data is provided in five major categories. All the respondents were male, which, in South Africa is the norm on commercial farms.

3.3.1 Farmers' experience in maize cultivation

Farming experience with maize cultivation ranged between one and 64 years with the majority of farmers cultivating maize for 16 to 30 years (Table 3.1). Farmers started adopting yellow Bt maize during the 1998/99 season when approximately 47 % of them planted Bt maize for the first time (Fig. 3.1). Since 1999, the number of farmers planting Bt maize for the first time increased with an average of 5.9 % per year until 2007 when all farmers planted Bt maize. Fifty-four percent of farmers prefer to plant yellow maize, while 40 % plant both white and yellow maize and 6 % plant only white maize. Farmers started adopting white Bt maize during the 2001/02 season (Gouse, 2005).

Although the irrigation scheme is subdivided into 25 ha farm units, many farmers farm larger areas of land by renting or owning several farm units. Few farmers plant Bt maize as well as large conventional maize fields as was common practice before the introduction of GM maize. The number of hectares per farmer planted to Bt maize ranges from 4 to 400 ha. The majority of farmers (28 %) plant between 71 and 80 ha while only 23.5 % of farmers plant more than 100 ha (Table 3.1).

There are currently only two different Bt maize events available in South Africa. At the Vaalharts irrigation scheme 96 % of farmers plant hybrids deploying event MON810 (available in many hybrids from different seed companies) and 4 % plant hybrids with event Bt 11. The majority of farmers (53.3 %) plant seed supplied by two seed companies and less than 7 % make use of three companies' seed, in order to decrease the risk of cultivar / crop failure. These companies include Monsanto, Pannar, Pioneer Hybrid International, Afgri and Syngenta.

3.3.2 Farmers' perceptions of Bt maize and its benefits

Farmers indicated the two greatest advantages associated with Bt maize to be convenient management (88 %) and increased productivity (61.3 %) while 42.5 % indicated that they perceived Bt technology to be environmentally friendly. Although growing Bt maize increased seed costs, 91.3 % of farmers still considered it economically worthwhile to plant. The majority of farmers (87.5 %) were positive

towards Bt maize technology and deemed it advantageous to plant while 92 % did not think GM crops had a negative effect on the environment.

The greatest benefit associated with Bt maize in this study was indicated to be the convenience of management. In a similar study in the US, Wilson *et al.* (2005) reported that 69 % of farmers also considered ease of management and reduced insecticide applications as the main benefits of Bt maize.

In South Africa farmers have benefited from the adoption of Bt maize since its deployment during 1998 (Gouse *et al.*, 2005). Despite paying more for seed, adopters enjoyed increased income over conventional maize varieties through savings on pesticides and increased yield due to better pest control. Irrigation and dryland commercial farms surveyed in the Mpumalanga, Northern Cape and the North-West provinces enjoyed statistically significant yield increases of 11 % and 10.6 % respectively during the 1999/00 and 2000/01 seasons (Gouse *et al.*, 2005). However, Bt maize is only an advantage when target pests are present and there is no advantage to plant seed with the Bt-gene in areas where infestation pressure is generally low. Stem borer populations can vary in abundance from year to year and their pest status is not predictable. This is evident from research done by Van Rensburg *et al.* (1985) on *B. fusca*, which indicated large scale variation in infestation levels over seasons.

An important advantage in the growing of Bt maize is the reduction in number of insecticide applications. Reduction in pesticide application also reduces the potential pesticide drift onto other crops or environmentally sensitive areas (Meeusen & Warren, 1989). Because the active Bt toxin is produced directly in the crop tissue, concerns such as spray drift and groundwater contamination are obviated (Meeusen & Warren, 1989). The use of transgenic crops reduces the use of insecticides and minimizes the impact of these chemicals on non-target organisms and has positive health consequences for farm workers (Barton & Dracup, 2000).

3.3.3 Compliance to refuge requirements

The Genetically Modified Organisms Amendment act (Act 23 of 2006) guides the use of Bt maize in South Africa and obligate seed companies to sign contracts with farmers that purchase seed of GM crops. These contracts prescribe the use of the product user guide (Monsanto, 2007) and specify aspects such as the implementation of an insect resistance management program.

Results from this study indicated that initial refuge compliance was low. Even though farmers were obligated to plant a refuge area for each Bt maize field only 7.7 % of farmers planted refuges during 1998 (Fig. 3.1). The level of compliance increased with an average of 9.3 % per year until it reached 92.3 % during the 2007/08 season (Fig. 3.1). The number of farmers that signed contracts with seed companies for the first time slowly increased after 2003. The increase in the number of farmers that planted refugia for the first time increased significantly after 2004, possibly due to insect resistance reported from Christiana area (Van Rensburg, 2007). The cumulative percentage of farmers planting refugia followed the same tendency as the cumulative percentage of farmers signing 1st-time contracts. Survey data showed that no 1st-time contracts were signed during 1999, 2002 and 2004.

Farmers that planted refugia indicated that they used the prescribed refuge layout options (Fig. 3.2) (Monsanto, 2007). Forty-eight percent of farmers used the separate refuge field which for example includes a strip of conventional maize through the central area of a centre-pivot field. Perimeter-, block- and strip-refuge layouts were used by 14.3, 24.7 and 12 % of farmers respectively.

Approximately 70 % of farmers did, however, indicate that they did not read the contract or the user guide themselves, and many farmers (90.7 %) relied on the seed merchant for information pertaining to refuge area layout and requirements. Only a few farmers took time to read the documentation making sure what was expected from them. Sources of information with regard to Bt maize included farmers' days organised by

seed companies (16 %) while less than 10 % of farmers used agricultural advisors, printed media and insecticide companies.

Eight percent of farmers indicated that they did not plant a refuge field for each Bt maize field and justified this in terms of the patchy distribution and small field size on their farms. The spatial arrangement and the presence of many small fields, however, results in Bt maize fields usually being within the prescribed 400 m distance from the closest conventional maize field. During visits to the irrigation scheme incorrect refuge layout designs were, however, observed and it is doubted whether the prescribed refuge layouts were used prior to 2005 when the increased awareness of the importance of planting refugia was recognised. A few farmers indicated that farming systems with very small fields do not facilitate easy planting of refugia and questioned whether these small fields warranted the presence of refugia.

Nearly all farmers (99.8 %) allowed no spatial separation between the Bt maize field and adjacent refuge area. This practice may have allowed for exposure of migrating larvae to sub-lethal doses of the Bt toxin since *B. fusca* larvae migrate to adjacent plants from approximately 14 days after egg hatch. This may result in the survival of larger larvae that start feeding on Bt maize after they have grown to the 3rd and 4th instar on conventional maize inside the refugia. Since the Bt toxin concentration in certain plant parts may not be high enough to kill migrating larvae (Van Rensburg, 2001) these survivors may contribute to resistance development. Although the refuge planting is usually done directly adjacent to the Bt maize field, the refuge area may, however, be at any given distance within 400 m as stipulated in the user guide for the production of YieldGard maize (Monsanto, 2007). All surveyed farmers that planted refuge areas selected to plant the separate 5 % option instead of the 20 % option.

The very high adoption rate of Bt maize at the irrigation scheme implies that only 5 % of the approximate 9000 ha planted each year, is planted to conventional maize. Few “unstructured” refugia in the form of conventional maize fields are present at the scheme. The high incidence of Bt maize together with non-compliance to refuge

requirements during the past, could have contributed to resistance development in *B. fusca*. Bates *et al.* (2005) indicated that social and economic factors that result in less than 100 % adoption of Bt crops may result in regional “unstructured” refuges that may compensate in part for some violations of refuge requirements. This was, however, not the case at the Vaalharts irrigation scheme where high-input irrigation farming is practiced. Risk of economic damage to refugia may be the reason for the lack of compliance amongst farmers growing Bt crops and as noted by Bates *et al.* (2005) remains a major drawback for the high-dose/refuge strategy.

Bourguet *et al.* (2005) observed that non-compliance with requirements under the refuge strategy is due to the perception that Bt-susceptible individuals are common property shared by Bt maize growers. In other words, no one wants to take responsibility for protecting the resource of susceptible moths. By planting Bt crops, each grower increases personal short-term benefits but favors the selection of resistant pests, which may negatively affect the long term benefits of neighbouring farmers (Vacher *et al.*, 2006). The observed tendency of non-compliance to refuge requirements at the Vaalharts irrigations scheme, especially during the first few years could probably be ascribed to the short-term benefits that farmers expected and to the perception that many other farmers were still planting non-Bt maize. Rapid adoption of Bt-technology, up to a level of nearly 100 % over a short period of nine years probably created an environment in which farmers and seed companies did not foresee or realize the absence of refugia and the problem this was creating. A lesson that could be learned from this is that in areas where Bt-technology adoption rate is very high, refuge compliance should be followed-up and enforced.

In a study on how to induce farmers to plant refugia Vacher *et al.* (2006) concluded that the “refuge strategy” could provide sustainable resistance management as compared to a “fee-strategy” where Bt maize seed is made more expensive and the revenue is used to develop new varieties or management strategies. The latter strategy does not provide farmers with the incentive to select efficient actions to preserve the resource (Vacher *et al.*, 2006).

Although the initial level of compliance to refuge requirements for planting of Bt maize was low, it increased drastically over the past few years. Farmers are aware of resistance development of *B. fusca* and the regulatory requirements relating to Bt maize.

Stewardship programs managed by seed companies will contribute significantly to improved future management of GM technology and to the limiting of resistance development in target pests in other areas of the country. In South Africa, as part of the Bt maize stewardship program seed deliveries to farmers in certain areas include consignments of non-Bt maize seed to plant a 5 % refuge area. In South Africa stewardship programs instituted during the 2008/09 growing season, involve grower education programs as well as the signing of contracts between companies and farmers that contractually bind them to comply with refuge requirements. Companies furthermore do on-farm inspections, investigate cases of possible non-compliance and also institute punishment measures for farmers that do not comply.

3.3.4 Pest management practices

All farmers indicated that they applied only herbicides on the refuge areas and that no pesticides were used to control other pests on Bt or conventional maize. *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and *Astylus atromaculatus* (Blanchard) (Coleoptera: Melyridae) were two of three non-target pest species reported to attain pest status on Bt maize (Table 3.2). The red spider mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae) was reported by 72.3 % as a problem during the 2007/08 growing season and was a concern to farmers.

During the past nine years Bt maize production was perceived to provide effective control of stem borers at the Vaalharts irrigation scheme. Farmers indicated that stem borers were effectively controlled with Bt maize but that they already observed an increase in damage levels during the 2005/06 season. These levels of borer infestation on Bt maize increased towards the 2007/08 season, when nearly 50 % of farmers

observed stem borer damage on Bt maize for the first time (Fig. 3.3). During the 2007/08 growing season farmers experienced high stem borer infestation levels on Bt maize (Table 3.2) and were compelled to use insecticides to prevent economic crop losses (Fig. 3.4).

Since farmers were confident that Bt-technology would provide continuous protection against stem borers, they did not foresee that resistance development would happen over such a short time. Because of this farmers indicated that they did not consider scouting a necessity any more and fields were consequently not scouted as regularly as in the past. According to farmers the increasing pest pressure of stem borers on Bt maize was realised too late, possibly contributing to the rapid development of resistance of *B. fusca* to Bt maize. Wilson *et al.* (2005) indicated farmers' perceptions of Bt maize in the US showed that farmers were significantly less inclined to scout maize fields for the presence of pest damage. The importance of scouting on Bt maize should therefore be emphasized in awareness programs in order to have effective insect resistance management strategies, especially in areas where Bt maize is planted for the first time.

Seventy percent of farmers confirmed that insecticide applications would be necessary against stem borers if Bt maize was not planted, while 61.9 % indicated that insecticide sprays will have to be applied twice per growing season on conventional maize. No insecticides for *B. fusca* control were applied on Bt maize prior to the 2007/08 growing season when 55 % of farmers applied insecticides on both their Bt-maize and refuge areas in an effort to protect their crops against stem borer damage. During the 2007/08 cropping season, Monsanto carried spraying costs against stem borers for those farmers who planted early in the season and on whose Bt maize fields the incidence of stem borer infestation was higher than the economic threshold level for control (10 % infested plants).

3.3.5 Farming practices

In South Africa grain is graded according to quality and the presence of yellow grains in white grain consignments results in downgrading and loss of income. Because of the

small size (4-10 ha) of many maize fields at the Vaalharts irrigation scheme some farmers coordinate cultivar selection with that of neighbours to limit gene flow and contamination of white by yellow maize.

Farmers that did not utilise their fields for winter crops, tended to plant maize early in the season, between September and the first week of November. Late planting of maize which followed the harvest of a winter crop, was done between mid-November and the first week in January. The incidence of farmers indicating that they always planted early was low (8.6 %) while 62.3 % of farmers preferred to plant late. The other 19 % of farmers indicated that the planting date of maize was influenced annually by their respective crop rotation systems. Late planting of maize as well as the continuous availability of maize of younger growth stages results in the build-up of stem borer populations towards the end of the growing season (Van Rensburg *et al.*, 1987).

In the Vaalharts irrigation scheme three crops can be harvested within two years on a single field. Two methods of irrigation are used, i.e. flood- and centre-pivot irrigation. Forty-three percent of farmers use both irrigation methods while 43 % use centre-pivots only. Maize is rotated with cotton or groundnuts and in the winter wheat can be rotated with barley. The majority (54.8 %) of farmers make use of the following rotation system: maize, wheat/barley, maize, fallow field, cotton/groundnut. A cropping sequence of maize, wheat, maize, wheat/barley was followed by 40.2 % of farmers. Two percent of farmers used the following cropping sequence: wheat, maize, groundnut, watermelon and lucerne while 3 % followed maize with cotton, wheat/barley and groundnut. The most common soil cultivation strategy after harvesting maize is the burning of crop residues followed by a mouldboard plough (42.1 % of farmers). This or any cultivation method that destroys maize stubble results in death of overwintering *B. fusca* larvae that diapause inside the bases of maize stems. Soil cultivation can be used to suppress stem borer populations (Van den Berg *et al.*, 1998). However, many farmers (13.7 %) leave maize stubble in the soil and only burn maize crop residues before they plant the follow-up crop. Burning of crop residues followed by chisel plough is practiced by 17.9 % of farmers. Burning followed by ripping and a mouldboard plough was done by 10.2

% while 12.3 % of farmers used ripper and disc after burning. A minority of farmers use combinations of the above mentioned cultivation methods.

Except for the use of mouldboard ploughs, all other farming practices are conducive to the build-up and survival of stem borer populations at the Vaalharts irrigation scheme. Although crop rotation with non-host plants could be an effective method for stem borer control, its benefits will not realize in areas such as Vaalharts where farms are very small. The burning of crop residues or leaving of stubble in the fields where follow-up crops are planted, facilitates over-wintering of larvae of *B. fusca* which go into diapause at the base of the stem below soil level (Van den Berg *et al.*, 1998).

3.4 Conclusions

It appears that stem borer resistance to Bt maize in the Vaalharts area resulted from a combination of a late generally planting date with consequent increased levels of infestation and variance in time of planting providing a continuous supply of moths. This increased pest pressure, combined with non-compliance with refuge requirements, probably contributed to selection pressure that resulted in increased levels of insect tolerance to the Cry 1Ab toxin.

Closer collaboration between farmers, seed companies and research/extension services is needed to develop methods to delay resistance development, to emphasise the importance of compliance to requirements and to monitor compliance levels. The possible role of the unique spatial arrangement of Bt and non-Bt maize fields in the development of resistance needs to be further investigated. One possible insect resistance management strategy is the deployment of Bt events with different modes of action. The special arrangement of refuges needs further investigation with the emphasis on edges of fields where pest infestation levels are highest, instead of the current emphasis on spatial distances.

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Table 3.1 Farmers' experience in maize cultivation.

Farmer experience in maize cultivation (years)	Percentage of farmers
1-5	13.9
6-10	17.6
11-15	11.4
16-20	21.7
21-30	19.1
31-40	9.0
> 40	8.3
Area planted with Bt maize (hectares / farmer)	
1-10	9.3
11-20	15.4
21-30	12.7
31-40	12.5
41-50	5.0
51-60	12.5
61-70	18.9
71-80	28
81-90	9.3
91-100	8.5
101-200	2.8
201-300	11.4
301-400	9.3
Area planted with conventional maize (hectares / farmer)	
11-20	37.5
31-40	25.0
41-50	12.5
61-70	12.5
> 100	12.5

Table 3.2 Farmer perceptions of pests of Bt maize and their severity.

Pest incidence in Bt maize (excluding stem borers)		Percentage of farmers		
<i>Astylus atromaculatus</i>		9.8		
<i>Helicoverpa armigera</i>		35.7		
<i>Tetranychus urticae</i>		72.3		
Severity of stem borer damage to Bt maize during different cropping seasons?		2005/06	2006/07	2007/08
Absent		81.3	33.8	3.8
Light		16.3	37.5	37.5
Medium		2.5	17.5	26.3
Severe		0.0	5.0	32.5

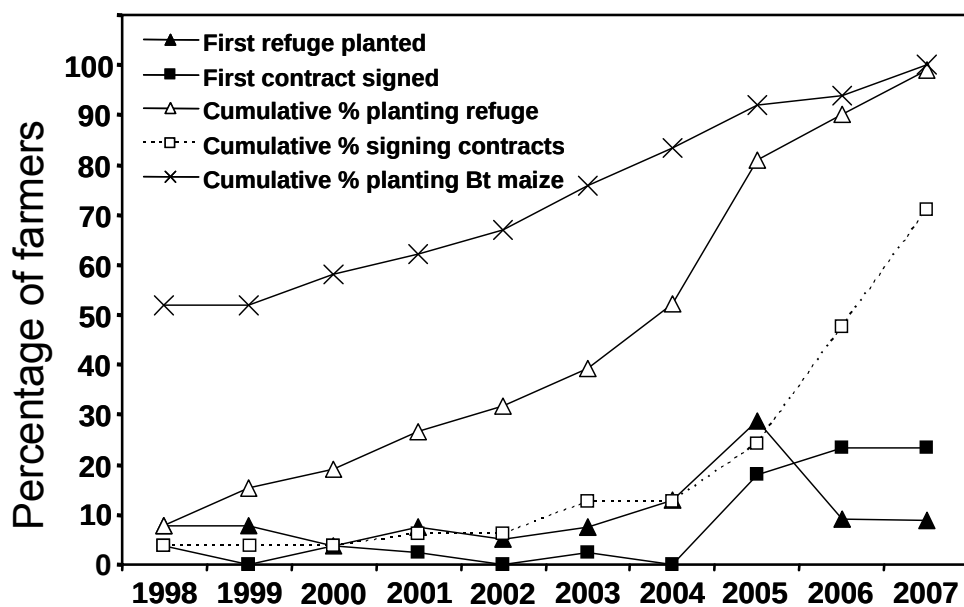


Fig. 3.1 The incidence of farmers signing contracts and complying to refuge requirements for the first time between the 1998 and 2007 growing seasons.

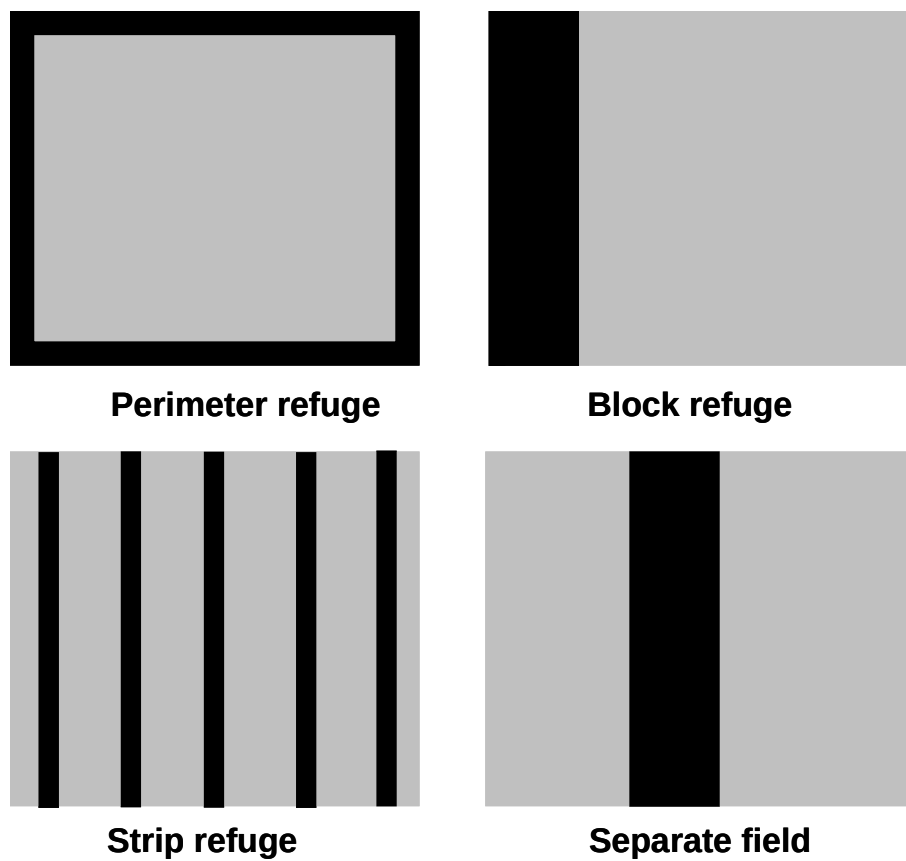


Fig. 3.2 Lay-out options for refuge areas. Legend: Black areas indicate conventional maize as refuge, grey areas indicate Bt maize (Source: Monsanto, 2007).



Fig. 3.3 *Busseola fusca* larvae damaging a maize ear.



Fig. 3.4 Stem borer damage observed by farmers on Bt maize.

Chapter 4

Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa

Abstract

Based on surface area, South Africa is currently ranked the 8th in planting genetically modified (GM) crops in the world. Bt maize (MON810) has been grown to control lepidopterous stem borers in South Africa since its first release during 1998. The first report of resistance of the African maize stem borer, *Busseola fusca* (Lepidoptera: Noctuidae) and poor compliance to refuge requirements that could have contributed to resistance development prompted this study which was done in the main maize production area of South Africa. Objectives were to evaluate farmer's perceptions of the regulatory aspects guiding the planting of Bt maize and refugia and how they were applied between 1998 and 2010. This study involved 105 commercial farmers covering approximately 87 778 ha of maize throughout the main maize production region on the Highveld of South Africa. A questionnaire survey was conducted and addressed signing of technical agreements upon purchasing GM seed, refuge compliance, pest management practices, perceived benefits and risks relating to Bt maize. Compliance with refugia requirements was low especially during the initial 5-7 years after release. An alarmingly high number of farmers applied insecticides as preventative sprays on Bt maize and refugia irrespective of stem borer infestation levels. A large proportion of farmers reported significant borer infestation levels on Bt maize and between 5 – 93 % farmers in all districts applied insecticides to Bt maize to limit borer damage, indicating that the occurrence of resistance is more widespread in the country than previously thought. This study shows irresponsible management of GM crop technology by farmers, chemical- and seed companies. South African farmers perceived little, if any, negative impact on non-target organisms and remain positive about the technology in spite of resistance development.

4.1 Introduction

Large-scale planting of Bt crops in the world began during 1996 with South Africa quickly adopting Bt maize and cotton for control of lepidopteran pests during 1998. Based on surface area, South Africa is currently ranked eighth in planting genetically modified (GM) crops in the world (James, 2009). Bt maize has been grown in the main maize production region of South Africa (Highveld region) since its first release in 1998, specifically to control the target stem borers *Busseola fusca* (Lepidoptera: Noctuidae) and *Chilo partellus* (Lepidoptera: Crambidae).

Field resistance of a target pest (*B. fusca*) to Bt maize (MON810) was reported for the first time in the Christiana area in South Africa (Van Rensburg, 2007). A follow-up study during the 2008/09 season confirmed this resistance approximately 50 km away from the original site and indicated that even stem borers that occurred in the refuge areas at the Vaalharts irrigation scheme were also resistant (Kruger *et al.*, accepted, Chapter 2). A survey conducted during 2008/09 in the Vaalharts irrigation scheme (Kruger *et al.*, 2009, Chapter 3), showed that poor refuge compliance especially during the first 5 – 7 years after release of Bt maize could have been the main reason for resistance development. Non-compliance was largely ascribed by farmers to their small farming units (25 – 50 ha) and the impracticality of refuge designs for centre pivot irrigation systems which complicate correct management of refugia (Kruger *et al.*, 2009, Chapter 3).

No information exists on the level of refuge compliance and resistance management in the main maize production areas of the South Africa, where resistance has not been reported before (Fig. 4.1). Maize is produced under flood and centre pivot irrigation at Vaalharts and Christiana, the localities where resistance has previously been reported. Both these localities lie outside the main maize cropping area (maize triangle) where the crop is largely produced by commercial farmers under dryland conditions. The possibility exists that non-compliance to refuge requirements in the Highveld region could play a role in evolution and spread of resistance in these areas. A unique opportunity exists after 12 years of cultivation of Bt maize in South Africa to gain insight from farmers into its use as well as prescribed regulatory aspects/requirements. Knowledge regarding management aspects as well as farmers' perceptions and understanding of Bt-maize technology will be beneficial to

extension specialists, crop consultants and industry in developing educational information for farmers (Pilcher & Rice, 1998). It will also determine levels of compliance to regulatory requirements such as planting of refugia and highlight possible areas where resistance development may be a risk.

The current refuge requirements are either a 20 % refuge planted to conventional maize which may be sprayed with insecticides, or a 5 % refuge area that should not be sprayed. The high dose/refuge strategy, involves the expression of Bt toxin at a level high enough to ensure that individuals heterozygous for resistance are killed (Bates *et al.*, 2005). The purpose of the high dose of toxin is to kill as many individuals of the target pest as possible, whereas the purpose of the refuge is to maintain susceptible (SS) pest individuals that survive on non-Bt crops (Renner, 1999; Gould, 2000). The aim is to ensure that those rare, Bt-resistant individuals that survive on the Bt crop do not produce completely resistant offspring (RR) by mating with other toxin-resistant individuals. Instead, susceptible individuals from the refuge supposedly mate with initial RS-resistant individuals that survive on the Bt plants in order to dilute the R- allele in the population. Offspring from the combination of susceptible and resistant individuals are expected to have only a low to moderate level of toxin resistance. These individuals should not be able to survive on plants with high Bt toxin levels (Renner, 1999; Gould, 2000), thereby delaying the rate of resistance evolution in the population.

When assessing farmers' perceptions of innovations such as transgenic crops, surveys can be useful tools (Grieshop *et al.*, 1988). Surveys have for example been used to determine the success or failure of GM maize used for European corn borer (*Ostrinia nubilalis*) and corn root worm (*Diabrotica* spp.) control after this technology has been introduced and used by farmers in the USA (Pilcher & Rice, 1998). In Sweden, surveys amongst GM crop farmers showed a negative attitude towards the technology (Lehrman & Johnson, 2008), while South African farmers were very positive towards the technology, mainly due to ease of management.

The objectives of this study were to evaluate farmers' perceptions of the regulatory aspects guiding the planting of Bt maize and refugia, its benefits and disadvantages as well as their perceptions of the pest status of *B. fusca* in the Highveld region of South Africa.

4.2 Material and methods

4.2.1 Study areas

This study was conducted in six different geographical areas in the main maize production region of South Africa, a large proportion of which can be considered to be semi-arid (Table 4.1). One hundred-and-five farmers in six towns (districts) were interviewed. Ten farmers were interviewed in the town of Christiana (North-West province), which is mainly a potato production area where maize is used in crop rotation systems (Table 4.1 and Figure 4.1). Hoopstad, Bothaville and Reitz are situated in the Free State province (Table 4.1 and Figure 4.1) where 14, 20 and 21 farmers were respectively interviewed out of a total of approximately 64, 130 and 120 farmers in these districts. Twenty of the approximately 120 maize farmers in each of the Delmas and Standerton districts of Mpumalanga, were interviewed. Information on number of farmers per district was provided by maize seed company representatives in each region. In both Christiana and Standerton one farmer indicated that they have never planted Bt maize.

4.2.2 Farmer survey

The survey was conducted between March and May 2010 and was designed as a self-administered questionnaire. For comparative reasons the same questionnaire was used as during the farmer's survey at the Vaalharts irrigation scheme (Kruger *et al.*, 2009, Chapter 3). Prior to this survey the names of farmers were randomly selected from a list of maize producers provided by seed companies in the different areas. Each farmer was therefore given an equal probability of being selected.

The questionnaire addressed basic questions on maize farming and the importance of GM maize as a crop in their specific farming system. The questionnaire was divided into five major categories that addressed the history of maize production in the area, farmer's perceptions of Bt maize as a tool and its possible environmental

impact, regulatory aspects and compliance to refuge requirements. Other questions addressed farming practices and pest management practices.

4.2.3 Data analysis

Data were summarized in Excel and expressed as percentages that were calculated based on the total number of farmers who responded to a particular question. Farmers who did not respond to a particular question, or if it was not applicable, were excluded from the calculation of percentage values for that question. When a farmer selected more than one answer to a question, percentages were calculated using all answers given.

4.3 Results

A summary of the survey data is provided below in five major categories. All the respondents were male, which, in South Africa, is the norm on commercial farms.

The total area planted to maize by the 105 farmers interviewed was approximately 87 778 hectares of which 71 405 ha were planted to Bt maize including the associated 3 780 ha of non-Bt maize refugia. 16 373 ha were planted to non-Bt maize. The surveyed area is approximately 4.5 % of the total maize production area in South Africa.

4.3.1 Farmers' experience in maize cultivation and adoption of Bt maize

In the six study areas, farming experience with maize cultivation ranged from one to 70 years with the majority of farmers cultivating maize for less than 20 years (Table 4.2). Bt maize was adopted in 1998/99, the first year it was released, in five of the six districts included in this study. The highest incidence of adopters in that year was at Bothaville (35 %) (Table 4.2). The annual rate at which farmers adopted the new Bt maize technology ranged between 5 and 35 % per year and at the time of this survey (2010) nearly 100 % of farmers in all surveyed districts except at Standerton and Christiana planted Bt maize. Mean adoption rate in the Delmas district was lower than in other districts. This could probably be ascribed to the fact that farmers in this area still planted non-Bt maize for export purposes. Although few farmers in Hoopstad (28 %) and Standerton (75 %) planted Bt maize fields with their refuge

areas, the largest part of the farming area was still planted with non-Bt maize as was common practice before the introduction of GM maize (Table 4.2).

Forty seven percent of the farmers interviewed planted herbicide tolerant maize and 53 % indicated that they plant the stacked gene which is a combination of the Bt-gene and herbicide tolerance gene.

4.3.2 Farmer's perceptions of Bt maize and its benefits

Farmers indicated the two greatest advantages associated with Bt maize to be convenient management (65 %) and increased productivity (64 %) (Table 4.3). While more than 15 % indicated that they perceived Bt technology to be environmentally friendly and 93 % indicated that they did not perceive GM crops to have a negative effect on the environment (Table 4.3). The majority farmers were positive towards Bt maize technology and deemed it advantageous to plant (79 %) (Table 4.3). In Christiana there was strong opinion amongst farmers (50 %) that Bt maize technology was of no benefit anymore and that the advantage associated with planting Bt maize was diminishing (Table 4.3). Farmers in Christiana (10 %), Hoopstad (21 %), Bothaville (5 %) and Standerton (5 %) indicated that Bt maize could be an advantage “provided it was effective against the target pest” (Data not shown). This can be ascribed to the fact that between 70 and 100 % of farmers in the different districts were aware of reports of resistance development (Table 4.3) and a large proportion of them, especially at Christiana, observed high incidences of stem borer damage on their Bt maize (Table 4.5). Although Bt maize seed is more expensive than conventional maize seed, more than 84 % of farmers interviewed still considered it economically worthwhile to plant Bt maize. Only 11 % of farmers in Christiana agreed with this statement (Table 4.3) which can be ascribed to the high levels of resistance of the target pests to the Bt gene in this area.

The number of farmers that were not aware of stem borer resistance development against Bt maize was lowest in Christiana (10 %) and highest in Delmas (30 %) (Table 4.3). On the question if they would continue planting Bt maize in the future, the incidence of farmers confirming that they would, ranged between 30 % – 100 % (Table 4.3). The majority of farmers who indicated that development of resistant target pests will prevent them from cultivating Bt maize ranged between 30 % - 92

%. The incidence of farmers indicating that the price of the produce or grain marketing and export markets would affect their choice of Bt maize was low (Table 4.3).

4.3.3 Compliance to refuge requirements

Results from this study indicated that initial refuge compliance in all six survey areas was low. Even though farmers were obligated to plant a refuge area for each Bt maize field only 10 % of farmers in the Christiana area and 22 % in the Hoopstad area planted refuges during 1998 (Fig. 4.2 and Table 4.4). The level of compliance in Hoopstad, Bothaville, Reitz and Delmas increased slowly until it reached 100 % during the 2009/10 season (Fig. 4.2).

The cumulative percentage of farmers planting refugia followed the same tendency as the cumulative percentage of farmers signing first time technical agreements in all six study areas (Fig. 4.2). This shows that the signing of technical agreements contributed to awareness and compelled farmers to comply with refuge requirements. Survey data showed that no first time technical agreements were signed between 1998 and 2006 in Christiana although the farmers planted Bt maize from 1998. The number of farmers that signed technical agreements with seed companies for the first time increased after the 2006/07 growing season, however, 80 % of farmers in Christiana indicated that they did not sign any technical agreements with seed companies (Table 4.4). All the farmers indicated that seed representatives provide oral information with regard to Bt maize and planting of refugia.

Farmers in Christiana (20 %) and Standerton (16 %) indicated that they did not plant a refuge field for each Bt maize field (Table 4.4) and justified this as being too labour intensive and time consuming. The majority (95 %) of farmers that planted refuge areas preferred to plant the separate 5 % option instead of the 20 % option. These farmers indicated that they cultivated maize under centre-pivot irrigation (22.8 %), dryland (55 %) or both (22.2 %). Of the four refuge layout options, the separate field (70 %) and strip refuge (95 %) was the most common practice used in Christiana and Bothaville respectively (Table 4.4). Seventy percent or more of farmers indicated that they planted herbicide tolerant maize in the refuge area while the main planting

consisted of hybrids with stacked gene. In Hoopstad and Reitz 7 and 15 % of farmers respectively planted refuges on the corners of square fields that were not reached by the pivot irrigation system. This practice is not in accordance with the high/dose refuge legislation, since growing conditions outside the irrigation pivot differ from that of the main crop and may result in preferential oviposition of stem borer moths inside the irrigated field.

4.3.4 Pest management practices

Farmers indicated that stem borers were effectively controlled with Bt maize but farmers at Christiana, Hoopstad, Bothaville and Reitz indicated that they already observed stem borer damage on Bt maize during the 2003/04, 2006/07 and 2007/2008 production seasons respectively (Table 4.5). Although these levels of borer infestation on Bt maize increased towards the 2009/10 season between 40 and 95 % of farmers indicated that they have never observed stem borer damage on their Bt maize (Fig. 4.3). The majority of the farmers in the Hoopstad, Bothaville and Reitz areas indicated that stem borer damage on Bt maize was lower than 10 % between the 2008/09 and 2009/10 growing seasons (Table 4.5). During the 2007/08 growing season 20 % farmers experienced high stem borer infestation levels of more than 50 % on Bt maize (Table 4.5) and were compelled to use insecticides to prevent economic crop losses. Ten percent of farmers in Bothaville and 25 % in Standerton applied insecticides preventatively on Bt maize and the refuge area irrespective of the presence of stem borer damage. Results further indicate that insecticides were applied preventatively on Bt maize and the refuge planting by 6.6 % of farmers, covering 10.4 % of the surface area in this study. Since these sprays were applied to all maize on these farms, farmers therefore did not comply with the requirement of not spraying on the 5 % refuge with selection of this option compared to the 20 % refuge option.

In Christiana the farmers indicated that they scouted for stem borer damage in Bt maize once or twice a week (Table 4.5) and pointed out that this was not the norm before the first report of resistance by Van Rensburg (2007). In areas of South Africa where stem borer pressure on Bt maize is low, some farmers scout their fields once a season or never which corresponds to the percentage of farmers who never observed any stem borer damage on their fields (Table 4.5). Private companies

carried costs of insecticides (excluding application costs, e.g. airplane cost) against stem borers for those farmers that planted early in the season and on whose Bt maize fields the incidence of stem borer infestation was higher than the economic threshold level for control (10 % infested plants) (Monsanto 2007).

All farmers indicated that insecticide applications would be necessary against stem borers if Bt maize was not planted (data not shown), while only 20 % of the farmers in Bothaville indicated that insecticide sprays would have to be applied more than twice per growing season on conventional maize.

The number of farmers who applied insecticides for *B. fusca* control on Bt maize in the 2009/10 growing season was 50 % in Christiana, 93 % in Hoopstad, 30 % in Bothaville, 5 % in Reitz, 10 % in Delmas and 10 % in Standerto (These percentages include the number of farmers who apply insecticides preventatively).

In the six survey areas more than 60 % of farmers indicated they did not think that Bt maize could control any other insect pests (excluding stem borers). Between 8 – 40 % of farmers in Christiana, Hoopstad, Bothaville and Reitz indicated that Bt maize suppressed bollworm (*Helicoverpa armigera*) numbers (Table 4.5) on maize ears.

4.4 Discussion

4.4.1 Farmers' experience in maize cultivation and adoption of Bt maize

South African farmers have benefited from the adoption of Bt maize since its deployment during 1998 (Gouse *et al.*, 2005). Although a relatively high number of farmers (between 5 and 35 %) planted Bt maize during the first season when it became available, farmers in the Delmas district adopted Bt maize for the first time during the 2003/04 growing season but 30 % of them also continued to plant large areas of conventional maize (Table 4.2). The reason for this is that these farmers plant maize on a technical agreement basis for private companies for export to other African countries, which only accept certified GM-free maize. Neighbouring farmers in the Delmas district communicate and negotiate with each other to prevent cross pollination between Bt- and conventional maize. Co-existence between farmers that plant GM or non-GM crops is also a concern in other GM producing countries such as Spain (Brooks & Barfoot, 2010). Co-existence can be defined as an issue related to the economic consequences of the adventitious presence of material from one

crop inside another as well as the principle that farmers should be able to cultivate freely the agricultural crop they choose (Brooks & Barfoot, 2010). Farmer concerns are largely related to marketability of maize for export to other African countries where GM crops are not allowed. Although it is possible to limit cross-pollination between GM and non-GM fields through temporal separation, it is not always practical in dryland farming systems due to limited planting time. Farmers in the Standerton region (75 %) who also planted large areas to conventional maize (Table 4.2) indicated that they have no problem with co-existence of Bt and conventional maize.

4.4.2 Farmer's perceptions of Bt maize and its benefits

Pilcher *et al.* (2002) reported the main reason for farmers planting Bt maize in the USA was to reduce yield losses. These farmers reported on average higher yields, less need for scouting and reduced pesticide use (Pilcher *et al.*, 2002). A survey conducted among Swedish farmers, indicated that they were predominantly negative towards GM crops. A large proportion of Swedish farmers did not anticipate any benefits that could result from the adoption of Bt maize with their major concerns being market acceptance and environmental risk. These farmers did, however, not have the opportunities to use transgenic technology (Lehrman & Johnson, 2008) as was the case with farmers in Spain and South Africa. In Europe, farmers are largely concerned about consumer acceptance of the product, non-target effects and resistance development. Concerns among farmers in Spain where Bt maize has been planted for a number of years and where grain is sold as food into the human sector (Brookes & Barfoot, 2010), seem to be similar to those of South African farmers.

In spite of Bt-resistant stem borer caused that causes damage to Bt maize in Christiana, 70 % of these farmers together with more than 90 % of farmers in the other five study areas, indicated that they thought that GM maize had no negative effect on the environment (Table 4.3). Although 90 % of the farmers were aware that stem borer resistance to Bt maize had been reported they still considered it advantageous to plant (Table 4.3).

A study on agronomic benefits experienced by Bt maize farmers in South Africa, indicated that, despite paying more for seed, adopters enjoyed increased income over conventional maize varieties through savings on pesticides and increased yield due to better pest control (Gouse *et al.*, 2005). Irrigation and dryland commercial farms surveyed in the Mpumalanga, Northern Cape and the North-West provinces enjoyed statistically significant yield increases of 11 % and 10.6 % respectively during the 1999/2000 and 2000/01 seasons (Gouse *et al.*, 2005). Farmers in the United States expected that the primary benefit of planting Bt maize would be less insecticide exposure for them and a reduction of insecticides in the environment (Wilson *et al.*, 2005).

An important advantage in the cultivation of Bt maize is the reduction in number of insecticide applications. However, Bt maize is only an advantage when target pests are present and there is no advantage in areas where infestation pressure is generally low. Stem borer populations can vary in abundance from year to year and their pest status is not predictable. This is evident from research done by Van Rensburg *et al.* (1985) on *B. fusca*, which indicated large scale variation in infestation levels over seasons.

4.4.3 Compliance to refuge requirements

The Genetically Modified Organisms act (Act 23 of 2006) guides the use of Bt maize in South Africa and compel seed companies to sign technical agreements with farmers that purchase seed of GM crops. These technical agreements prescribe the use of the product through a user guide (Monsanto, 2007) and specify aspects such as the implementation of an insect resistance management program, including the planting of refuge areas.

The number of farmers that planted refugia for the first time increased significantly after 2004 at all survey-areas. This could possibly be due to insect resistance reported from the Christiana area (Van Rensburg, 2007). The low incidence of signing of technical agreements was similar to previous observations reported in the Vaalharts irrigation scheme (Kruger *et al.*, 2009, Chapter 3). Legally binding technical agreements (contracts) have to be made between farmers and seed companies and compel the farmer to adhere to all prescriptions regarding refuge

compliance, intellectual property rights, and general management of GM crops. The low level of compliance observed on small intensively managed plots at the Vaalharts irrigation system reported by Kruger *et al.* (2009, Chapter 3) was therefore also the case on extensive farms.

Incorrect refuge layout designs were also observed in a previous study in the Vaalharts irrigation scheme (Kruger *et al.*, 2009, Chapter 3) and it is doubted whether the prescribed refuge layouts were used prior to 2005 when the increased awareness of the importance of refugia was recognized. Bates *et al.* (2005) indicated that social and economic factors that result in less than 100 % adoption of Bt crops may result in regional “unstructured” refuges that may compensate in part for some violations of refuge requirements. Risk of economic pest damage to refugia may be the reason for the lack of compliance amongst farmers growing Bt crops and remains a major drawback for the high-dose/refuge strategy (Bates *et al.*, 2005). A study conducted by Bourguet *et al.* (2005) observed that non-compliance with requirements under the refuge strategy is because of a possible reluctance of farmers who consider susceptible pest populations as common property shared by Bt maize growers. However, by planting Bt crops, each grower increases personal short-term benefits but favours the selection of resistant pests, which may negatively affect the long term benefits of neighbouring farmers (Vacher *et al.*, 2006). Rapid adoption of Bt-technology over a short period as was the case at the Vaalharts irrigation scheme where resistance was reported, therefore necessitates enforcement of refugia requirements (Kruger *et al.*, 2009, Chapter 3).

A lesson that could be learned from this is that in areas where Bt-technology adoption rate is very high, refuge compliance should be followed-up and enforced. Although the initial level of compliance to refuge requirements was low, it increased drastically over the past few years, which is largely ascribed to awareness of resistance development. Bt maize stewardship programs managed by seed companies will contribute significantly to improved future management of GM technology and to limiting of resistance development in target pests in other areas of the country. As part of the Bt-maize stewardship program seed deliveries to farmers in certain areas automatically include consignments of non-Bt maize seed to plant a 5 % refuge area. These programs instituted during the 2008/09 growing season, involve grower

education programs as well as the signing of technical agreements between companies and farmers. Companies furthermore do on-farm inspections, investigate cases of possible non-compliance and also institute punishment measures for farmers that do not comply (Kruger *et al.*, 2009, Chapter 3). For repeat offenders, this implies that GM technology of the relevant crops (maize, cotton and soybeans) cannot be purchased prior to the completion of a training program/rehabilitation (Monsanto, 2009).

In this study 15 % of the 105 farmers surveyed indicated that they received a visit from inspectors and only one farmer was found not to adhere to requirements. These stewardship programs are a positive contribution to protect GM technology and delay target pest resistance development. There is, however, room for improvement since several farmers still do not comply with refuge requirements (Table 4.4).

4.4.4 Pest management practices

During the 12 year history of Bt maize production the crop was perceived to provide effective control of stem borers in South Africa. In Christiana, levels of borer infestation on Bt maize increased towards the 2005/06 season, when nearly 50 % of farmers observed stem borer damage on Bt maize for the first time. A similar trend was observed in the Vaalharts irrigation scheme where resistance was confirmed (Kruger *et al.*, 2009, Chapter 3).

Preventative insecticide sprays for stem borer control have been a common practice since the 1960's. Before the advent of GM maize, farmers combined the spray of a post emergence herbicide, five weeks after crop emergence with a preventative insecticide spray. This practice was justified as contributing to ease of management, but did not take economic threshold levels that do exist for stem borer control into account. From this study it seems that these practices still continue in spite of the fact that Bt maize effectively controlled stem borers in most of these areas.

The use of these unacceptable practices leads to several questions regarding the use of this technology. For example, with whom does the responsibility to see that insecticides are not sprayed preventatively on Bt maize lie? Although management

of the target pest is effective (except in areas where resistance occurs), do seed companies have a responsibility in this case?

As was the case in the Vaalharts farmers were confident that Bt-technology would provide continuous protection of maize crops against stem borers, they did not foresee that resistance development would happen over such a short time. Farmers therefore indicated that they did not consider scouting a necessity any more and fields were consequently not scouted as regularly as in the past (Kruger *et al.*, 2009, Chapter 3). Although *H. armigera* is a non-target species for Bt maize in South Africa, Van Wyk *et al.* (2008) observed that at field level, the incidence of damage caused by *H. armigera* was always significantly lower on Bt maize than on non-Bt maize. The Monsanto user guide for the production of YieldGard (MON810) maize indicates that MON810 suppresses bollworm numbers in Bt maize fields (Monsanto, 2007). The fact that *H. armigera* numbers are only suppressed by Bt maize, could result in this pest becoming an important secondary pest.

4.5 Conclusions

This study which made use of farmer questionnaires was effective in recording previously undocumented cases of resistance of the target pest to Bt maize. Although questionnaires were effective to collect relevant data, it is most likely that by the time resistance was recorded it was already too late to prevent harm or to take measures to reverse or delay the process of resistance evolution. Based on results of this study and the overwhelming evidence of resistance evolution in South Africa (Kruger *et al.*, 2009, Chapter 3), case specific monitoring to manage an identified risk, such as resistance development, should be seriously considered when and if new GM maize events are deployed in South Africa. Based on the reported incidence of farmers that spray insecticides for borer control on Bt maize, it can be presumed that *B. fusca* resistance to Bt maize (MON810) is wide spread in the country. This study shows irresponsible management of GM crop technology by the maize industry. Future research should investigate farmer's perceptions, adoption rates and compliance to legal requirements in rural areas where enforcement of requirements will be even more difficult than in large scale commercial farming systems.

Although it was generally regarded that stem borer resistance to Bt maize only occurred in an isolated area around the Vaalharts irrigation scheme and Christiana area, this survey showed that resistance also occurred in several other areas in the main maize production region of the country. Although this resistance was not confirmed in laboratory bioassays, the mere fact that farmers sprayed insecticides to control stem borers on Bt maize was taken as indication of resistance.

4.6 References

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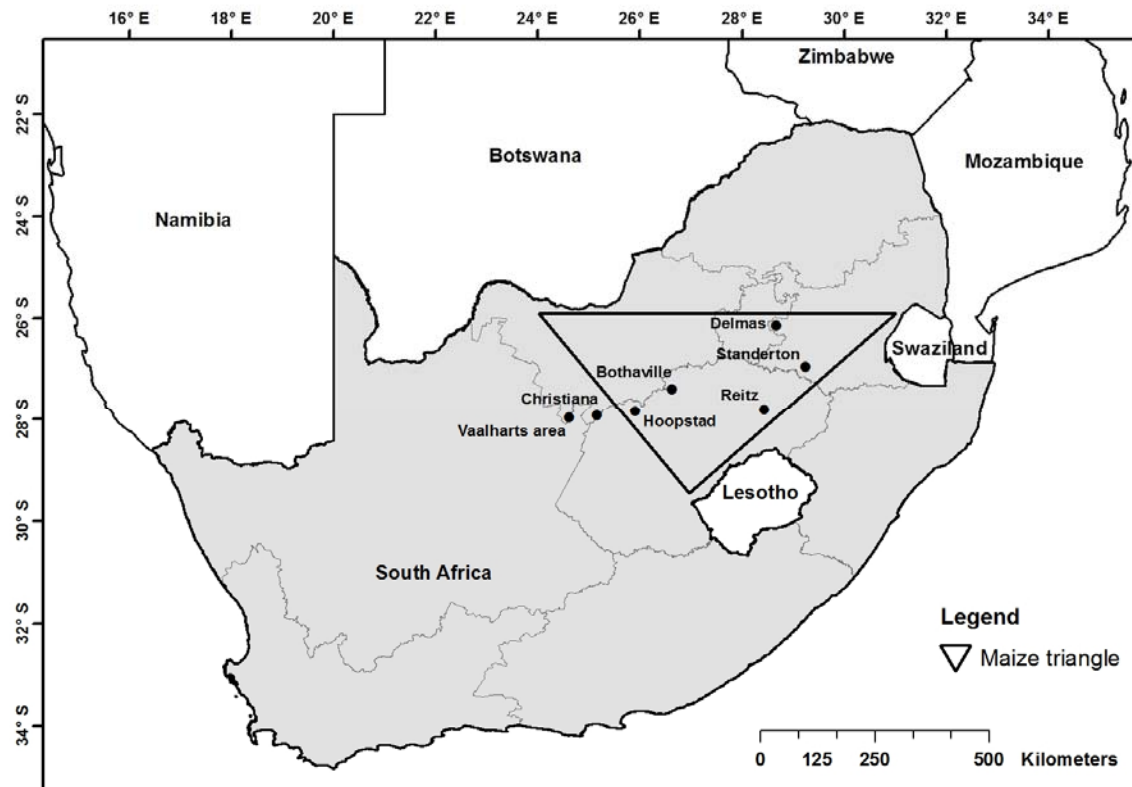


Fig. 4.1 Map of South Africa indicating the different survey areas inside the maize triangle on the Highveld region of South Africa.

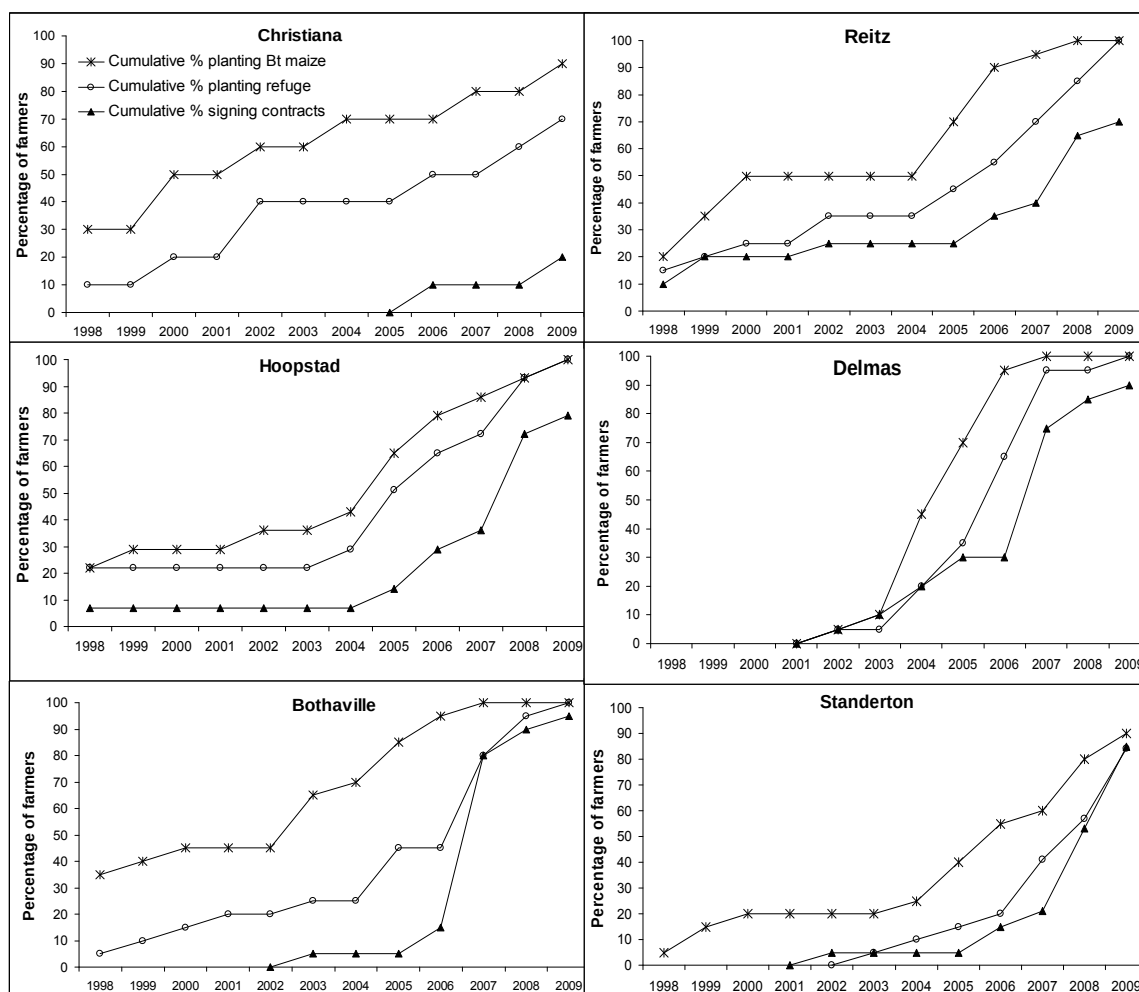


Fig. 4.2 The cumulative incidence of farmers planting Bt maize complying to refuge requirements and signing technical agreements (contracts) for the first time between the 1998 and 2009 growing seasons.



Fig. 4.3 Stem borer damage on Bt maize.

Table 4.1 Geographical information of the six survey areas in the Highveld and western maize production regions of South Africa.

Geographical information	Christiana	Hoopstad	Bothaville	Reitz	Delmas	Standerton
Grid reference GPS	S 27° 54.726 E025° 10.138	S 27° 50.149 E025° 54.306	S 27° 23.587 E026° 36.971	S 27° 48.005 E028° 25.760	S 26° 09.012 E028° 40.965	S 26° 57.236 E029° 14.599
Altitude (meter above sea level)	1180	1239	1276	1633	1560	1499
Mean maximum daily temperatures (C°)	27	25	24	21	22	22
Mean minimum daily temperatures (C°)	10	10	8	7	8	8
Mean annual rainfall (mm)	400 - 600	400 - 600	400 - 600	600 - 800	600 - 800	600 - 800

Table 4.2 Farmer's experience in maize cultivation and area planted to maize in six geographical areas in South Africa.

		Percentage of interviewed farmers					
		Christiana	Hoopstad	Bothaville	Reitz	Delmas	Standerton
Farmer experience in maize cultivation (years)	1 – 10	40	0	0	25	15	20
	11 – 20	40	29	50	20	50	30
	21 – 30	20	43	20	25	10	25
	31 – 40	0	7	25	5	25	10
	41 – 50	0	14	5	10	0	15
	51 – 60	0	7	0	10	0	0
	61 – 70	0	0	0	5	0	0
Planting Bt maize for the first time (year)	Never	10	0	0	0	0	5
	1998	30	22	35	20	0	5
	1999	0	7	5	15	0	10
	2000	20	0	5	15	0	5
	2001	0	0	0	0	0	0
	2002	10	7	0	0	0	0
	2003	0	0	20	0	5	0
	2004	10	7	5	0	5	10
	2005	0	22	15	20	35	15
	2006	0	14	10	20	25	15
	2007	10	7	5	5	25	5
	2008	0	7	0	5	5	20
	2009	10	7	0	0	0	10
Area planted with Bt maize (hectares / farm)	< 100	20	14	0	5	5	16
	101 – 250	40	0	10	60	0	21
	251 – 500	40	0	15	10	45	37
	501 – 1000	0	21	30	10	25	10
	1001 – 2000	0	37	35	5	25	16
	> 2000	0	28	10	0	0	0
Area planted with conventional maize (hectares / farm)	< 100	0	7	10	25	15	5
	101 – 250	30	7	5	20	0	20
	251 – 500	0	7	15	0	20	20
	501 – 1000	0	0	0	0	10	20
	1001 – 2000	0	7	0	0	0	10

Table 4.3 Farmer's perceptions of the benefits and potential environmental impact of Bt maize.

		Percentage of interviewed farmers					
Question posed to farmers		Christiana	Hoopstad	Bothaville	Reitz	Delmas	Standerton
Can GM maize have a negative effect on the environment?	Yes	30	7	5	5	10	0
	No	70	93	95	95	90	100
Is it advantageous to plant Bt maize?	Yes	0	79	100	100	100	89
	No	50	7	0	0	0	11
	Not sure	50	14	0	0	0	0
Advantages associated with Bt maize:							
Increased farm productivity	Yes	10	64	75	95	75	60
	No	90	36	25	5	25	40
Environmentally friendly	Yes	0	15	70	35	75	60
	No	100	85	30	65	25	40
Convenient management	Yes	30	92	100	100	90	70
	No	70	8	0	0	10	30
Is it economically worthwhile to plant Bt maize in spite of increased seed costs?	Yes	11	86	85	80	90	84
	No	89	14	15	20	10	11
	Not sure	0	0	0	0	0	5
Are you aware of stem borer resistance to Bt maize?	Yes	90	100	100	90	85	70
	No	10	0	0	10	15	30
Will you plant Bt maize in the future?	Yes	34	92	100	100	100	85
	No	66	8	0	0	0	15
What may prevent cultivation of Bt maize in future?	Resistance to stem borer	30	92	75	55	45	45
	Grain marketing/export	20	8	15	20	25	45
	Price of produce	0	0	10	25	30	10

Table 4.4 Compliance to Bt maize refuge requirements in six maize producing districts in South Africa.

		Percentage of interviewed farmers					
		Christiana	Hoopstad	Bothaville	Reitz	Delmas	Standerton
Layout options for refuge areas	Separate field	70	7	5	30	5	5
	Block refuge	0	0	0	10	5	26
	Strip refuge	30	93	95	60	65	47
	Border refuge	0	0	0	0	25	22
Year refuge area first planted	Never	20	0	0	0	0	16
	1998	10	22	5	15	0	0
	1999	0	0	5	5	0	0
	2000	10	0	5	5	0	0
	2001	0	0	5	0	0	0
	2002	20	0	0	10	5	0
	2003	0	0	5	0	0	5
	2004	0	7	0	0	15	5
	2005	0	22	20	10	15	5
	2006	10	14	5	10	30	5
	2007	10	7	35	15	30	21
	2008	10	21	15	15	0	16
	2009	0	7	0	15	5	27
Year technical agreements first signed	Don't know	0	21	0	25	0	0
	Never	80	0	5	0	10	5
	1998	0	7	0	10	0	0
	1999	0	0	0	10	0	0
	2000	0	0	0	0	0	0
	2001	0	0	0	0	0	0
	2002	0	0	0	10	5	5
	2003	0	0	5	0	5	0
	2004	0	0	0	0	10	0
	2005	0	7	0	0	10	0
	2006	10	15	10	10	0	10
	2007	0	7	65	5	45	16
	2008	10	36	10	25	10	32
	2009	0	7	5	5	5	32

Table 4.5 Farmers observations of stem borer damage and severity (for the first time) on Bt maize.

		Percentage of interviewed farmers					
		Christiana	Hoopstad	Bothaville	Reitz	Delmas	Standerton
Estimated infestation level (%) of stem borers in Bt maize fields during the 2008/2009 cropping season.	< 10 %	10	21	10	5	5	5
	11 – 20 %	10	7	10	10	10	0
	21 – 30 %	60	7	5	0	0	0
	60 – 70 %	10	0	0	0	0	0
	80 – 90 %	10	0	0	0	0	0
Estimated infestation level (%) of stem borers in Bt maize fields during the 2009/2010 cropping season.	< 10 %	10	43	20	10	10	0
	11 – 20 %	0	14	10	0	0	0
	21 – 30 %	10	29	5	0	0	0
	31 – 40 %	10	7	5	0	0	0
	50 – 60 %	30	0	0	0	0	0
	80 – 90 %	10	0	0	0	0	0
Year stem borer damage was first observed on Bt maize	Never	0	0	50	40	75	95
	2003	10	0	0	0	0	0
	2004	0	0	0	0	0	0
	2005	40	0	0	0	0	0
	2006	10	0	0	0	0	0
	2007	20	0	15	10	0	0
	2008	10	43	10	30	15	5
	2009	0	57	25	10	10	0
How often are fields scouted for stem borer damage to Bt maize?	Once a week	70	64	55	30	50	63
	Twice a week	20	36	0	0	30	21
	Once every two weeks	0	0	15	10	0	0
	Once a month	0	0	0	10	10	0
	Twice a month	0	0	0	10	10	0
	Once a season	0	0	0	30	0	0
	Never	10	0	15	10	0	16
In your opinion, which other pests can be controlled by Bt maize? (excluding stem borers)	None	60	92	80	100	90	100
	Bollworm	40	8	20	0	10	0

Chapter 5

Development and reproductive biology of Bt-resistant and susceptible field-collected larvae of the maize stem borer *Busseola fusca* (Lepidoptera: Noctuidae)

Abstract

The maize stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is of economic importance throughout sub-Saharan Africa. The seasonal activity pattern of this pest is characterised by two to three distinct generations during spring and summer followed by a diapause period that lasts for several months. Bt maize (MON810) has been deployed in South Africa since 1998 to control stem borers and the first report of field resistance of *B. fusca* to Bt-maize was made during the 2006 cropping season. It is expected that resistant strains of this pest have reduced fitness compared to susceptible individuals. Information regarding fitness of individuals that survive on Bt maize could contribute to the understanding of resistance evolution as well as to development of improved resistance management strategies. Life history parameters of different *B. fusca* populations were compared in a laboratory study using diapause (spring) as well as second-generation (summer) populations. Pupal characteristics, fecundity and longevity of moths of field-collected Bt-resistant and susceptible *B. fusca* populations were compared. Slight adverse effects of Bt maize on fitness of the resistant summer-population were observed. There was a tendency that the sex ratio was biased towards males in resistant populations and females in susceptible populations. The resistant population had a lower mean pupal mass, shorter longevity of moths and moths also laid fewer eggs.

5.1 Introduction

The stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is of economic importance throughout sub-Saharan Africa and causes serious yield losses in maize (Van den Berg and Van Rensburg, 1996). Its biology has been studied by several authors (Swaine 1957; Ingram, 1958; Usua, 1967, 1968, 1970).

In South Africa *B. fusca* larvae go into diapause during winter months (between May – September) (Van Rensburg *et al.*, 1987). Moths emerge from crop residues during October and the subsequent moth flight pattern is characterized by two to three distinct peaks (Van Rensburg *et al.*, 1985). Depending on the planting date the first generation (spring moths) generally attacks pre-flowering stages of the maize crop while second-generation moths (summer moths) infest plants during reproductive growth stages. It is especially with latter infestations that damage is observed on maize (Van Rensburg *et al.*, 1985).

Bt maize (MON810) has been deployed in South Africa since 1998 to control stem borers and adoption of this technology is high (Kruger *et al.*, 2009, Chapter 3; Kruger *et al.*, accepted, Chapter 2). Since the first deployment of Bt crops to control lepidopterous pests of maize and cotton there has been concern with regard to the evolution of resistance in target pests (Tabashnik 1994; Gould, 1998). As the use of Bt crops continues to grow, so does selection pressure on various pests of these crops (Bates *et al.*, 2005). The first report of field resistance of *B. fusca* to Bt-maize was made by Van Rensburg (2007) who showed that the larvae on Bt maize at certain locations survived in the presence of the Bt-toxin (Van Rensburg, 2007). Van Wyk *et al.* (2008) also reported increased incidences of *B. fusca* larvae on Bt-maize during the post-flowering period in the Highveld region of South Africa and also accurately predicted the evolution of resistance at irrigation systems in the country (Van Wyk *et al.*, 2007).

Field-evolved resistance can be described as a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field (Tabashnik, 1994). In contrast, laboratory-selected resistance occurs when

exposure to a toxin in the laboratory causes a heritable decrease in susceptibility of a laboratory strain. Because both field-evolved and laboratory-selected resistance entail changes in gene frequency across generations, they exemplify evolution (Tabashnik *et al.*, 2009). Although the terms “field-evolved resistance” and “evolution of resistance” refer to a process that occurs at population level, the word “resistance” is used to indicate the heritable, lower susceptibility of an individual relative to conspecific individuals (Tabashnik *et al.*, 2009).

Strains of pests resistant to Bt-sprays are often reported to show adversely affected life-history characteristics (Roush & McKenzie, 1987). To assess these disadvantages associated with evolution of resistance, various components of fitness, such as mating, fertility, fecundity and development time must be measured (Roush & McKenzie, 1987). For example, Kruger *et al.* (accepted, Chapter 2) showed that larval mass and development rate of Bt-resistant larvae were adversely affected when feeding on Bt maize.

Knowledge regarding the fitness of resistant insects is important in development of effective resistance management strategies (Wu *et al.*, 2009) especially in cases where resistance has already been reported. The aims of this study were to compare pupal characteristics as well as fecundity and longevity of moths of field-collected Bt-resistant and susceptible *B. fusca* populations.

5.2 Material and methods

The life history parameters of a known resistant Bt-population (Kruger *et al.*, 2009, Chapter 3; Kruger *et al.*, accepted, Chapter 2) and a susceptible population were compared in a laboratory study using diapause (spring) as well as second-generation (summer) populations collected in maize fields.

5.2.1 Collection sites

5.2.1.1 Diapause larvae (spring moths)

Two geographically separate populations of diapausing *B. fusca* larvae were used. The first population was collected during August 2008 in the Vaalharts irrigation scheme (S27°41.144 E024°41.639) (Fig. 5.1). This Bt-resistant population (designated R-VHdp) was collected after harvest by uprooting and dissecting the lower parts of maize stalks. A Bt-susceptible population was also collected on non-Bt maize during August 2008 at Fochville (designated S-Fdp) (S27°34.582 E026°29.634) approximately 400 km from the Vaalharts irrigation scheme.

5.2.1.2 Second-generation larvae (summer moths)

Second-generation larvae were collected from two distant areas during January 2009. Bt-resistant larvae were collected from three different farms inside the Vaalharts irrigation scheme (designated R-VH2gen) (farm 1: S28°04.970 E024°66.797, farm 2: S27°81.648 E024°76.029, farm 3: S28°16.860 E024°47.641). Larvae were collected from late-planted 4-7 week-old Bt maize plants that exhibited symptoms of borer damage. One hundred plants per locality were collected and dissected inside the respective Bt maize fields. Approximately 90 larvae per locality were collected and these larvae were reared on Bt maize in the laboratory until the pupal stage. Rearing was done in plastic containers (40 x 20 x 15 cm) with aerated lids. Larvae inside these containers were provided with freshly cut Bt maize stems at 3 to 4-day intervals. Rearing was done on laboratory desks under ambient conditions and a natural day/night photoperiod.

A Bt-susceptible population (designated S-Vil2gen) was collected at a locality in the Viljoenskroon area (S27°12.575 E026°56.728) during January 2009. Larvae were collected from maize ears as well as inside the stems of non-Bt maize plants. One hundred-and-fifty plants exhibiting stem borer damage were dissected and approximately 120 individuals collected and reared on non-Bt maize as described above.

5.2.2 Pupal characteristics and fertility, fecundity and longevity of moths

Before initiation of the experiment larval diapause was terminated using methods described by Van Rensburg and Van Rensburg (1993). Pupae (Fig.5.2) from all populations were weighed and their sex determined based on differences in external appearance of male and female pupae as described by Blair and Read (1969).

The sex ratio of the four different populations was determined using 253 and 115 pupae for the R-VH2gen and S-Vil2gen populations respectively. The numbers of pupae sexed for the diapause populations were 127 for the R-VHdp and 245 for the S-Fdp population. Pupae were then placed individually in 30 ml plastic bottles covered with mesh until moths appeared.

A bioassay was conducted to determine ovipositional fitness (fecundity and fertility) of moths. After emergence of moths, pairs of males and females were kept in oviposition chambers, using 30-70 breeding pairs per population. Oviposition chambers (Fig. 5.3) were 30 cm high and 15 cm in diameter and covered with a fine gauze mesh to prevent escape of moths. A 20 cm-long piece of maize stem (25-30 m diameter) with bases of leaves intact was placed in an upright position in the container. Plastic containers were filled up to a height of approximately 5 cm with crusher stone (7 mm diameter) as substrate to keep the maize stems upright. Stems were inserted 3-4 cm into the substrate. Water was added up to a level three-quarters of the height of the substrate to provide humidity to moths and to keep the stems fresh.

Stems were checked for eggs on a daily basis. Egg batches (Fig. 5.4) were carefully removed from each stem at 2-day intervals by cutting off a small piece of the leaf with the egg batch attached to it. The number of eggs per batch was counted and a record kept of the number of eggs laid by each female. In order not to inhibit oviposition because of the presence of large numbers of eggs on stems, stems were removed and replaced with a freshly-cut stem if there were two or more egg batches on it every day. After removal, each egg batch was placed separately in a test-tube and the opening covered with damp cotton wool to prevent desiccation. Eggs were kept in an incubator

at 26 ($\pm$ 1°C). Oviposition chambers were maintained as described until both moths died. Moth longevity was determined as the number of days from eclosion until death.

The total number of eggs and egg batches were determined for each female (Fig. 5.5). In order to determine fertility, the number of eggs that developed to the black head stage was determined in each batch 10 days after removal from the plant and expressed as a percentage of the total number of eggs per female. An attempt was made to have at least 30 individuals from each population of which the different parameters were measured.

5.2.3 Data analyses

Data were analysed using STATISTICA version 8.1 (STATISTICA version 8.1, StatSoft). The life history parameters of the different populations were compared by means of t-tests. The 2-sided Fisher-test was used to determine if the observed sex ratio deviated significantly from the expected 1:1 ratio. A Chi-square analysis was used to determine if the sex ratio differed between populations. Where relevant, simple correlation analyses were done to determine relationships between different life history parameters within populations (e.g. female mass and longevity).

5.3 Results and discussion

Diapause larvae are subjected to environmental changes and harsh environmental conditions for a prolonged period (April - September). Furthermore, the feeding status of diapause larvae may be affected by environmental conditions as well as status of the host plants that prevailed during the larval feeding period prior to entering diapause. The condition of larvae towards the end of diapause can also be influenced by photoperiod and planting dates of the host crop (Van Rensburg *et al.*, 1987). The influence of contact moisture on the termination of diapause in *B. fusca* is well documented and the onset of rain in the field or artificial wetting in the laboratory contributes to diapause termination (Kfir, 1991). For these reasons it would have been inappropriate to compare fitness parameters between the Bt-susceptible populations of

diapause larvae collected from Fochville and Viljoenskroon since these collection sites were geographically separated.

It was, however, appropriate to compare fitness parameters between field-collected second-generation summer populations of the Bt-resistant and susceptible populations collected at different sites. Although the summer generation of larvae used in this study, which were the offspring of moths originating from diapausing larvae, were also exposed to different environmental conditions that could affect their general fitness, the laboratory conditions under which they were reared for approximately four weeks of their 5-6 week life cycle were identical. Conditions under which pupae were kept and where oviposition bioassays were conducted were also identical.

5.3.1 Sex ratio

The Fisher-test indicated a highly significant difference ($p=0.0001$) in sex ratio of populations. There was a tendency that the sex ratio was biased towards a certain sex with the Bt-resistant populations having fewer females than males while the opposite was observed for the Bt-susceptible populations (Table 5.1). However, significant deviations from an expected 1:1 sex ratio were only observed in the R-VH2gen ($p=0.008$) and S-Fdp populations ($p=0.007$) (Table 5.1).

The observation that the sex ratio of Bt-resistant populations was biased towards males may impact on further resistance evolution as well as insect resistance management strategies. Under field conditions this tendency could imply that males will be inclined to leave Bt maize fields where they occur in relatively high numbers, to mate with the calling females which may be relatively more abundant in refugia of non-Bt maize. This could also imply that males will be present for a comparatively longer period than females and that there will be an increased incidence of Bt-resistant males in refugia, resulting in possible increased competition for females in the refuge. If this is the case, this could result in the further development of populations in which the frequency of resistant alleles is high. This aspect needs further investigation using larger samples of pupae from more localities. Little information is available on the sex ratio in populations

of *B. fusca* and whether it could be affected by biotic or abiotic stresses. Earlier studies done by Kaufmann (1983) on *B. fusca* in Nigeria indicated that the sex ratio was about equal in both spring and summer moths. A study conducted by Wu *et al.* (2009) on *Diatraea saccharalis* (Fuller) (Lepidoptera: Crambidae) showed that sex ratio was not affected after exposure of larvae to a Cry1Ab-treated diet.

5.3.2 Pupal mass

Highly significant differences between the mass of pupae of resistant and susceptible populations were observed (Table 5.2). Pupae of both males and females of the Bt-resistant summer-collected larvae (R-VH2gen) weighed significantly less than those of the susceptible population (S-Vil2gen) (Table 5.3). Since it is known that pupal mass is related to moth size and also their fitness to fly, mate and lay eggs, this study indicated that fitness of the adult stage of Bt-resistant *B. fusca* was adversely affected in Bt maize. However, this reduced level of fitness does not prevented development of economically important infestations of this pest under field conditions (Kruger *et al.*, 2009, Chapter 2).

As expected the mean mass per pupa was lower for the spring-collected individuals than summer-collected populations (Table 5.3). Similar differences between mass of spring and summer pupae were observed by Kfir (1991). The higher mass of summer pupae, compared to spring pupae which formed after an extended period in diapause, is ascribed to the utilization of food reserves during diapause.

Observations in a laboratory study on diamondback moth larvae (*Plutella xylostella*) (L.) (Lepidoptera: Plutellidae) treated with a Bt spray showed that there were no significant differences in the pupal weight between pupae reared on treated and untreated diets (Groeters *et al.*, 1994). Wu *et al.* (2009) reported that the female pupae of *D. saccharalis* generally weighed more than males, but that consumption of Cry1Ab protein did not affect their mass.

5.3.3 Fecundity

The number of eggs laid by moths of the Bt-resistant population (R-VH2gen) was significantly (Table 5.2) lower than that of the susceptible population (S-Vil2gen) (Table 5.3). The susceptible population had an almost two-fold higher mean number of eggs per female than the resistant population indicating that ovipositional fitness was negatively affected in resistant moths. However, compared to the number of eggs per female of Bt-resistant spring moths observed in this study, numbers were still twice as high as reported for *B. fusca* under field conditions by Usua (1967). Previous observations also indicated that the number of eggs laid by females from summer moths was higher than that of spring moths. The number of eggs from the non-diapausing generation of moths (mean = 353 eggs per female) was nearly three times that laid by moths originating from diapause larvae (mean = 131 eggs per female) (Usua, 1967) and almost twice that of the spring moths (mean = 538) (Kfir, 1991). Harris (1962) reported that moths of non-diapause larvae lay approximately 400 eggs per female.

As expected the number of eggs laid by summer moths was higher than that of spring moths. Little variation was observed in the mean number of eggs laid per female for three of the populations (R-VHdp, R-VH2gen and S-Fdp) and numbers ranged between 242.7 – 286.4 eggs per female (Table 5.3). The mean egg batch size of spring moths of the different populations were nearly identical (Table 5.3) while the R-VH2gen moths had the lowest number of eggs per batch (43.2) with the S-Vil2gen the highest number (58.8). There was, however, no significant difference any of the populations ($p > 0.15$) (Table 5.3). The mean number of eggs per batch observed in this laboratory study (Bt-resistant and Bt-susceptible populations) was similar to that reported under field conditions by Van Rensburg *et al.* (1987).

Even though fecundity were less of moths in this study with 2nd – generation larvae showed that moths originating from larvae that fed on Bt maize, this would probably not have an effect on population dynamics of this species under field conditions. In spite of females of susceptible moths laying more eggs, the number of egg batches was similar

to that of resistant moths and since females seldom lay more than one batch per plant, they can theoretically infest a similar number of plants under field conditions.

5.3.4 Longevity

Data in table 5.2 showed that both male and female moths of the summer-generation of Bt-susceptible populations lived significantly longer than those of the resistant populations (Table 5.3). Although this difference in longevity was only between 1 and 2 days, it indicates that exposure to Bt maize during the larval stages could possibly have a slight adverse effect on this life stage.

The average life span of moths ranged between eight and 10 days (Table 5.3). There were no differences between longevity of moths of the two diapause populations (R-VHdp and S-Fdp) (Table 5.2). Studies conducted by Usua (1970) indicated that the longevity of the male and female *B. fusca* moths under laboratory conditions were similar (average of 5-7 days), a result supported by Kaufmann (1983) (6 days). Longevity of moths of the Bt-resistant population in this study under laboratory conditions was within the general range of that described by other authors for *B. fusca* populations in other parts of Africa and the Bt-susceptible population used in this study. It is therefore expected that longevity of resistant moths under field conditions would be similar to that of susceptible moths and that it will not contribute to any changes in population dynamics or synchrony of emergence of stem borer populations between Bt-en non-Bt fields. Asynchrony in emergence patterns of moths from larvae that develop on non-Bt maize and those that derive from slower-developing larvae that survive on Bt maize could contribute to resistance evolution since this could result in non-random mating of individuals from Bt maize and those surviving in the non-Bt maize refugia. This asynchrony could result in mating and oviposition by individuals from refuges before adults from the Bt crop have emerged (Gould, 1998; Wu *et al.*, 2009). This situation would lead to reproductive isolation of Bt-selected adults and thus partially increase the rate of resistance development. On cotton for example, Bird and Akhurst (2004) reported that life history parameters of *Heliothis armigera* (Hubn.) (Lepidoptera: Noctuidae) larvae feeding on young cotton plants showed a significant developmental

delay of up to seven days for the resistant strain compared with the susceptible strain on non-Bt cotton.

No information is available on the comparative development rate of Bt-resistant and susceptible *B. fusca* larvae on Bt- and non-Bt maize. Although the fitness of resistant and susceptible field-collected individuals in this study did not differ markedly, it can be assumed that significant differences were present when the first individuals exhibiting tolerance to Bt-toxin survived on Bt maize, even prior to the observation of field-resistance in 2006 (Van Rensburg, 2007).

5.3.5 Relationships between life history parameters

Some generalisations regarding the biology and differences between some fitness parameters of the summer and spring generations of *B. fusca* can be made from this study. These generalisations refer only to certain parameters regarding biology and not the effects of Bt on *B. fusca*.

Only a few significant relationships existed between the different life history parameters that were recorded (Table 5.4). There were no significant relationships between female pupal mass and female moth longevity in any of the populations. Male mass and percentage fertilized eggs was correlated positively in only one of the four populations (Table 5.4).

Significant positive correlations were observed between the female mass and number of eggs laid per female for both the spring moth populations (R-VHdp, $p < 0.002$ and S-Fdp, $p < 0.010$) with larger moths laying more eggs. Larger moths also laid more egg batches (R-VHdp). No significant relationship was, however, observed between female mass and number of eggs laid per female in the summer moths (Table 5.4).

A significant negative correlation was observed between female mass and percentage fertilized eggs in the Bt-resistant summer moth population ($p < 0.025$, $r = -0.499$). There were no significant correlations between female mass and percentage fertilized eggs for

the spring (R-VHdp and S-Fdp) or summer susceptible moth population (S-Vil2gen) (Table 5.4). There were significant positive correlations between number of eggs per female and percentage fertilized eggs in the spring populations (R-VHdp, $p < 0.003$ and S-Fdp, $p < 0.033$) (Table 5.4).

5.4 Conclusions

This study indicated that the general fitness of a Bt-resistant summer-generation on Bt maize was poorer compared to that of a susceptible population on non-Bt maize. Although this was the case, fitness of the resistant population (pupal mass, fecundity, moth longevity) was still within the ranges reported in earlier studies on susceptible borer populations before the introduction of Bt maize. However, if fitness costs (reduced fitness of the Bt-resistant population on non-Bt maize) are associated with the evolution of resistance in *B. fusca*, then fitness of resistant individuals in non-Bt maize refugia may be lower than that observed for the Bt-resistant population on Bt maize as was observed in this study. This could contribute to a delay in further resistance development. However, if there is no cost, then resistance cannot be reversed and the refugia approach to resistance management in areas where field resistance is common would be of no further use. Further studies need to be conducted on the fitness costs associated with evolution of Bt-resistance in *B. fusca*. It is possible that the ultimate level of resistance to Bt has not been reached yet and that the observed differences might disappear with improved adaptation to Bt maize.

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Table 5.1 Sex ratios observed in various *Busseola fusca* populations collected on Bt and non-Bt maize.

Population	Sex ratio Male : Female	Observations (n)	Chi-square	p – value
R-VHd	1: 0.84	127	0.095	0.329
R-VH2gen	1: 0.65	253	11.102	0.008
S-Fdp	1: 1.55	245	11.46	0.007
S-Vil2gen	1: 1.13	115	0.426	0.513

* R-VHdp=Spring resistant Vaalharts diapause; R-VH2gen=Summer resistant Vaalharts second generation; S-Fdp=Spring susceptible Fochville diapause; S-Vil2gen=Summer susceptible Viljoenskroon second generation

Table 5.2 Comparison of life history parameters between Bt-resistant and susceptible spring and summer generations of *Busseola fusca* (t-test) (df =86)*.

Parameter	Population	t-value	p-value	F-ratio
Female pupal mass	Summer moths R-VH2gen vs. S-Vil2gen	-6.67777	0.000001	2.460
	Resistant moths Spring vs. summer	6.644250	0.000001	1.768
Male pupal mass	Summer moths R-VH2gen vs. S-Vil2gen	-7.42019	0.000001	1.018
	Resistant moths Spring vs. summer	4.751357	0.000008	2.444
Longevity of male moths	Summer moths R-VH2gen vs. S-Vil2gen	-4.20131	0.000065	1.911
	Resistant moths Spring vs. summer	-2.65869	0.009354	2.274
Longevity of female moths	Summer moths R-VH2gen vs. S-Vil2gen	-2.41136	0.018021	1.887
	Resistant moths Spring vs. summer	-1.26954	0.207597	1.660
Number of eggs laid per female	Summer moths R-VH2gen vs. S-Vil2gen	-2.92632	0.004387	3.235
	Resistant moths Spring vs. summer	2.453973	0.416146	1.051

*Actual numbers provided in Table 5.3
Resistant moths: spring = R-VHdp, summer = R-VH2gen

Table 5.3 Life history parameters of field collected Bt-resistant and Bt-susceptible *Busseola fusca* populations from different sites in South Africa (Mean $\pm$ S.E).

Parameter	Population			
	R-VHdp	S-Fdp	R-VH2gen	S-Vil2gen
Female pupal mass(g)	0.20 $\pm$ 0.01	0.29 $\pm$ 0.01	0.30 $\pm$ 0.01	0.40 $\pm$ 0.01
Male pupal mass (g)	0.17 $\pm$ 0.01	0.22 $\pm$ 0.01	0.23 $\pm$ 0.01	0.39 $\pm$ 0.01
Longevity female (days)	9.61 $\pm$ 0.44	9.42 $\pm$ 0.25	8.98 $\pm$ 0.28	10.23 $\pm$ 0.48
Longevity males (days)	9.70 $\pm$ 0.46	10.6 $\pm$ 0.26	8.48 $\pm$ 0.23	10.32 $\pm$ 0.41
Number of egg batches	5.13 $\pm$ 0.48	4.81 $\pm$ 0.39	7.68 $\pm$ 0.46	9.63 $\pm$ 0.66
Number of eggs per batch	47.55 $\pm$ 5.08	47.40 $\pm$ 3.76	43.29 $\pm$ 3.76	58.89 $\pm$ 7.84
Number of eggs / female	246.81 $\pm$ 32.59	286.40 $\pm$ 24.27	242.73 $\pm$ 25.21	506.56 $\pm$ 57.16

*Significant differences observed between summer moth populations (see table 5.2).

R-VHdp = Spring population from Vaalharts Bt-resistant

R-VH2gen = Summer generation population from Vaalharts Bt-resistant

S-Fdp = Spring population from Fochville Bt-susceptible

S-Vil2gen = Summer generation population Viljoenskroon Bt-susceptible

Table 5.4 Relationships between life history parameters of different field collected Bt-resistant and Bt-susceptible *Busseola fusca* populations.

Life history parameter		Population	p-value	r-value	F-value*	Regression equation
Female mass	Longevity of female	Spring moths R-VHdp	0.138	0.305	2.357	$Y = 6.11 + 14.79X$
		Summer moths R-VH2gen	0.065	0.252	3.543	$Y = 7.0 + 6.48X$
		Spring moths S-Fdp	0.480	0.103	0.505	$Y = 8.41 + 2.88X$
		Summer moths S-Vil2gen	0.398	0.149	0.733	$Y = 6.87 + 8.4X$
Male mass	Percentage fertilized eggs	Spring moths R-VHdp	0.568	- 0.138	0.334	$Y = 96.102 - 53.99X$
		Summer moths R-VH2gen	0.195	- 0.302	1.805	$Y = 111.92 - 52.48X$
		Spring moths S-Fdp	0.248	0.167	1.363	$Y = 79.84 + 50.86X$
		Summer moths S-Vil2gen	0.016*	- 0.648	7.963	$Y = 181.54 - 330.3X$
Female mass	Number of egg batches	Spring moths R-VHdp	0.009*	0.510	8.190	$Y = 0.27 + 24.43X$
		Summer moths R-VH2gen	0.114	0.217	2.577	$Y = 4.2 + 9.37X$
		Spring moths S-Fdp	0.546	0.088	0.368	$Y = 4.85 + 4.55X$
		Summer moths S-Vil2gen	0.268	0.195	1.269	$Y = 0.27 + 24.43X$
Female mass	Number of eggs laid per female	Spring moths R-VHdp	0.002*	0.613	12.166	$Y = (-128.1) + 245.4X$
		Summer moths R-VH2gen	0.056	0.261	3.811	$Y = 176.8 + 614.83X$
		Spring moths S-Fdp	0.010*	0.363	7.149	$Y = 31.13 + 1118.7X$
		Summer moths S-Vil2gen	0.606	0.091	0.269	$Y = 279.54 + 607.08X$
Female mass	Percentage fertilized eggs	Spring moths R-VHdp	0.755	0.054	0.992	$Y = 83.96 + 14.85X$
		Summer moths R-VH2gen	0.025*	- 0.499	5.974	$Y = 120.96 - 72.49X$
		Spring moths S-Fdp	0.508	- 0.096	0.443	$Y = 96.9 - 20.56X$
		Summer moths S-Vil2gen	0.805	0.076	0.639	$Y = 62.56 + 41.51X$
		Spring moths R-VHdp	0.413	- 0.144	0.685	$Y = 774.31 - 765.0X$
Number of eggs per female	Percentage fertilized eggs	Spring moths R-VHdp	0.003*	0.579	10.742	$Y = 72.91 + 0.45X$
		Summer moths R-VH2gen	0.828	0.051	0.048	$Y = 99.14 + 0.003X$
		Spring moths S-Fdp	0.033*	0.304	4.812	$Y = 83.50 + 0.21X$
		Summer moths S-Vil2gen	0.124	0.448	2.769	$Y = 42.37 + 0.71X$

*df for different populations: R-VHdp (1,22); R-VH2gen (1,52); S-Fdp (1,47); S-Vil2gen (1,32).

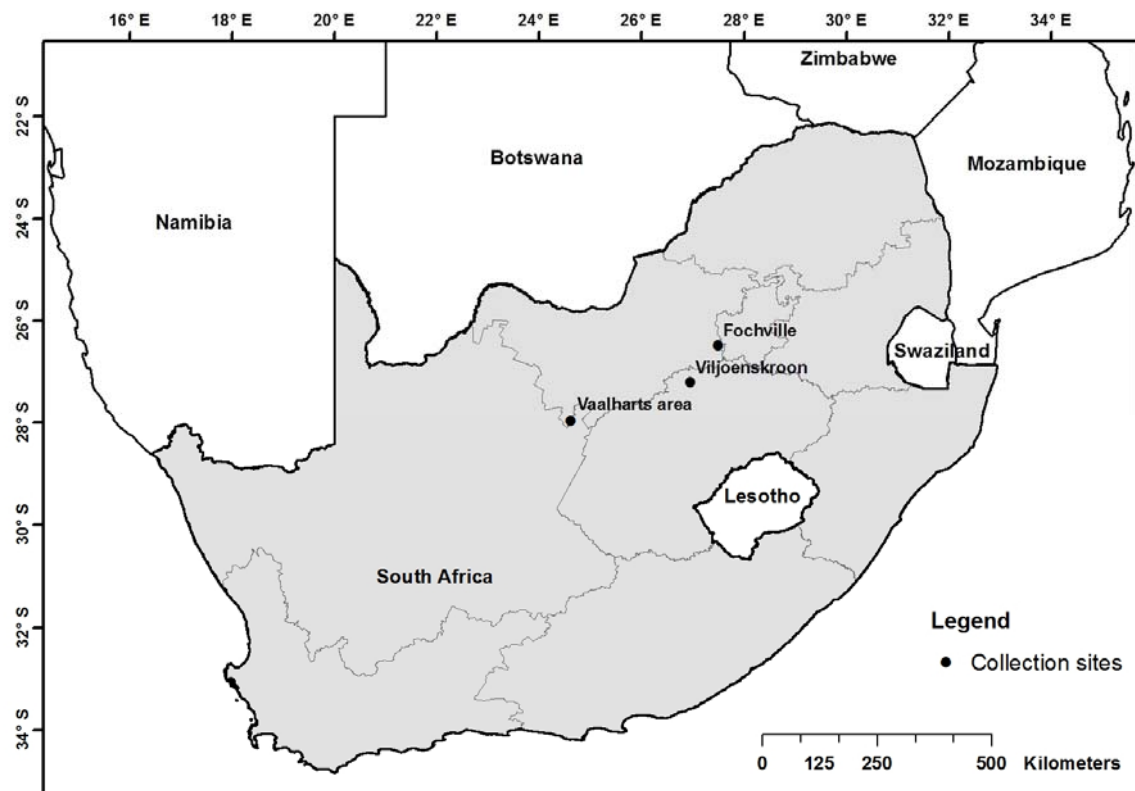


Fig. 5.1 A map of South Africa indicating the sites at which the different *Busseola fusca* populations were collected.



Fig. 5.2 *Busseola fusca* pupa.



Fig. 5.3 Oviposition chambers in which longevity, and fecundity of moths were determined.



Fig. 5.4 Egg batches of *Busseola fusca*.



Fig. 5.5 Female moth of *Busseola fusca*.

Chapter 6

Fitness costs associated with resistance of *Busseola fusca* (Lepidoptera: Noctuidae) to Bt maize

Abstract

One of the concerns regarding planting of Bt crops is that their widespread cultivation could lead to the evolution of insect resistance to Bt toxins. In South Africa, the maize stem borer (*Busseola fusca*), is resistant to Bt maize (MON810) which produces Cry1Ab protein. If fitness costs are associated with the evolution of resistance it adds a new dimension to the refuge strategy which is used in resistance management since the non-Bt maize refuge may select against resistance. Fitness costs occur when the fitness of individual's bearing resistant (R) alleles is less than that of homozygous susceptible (SS) individuals in the absence of toxin. The aim of the study was to determine if there are fitness costs associated with resistance of *B. fusca* to Bt maize. Different life history parameters were compared between field collected and laboratory reared individuals of a susceptible and known Bt maize resistant *B. fusca* population when feeding on Bt or non-Bt maize. The following parameters were compared: pupal mass, moth longevity, fecundity, fertility, larval mass and survival, and sex ratio. Except for moth longevity and LT50-values, no other fitness costs were observed to be associated with the resistance trait in the highly resistant *B. fusca* population used in this study. This implies that the fitness of the known Bt-resistant stem borer population that occurs at the Vaalharts irrigation scheme is not adversely affected when its larvae feed on non-Bt maize. The absence of fitness costs and presence of highly resistant populations of stem borers may promote the use of alternative Bt-resistance management strategies, such as a multi-gene strategy which would be expected to impact negatively on fitness.

6.1 Introduction

One of the main concerns regarding planting of Bt crops is that their widespread cultivation could lead to insect resistance evolution to Bt toxins and the subsequent reversal to the use of broad spectrum insecticides (Renner, 1999). High dose/refuge resistance management strategies may make theoretical sense, but the practical situation is still uncertain because there are huge gaps in knowledge about pest genetics and insect and plant ecology (Renner, 1999). The basic theory underlying the refuge strategy is to reduce the heritability of resistance by providing susceptible adults to mate with rare resistant adults surviving on Bt crops. More over the value of refuges is enhanced if the concentration of toxin in the Bt crop is high enough to kill the progeny from matings between resistant and susceptible insects.

Fitness of target pests is drastically affected by the insecticidal properties of Bt maize when the pest population is still susceptible to the Cry 1Ab protein produced by the plant. Fitness costs occur when the fitness of individuals bearing resistant (R) alleles is less than that of homozygous susceptible (SS) individuals in the absence of toxin. For example, because resistance alleles are rare in populations not previously exposed to insecticides (Bentur *et al.*, 2000), fitness of heterozygous individuals (RS) impacts strongly on the early dynamics of resistance evolution. If fitness costs are not recessive, RS individuals are less fit than SS individuals in refuges of non-Bt maize where the Bt toxin is absent. Yet with functionally recessive resistance, RS and SS individuals are equally fit in transgenic fields. This suggests that the spread of recessive R alleles with no recessive fitness costs could be prevented with an appropriate refuge/high dose strategy (Carrière & Tabashnik, 2001). Recognition of the prevalence and impact of fitness costs adds a new dimension to the refuge strategy in that a refuge may also select against resistance (Gould, 1998; Bourguet, *et al.*, 2000). The insect resistance management value that the presence of fitness costs may hold, was not addressed until resistance of a target pest became a reality, as was the case with *B. fusca* on Bt maize in South Africa (Van Rensburg, 2007; Gassmann *et al.*, 2009).

Random mating between susceptible and resistant insects is one of the two critical assumptions underlying the refuge strategy which is used for insect resistant management. The other assumption is that the inheritance of resistance is recessive. It is thus critical that the development rate of two strains of larvae developing in a Bt and adjacent non-Bt field is similar for the refuge strategy to be effective (Liu *et al.*, 1999). If developmental asynchrony occurs it will favour assortative mating among resistant moths from Bt plants. In the field, the extent of developmental asynchrony and assortative mating would be affected by variation in toxin expression, weather and overlap between generations. Assortative mating would generate a disproportionately high number of homozygous resistant insects, accelerating the evolution of resistance (Liu *et al.*, 1999).

Although the planting of refugia of non-Bt maize in close proximity to Bt maize with insecticidal properties is compulsory to delay the evolution of resistance, compliance levels are not always high. In South Africa for example the level of compliance between 1998 and 2006 was shown to be low in the region where resistance of the African maize stem borer, *B. fusca* to Bt maize was reported in (Kruger *et al.*, 2009, Chapter 3). Another study conducted by Kruger *et al.* (accepted; Chapter 2), showed larvae that occurred in the non-Bt maize refugia, which is supposed to sustain a Bt-susceptible population, were also resistant to Bt maize. Although evolution of resistance of *B. fusca* has been largely ascribed to non-compliance (Kruger *et al.*, 2009, Chapter 3) it is also possible that aspects of pest biology and low expression levels of the Cry1Ab protein in plants could have played a role and that a high frequency of resistant alleles could have initially been present in the population.

The phenomenon of resistance evolution to Bt has been studied in 14 species of moths and in all of these studies fitness costs were shown to be present (Gassmann *et al.*, 2009). It was observed that if fitness costs associated with resistance evolution was small or absent it could contribute to the rapid further development and spread of resistance, such as that reported in South Africa by Kruger *et al.* (accepted, Chapter 2). To date, no investigation has been done on frequency of resistant alleles in *B. fusca*

populations or fitness costs associated with resistance evolution. It is important to determine whether fitness costs are present since the management thereof may contribute to the delay of resistance evolution to Bt toxins when refugia are present (Carrière & Tabashnik, 2001). Various aspects of fitness of individuals during their immature and adult stages may be affected by biotic (e.g. host plant resistance) and abiotic (e.g. high temperature) stresses. Some of the parameters of fitness that may be indicative of possible adverse effects caused by certain stressors (e.g. Bt-toxin) on insects are the following: sex ratio, moth longevity, fecundity, fertility, larval development, larval survival as well as mortality during the pre-pupal stage and pupal mass.

The aim of the study was to determine if there are fitness costs associated with resistance of *B. fusca* to Bt maize.

6.2 Material and methods

In this study the life history parameters, larval mass and survival of a known Bt-resistant *B. fusca* population (Kruger *et al.*, 2009, Chapter 3) was compared to that of a susceptible population in a laboratory study using second generation (summer) populations.

6.2.1 Collection sites

Summer larvae (2nd-generation) (designated F₀-generation) were collected from two distant areas during January 2010. Bt-resistant larvae were collected from three farms inside the Vaalharts irrigation scheme where resistance is common (farm 1: S27°49.110 E024°45.577, farm 2: S27°79.736 E024°81.434, farm 3: S27°41.619 E024°42.893). Larvae were collected from late planted Bt maize plants that showed symptoms of stem borer damage. One hundred plants per locality were collected and dissected inside the respective Bt maize fields. Approximately 100 larvae per locality were collected and these larvae were reared in plastic containers (40 x 20 x 15 cm) with aerated lids, designated (VAA10Bt-Bt) and non-Bt maize (designated VAA10Bt-NBt) respectively in the laboratory until the pupal stage. Larvae inside these containers were provided with

freshly cut Bt or non-Bt maize stems at 3 to 4-day intervals. Rearing was done on laboratory desks under ambient conditions and a natural day/night photoperiod. Moths of these field-collected populations (F_0 -generation) and their offspring (F_1 -generation) were used in this experiment.

A Bt-susceptible population was collected at a locality in the Bronkhorstspuit area (S25°48.413 E028°44.744) during February 2010, approximately 550 km from the Vaalharts irrigation scheme. The Bronkhorstspuit site was selected because the planting of Bt maize in the area is not very high. Larvae were collected from late planted non-Bt maize plants that showed symptoms of damage. Two-hundred plants were collected and dissected. Approximately 150 larvae were collected and the larvae were reared to the pupal stage on non-Bt maize (designated BRO10Con-NBt). Moths of these field-collected populations (F_0 -generation) and their offspring (F_1 -generation) were used in this experiment.

6.2.2 Fitness costs in the life history parameters

Pupae from all field-collected populations (F_0) as well as their laboratory reared offspring (F_1) were weighed and their sex determined based on differences in external appearance of males and females as described by Blair and Read (1969). Individual male and female pupae were assigned numbers and placed in 30 ml bottles covered with gauze until moths appeared.

A bioassay was conducted to determine fecundity (ovipositional fitness) of moths. After emergence of moths, pairs of males and females were kept in oviposition chambers, using 30 breeding pairs per population. Oviposition chambers (Fig. 6.1) were 30 cm high and 15 cm in diameter and covered with a fine gauze mesh to prevent escape of moths. A 20 cm long piece of maize stem with bases of leaves intact was placed in an upright position in the container. Plastic containers were filled with approximately 5 cm of crusher stone (7mm diameter) as substrate to keep the maize stems upright. Stems were inserted 3-4 cm into the substrate. Water was added up to a level three-quarters of the height of the substrate to provide humidity to moths and to keep stems fresh.

Egg batches were carefully removed from each stem at 2-day intervals by cutting off a small piece of the leaf with the egg batch attached to it. The number of eggs per batch was counted and a record kept of the number of eggs laid by each female. In order not to inhibit oviposition because of the presence of large numbers of eggs on stems, stems were removed and replaced with a freshly-cut stem if there were two or more egg batches on it. After removal, each egg batch was placed separately into a test-tube and the opening covered with damp cotton wool to prevent desiccation. Eggs were kept in an incubator at 26 ($\pm$ 1°C). Containers were maintained as described until both moths died.

The total number of eggs and egg batches were determined for each female. Moth longevity was determined as the number of days from eclosion until death. In order to determine fertility, the number of eggs that developed to the black head stage was determined in each batch, 10 days after removal from the plant and expressed as a percentage of the total number of eggs per batch.

6.2.3 Larval growth and survival of F_1 -generation

Neonate larvae (F_1 -generation) obtained from the above mentioned experiment were used to study the possible fitness costs associated with survival of Bt-resistant larvae on non-Bt maize under laboratory conditions.

The experiment consisted of four treatments *viz.* two stem borer populations (VAA10Bt and BRO10Con) on each of a Bt- and non-Bt hybrid. The following two hybrids were used: DKC 78-15B (transgenic, MON810), CRN 3505 (non-Bt near iso-hybrid for DKC 78-15B).

Larvae were reared in glass test tubes (20 x 2.5 cm) containing a cut maize stem with the base of the whorl still intact (Fig. 6.2). Stems were cut from 4 – 6 weeks old potted plants that were grown in a greenhouse. Ten neonate larvae were used per test tube and were placed into the whorl of the cut stem. For the first 15 days freshly cut stems as

described above were provided every three days. Each stem was cut approximately 10 cm below the whorl of the plant and 5 cm of the base of the whorl was left intact. From 18 days onwards only a 15 cm long (10 – 15 mm diameter) cut stem was provided as food. All tubes were kept in a temperature controlled incubator at $25 \pm 1^\circ\text{C}$.

To ensure that the F_1 -individuals studied in this experiment was representative of each of the field collected populations (F_0), care was taken to use F_1 -1st instar larvae that originated from many different female moths. Larvae from 15 to 20 different moths were used and the offspring of each female put separately per test tube. There were 30 replications (test tubes), each containing 10 larvae, for each population.

The number and mass of live larvae per plant were determined at 3 – 5 day intervals up to 66 days after inoculation. The experiment was terminated on day 66 when larvae pupated or were considered to be in diapause. Larval survival was expressed as a percentage. Statistical analyses were done on larval mass determined on day 31 to avoid the effect that the change to pupae could have on mass. The number of live larvae of the different *B. fusca* populations on Bt and non-Bt maize was determined 66 days after inoculation and expressed as percentages.

6.2.4 Pre-pupa mortality and pupal development

Mortality during the pre-pupal stage was calculated for the Bt-resistant and susceptible populations of which larvae were reared on either Bt- or non-Bt maize, and expressed as percentage mortality during the pre-pupal stage. Cumulative numbers of male- and female pupae over time were determined for each *B. fusca* population. Pupal mass was also determined.

6.2.5 Data analyses

Data were analysed using STATISTICA version 9.1 (STATISTICA version 9.1, StatSoft, Inc. 2205, Tulsa, OK, USA). The life history parameters of the field collected Bt-resistant population on Bt and non-Bt maize and the susceptible population were compared by means of t-tests. One-way ANOVAs followed by post-hoc Tukey-tests were used.

Simple correlation analyses were used to determine if relationships existed between different life history parameters.

Mean larval mass of different F_1 -populations at day 31 after inoculation were analysed by means of ANOVA after which means were separated by means of the Tukey-test for significant differences. This was also done for the percentage survival 66 days after inoculation. Lethal time (LT50), indicating the time (number of days) until 50 % mortality was observed was calculated for each population using logistic regressions of larval survival over time.

Mass of male and female pupae of different F_1 -populations were compared by means of ANOVAs after which means were separated using the Tukey-test.

6.3 Results

Although the initial two field-collected populations from different localities were exposed to different environmental conditions at the respective sites that could have affected their fitness, the laboratory conditions under which they were reared for approximately four weeks were identical. Conditions under which pupae were kept and where oviposition bioassays were conducted were also identical.

6.3.1 Pupal mass, fecundity, fertility and longevity of moths

Pupal mass (F_0) as well as moth longevity (male and female) of the Bt-resistant population (VAA10Bt) were not affected when larvae were reared on Bt maize and the measured parameters did not differ from those of resistant individuals reared on non-Bt maize (Tables 6.1). Mean values are provided in Table 6.2. Pupal mass of the Bt-susceptible population (BRO10Con-NBt) reared on non-Bt maize was significantly higher than that of the resistant population reared on either Bt- or non-Bt maize (Tables 6.1 & 6.2). However, female moths of the Bt-resistant population that developed on non-Bt maize (VAA10Bt-NBt) lived significantly longer than those of the Bt-susceptible population on non-Bt maize (BRO10Con-NBt) (Tables 6.1 & 6.2).

Fecundity and fertility of resistant and susceptible moths was similar, irrespective of whether the larvae that they developed from were reared on Bt or non-Bt maize (Tables 6.1 & 6.2). The number of egg batches and eggs laid by moths that developed from Bt-resistant larvae that were reared on Bt maize (VAA10Bt-Bt) was not affected when compared to the same parameters from individuals that were reared on non-Bt maize (VAA10Bt-NBt) (Tables 6.1 & 6.2). However, moths that developed from Bt-resistant larvae that were reared on non-Bt maize (VAA10Bt-NBt) laid significantly fewer egg batches than that of the susceptible population (BRO10Con-NBt) (Tables 6.1). The mean number of eggs laid per female ranged between 283 – 324 and did not differ significantly between populations (Table 6.2). The mean number of unfertilized eggs per female ranged between 7.1 and 8.8 % and also did not differ significantly between populations (Table 6.2). Although there seemed to be large differences in the percentage egg hatch between Bt-resistant and susceptible populations (range between 14.6 % to 55.8 %), these differences were not significant. This is ascribed to large variation in hatch success and is most likely due to the difficulties experienced in handling and storing of eggs and optimising conditions for hatching.

6.3.2 Larval growth and survival

6.3.2.2 Larval survival

Survival of larvae from the resistant population (VAA10Bt) when feeding on Bt- or non-Bt maize was similar with no adverse effect resulting from feeding on Bt maize (Fig. 6.3). Larval survival of the resistant population was significantly higher compared to that of the susceptible population when larvae were feeding on non-Bt maize (Fig. 6.3). Although there was a rapid decrease in larval survival of the Bt-susceptible population (BRO10Con-Bt) on Bt maize over the first six days of feeding, mortality did not reach 100 % as expected in the first 12 days after inoculation. Survival of the susceptible population was 6 % (18 of 300 larvae), 66 days after commencement of feeding (Fig. 6.3). Larval survival of the VAA10Bt populations differed significantly from that of the BRO10Con populations (Table 6.3). Six percent larval survival was observed for the susceptible population (BRO10Con) feeding on Bt maize with 12 % survival observed for BRO10Con feeding on non-Bt maize. Though this difference was not significant at

$p=0.0001$ as shown in table 6.3 it was significant at $p=0.0215$. However, survival of Bt-resistant larvae was similar on Bt and non-Bt maize (Table 6.3). The period until 50 % larval mortality was observed (LT50) was significantly longer for the Bt-resistant population (35.64 days) when larvae were reared on Bt maize, compared to the resistant larvae reared on non-Bt maize (25.42 days) (indicated by fiducial limits, Table 6.3). The LT50 value was the shortest for the susceptible population on Bt maize (0.55 days) while the LT50 value for the susceptible population on non-Bt maize was 10.31 days (Table 6.3).

6.3.2.2 Larval mass

The mean mass of F_1 -larvae increased steadily up to day 18 after which a rapid increase was observed for all treatments (Fig. 6.4). The mass of larvae of the resistant population (VAA10Bt) on both Bt and non-Bt maize was similar 31 days after inoculation. The larval mass of the resistant population (VAA10Bt-Bt) did not differ significantly from that of susceptible larvae feeding on non-Bt maize (BRO10Con-NBt) (Table 6.3). Larval mass of the susceptible population feeding on Bt maize was the lowest of all treatments differing significantly from larvae of the resistant population.

6.3.2.3 Pupal mass and development

Pupae started to form on day 31 in all stem borer populations (Figs. 6.5 and 6.6). Mortality during the pre-pupal phase of the F_1 -generations was very low (between 0 - 6.7 %). The percentage mortality of the Bt-resistant population during the pre-pupa stage was not significantly affected by exposure to Bt-toxin during their larval stage and mortality during the pre-pupal stage did not differ between the Bt-resistant and Bt-susceptible populations (data not shown).

The rate of development and survival up to pupal stage was the highest for Bt-resistant female pupae of which larvae were reared on Bt maize (Female VAA10Bt-Bt) (Fig. 6.5). Bt-resistant male pupae showed the lowest survival (Fig. 6.5). When Bt-resistant larvae were reared on Bt maize (VAA10Bt-Bt), female pupae formed at a higher rate than male pupae of the same population (Fig. 6.6). When Bt-resistant larvae were reared on non-

Bt maize, female pupae formed at a higher rate. Data from the Bt-susceptible population (BRO10Con) reared on non-Bt maize showed that male pupae were quicker to develop. The cumulative number of pupae from larvae of the susceptible populations (BRO10Con) that were reared on Bt maize was very low after 59 days (Fig. 6.5).

Male and female pupal mass of the F_1 -population was also not significantly affected by exposure of the larvae that they developed from, to Bt maize (Table 6.4). The mass of pupae of the Bt-susceptible population that developed from larvae feeding on non-Bt maize (BRO10Con-NBt) was significantly higher than pupae of the resistant population (Table 6.4).

6.3.2.4 Sex ratio

The sex ratio of pupae did not deviate from the expected 1:1 ratio in any of the F_0 or F_1 -populations (Table 6.5) and was therefore not affected by exposure of larvae to Bt-toxin.

6.4 Discussion

This study indicates that there were no measurable fitness costs in terms of pupal and moth parameters and that the resistant population lived even longer than the susceptible population under laboratory conditions. There were, however, ovipositional fitness costs in the Bt-resistant population, measured in terms of fecundity as moths developing from larvae that were reared on Bt maize for their whole cycle laid significantly fewer eggs than moths originating from larvae reared on non-Bt maize.

No fitness costs in terms of percentage larval survival and growth (mass) was associated with resistance in the population that was collected at the Vaalharts irrigation scheme. The fitness of the resistant population, when larvae were reared on Bt maize was similar to when larvae were reared on non-Bt maize. The high LT_{50} value for the resistant population that developed on Bt together with the high percentage survival and mean larval mass found in this study, indicates the high level of resistance to Bt-toxin and fitness of the studied population. No fitness cost in terms of fertility or egg hatch were observed for the F_0 or F_1 -generations.

No studies have previously been conducted to quantify fitness costs that may be associated with Bt resistance development of *B. fusca*. However, laboratory studies with Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) concluded that there were modest fitness costs in terms of development time and survival in some Bt-resistant colonies but not in others (Oppert *et al.*, 2000). In another study Groeters *et al.* (1994) observed fitness trade-offs associated with evolution of resistance in the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Gelechiidae), with lower fecundity and fertility as well as reduced survival. Only slight fitness costs in term of development time (3 %) was determined by Carrière *et al.* (2001) for certain resistant pink bollworm strains (*Pectinophora gossypiella*) (Hubn.) (Lepidoptera: Gelechiidae) on cotton. Results showed that resistance to Bt cotton in pink bollworm had little or no effect on development rate on non-Bt cotton (Carrière *et al.*, 2001).

The spread of resistance to Bt maize (MON810) in South Africa has been surprisingly rapid and has been reported from several regions in the main maize production region (Kruger *et al.*, accepted, Chapter 4). Survival of larvae of the susceptible F₁-population collected at Bronkhorstspuit, although at a level of just below 10%, indicates that this population was not as susceptible to Bt-toxin as initially thought. Previous studies on survival of Bt-susceptible *B. fusca* larvae on Bt maize indicated that larvae die within 6 – 12 days after commencing feeding on Bt maize (Van Rensburg, 2007; Kruger *et al.*, 2009, Chapter 3). Survival levels in these studies were similar to those observed for another noctuid species, *Sesamia calamistis* (Van den Berg & Van Wyk, 2007) on Bt maize. The relatively high level of survival of the Bronkhorstspuit population could indicate that field-resistance is also developing in this geographical region approximately 550 km away from where the first report of resistance was made in 2007 (Van Rensburg, 2007; Kruger *et al.*, 2009, Chapter 3).

Rapid adaptation of insect pests could shorten the success of crops that are genetically modified to produce toxin from *Bacillus thuringiensis* (Tabashnik, 1994). As in the case with Bt-insecticides (Dipel), resistance alleles may have pleiotropic effects that could

compromise normal functions and impose a fitness cost in the absence of Bt toxin. However, while fitness costs were most likely present in the first *B. fusca* individuals exposed to Bt-toxin in South Africa, it is no longer the case after a relatively short evolutionary process of only 13 years at the Vaalharts irrigation scheme where the resistant population used in this study was collected. Although fitness costs may contribute to the delay of resistance evolution to Bt toxins when refugia are present (Carrière & Tabashnik, 2001), it is no longer the case with the highly resistant population in South Africa.

Results from the LT50-values may indicate some effect of Bt-toxin on the resistant population but does not translate into observable costs in terms of fecundity, larval mass and survival. The absence of fitness costs may promote the use of alternative Bt-resistance management strategies, such as a multi-gene strategy with non-overlapping targets, which would be expected to decrease fitness (Oppert *et al.*, 2000).

The results from this study differ from that of a previous study (Chapter 5) and showed no differences in sex ratio in any of the populations. Since sex ratio and the possibility of assortative mating could play a role in the sustainability of resistance of Bt maize to target pests, this aspect needs further investigation.

This study also provided information on which parameters to measure when doing fitness studies. The parameters measured in this study provided useful information for comparison of fitness of different populations. For most of the parameters no differences could be shown between individuals of the resistant population when feeding on Bt or non-Bt maize. These measured parameters were therefore not good indicators of fitness at such a late stage in the evolutionary process and with such a high level of resistance. However, significant differences were observed between several parameters when these were compared between resistant and susceptible populations. Female and male pupal mass as well as longevity of female moths differed between resistant and susceptible populations and can therefore be used as accurate indicators of fitness of stem borers on Bt maize. The number of eggs laid per female did

not highlight any differences between populations in this study but is most likely one of the most important measurements of the fitness of an individual. While a previous study (Chapter 5) indicated that the number of eggs laid per Bt-resistant female can be affected if the larva she developed from was reared on Bt maize, the number of eggs was within the general range of that laid by moths that have never been exposed to Bt maize. This study showed that unless the conditions under which eggs are kept until hatching is not optimal, this may not be a good parameter on which to base comparisons between treatments.

6.5 Conclusions

Minimal fitness costs associated with resistance of *B. fusca* to Bt maize (Cry1Ab) was observed in this study. Survival on Bt maize of the population collected at Bronkhorstspuit on non-Bt maize was low, but may indicate another resistant population in South Africa. The introduction of a stacked event such as MON89034 which produces more than one protein which is active against the resistant target pest, together with compliance to the refuge strategy, is most likely the only solution to managing Bt-resistant stem borer populations in South Africa.

6.6 References

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Table 6.1 Comparison of life history parameters between F₀-generations of a Bt-resistant and a Bt-susceptible population of *Busseola fusca*, reared on Bt or non-Bt maize. df = 58 except where otherwise indicated.

Parameters	Population	t-value	P-value	F-ratio
Female pupal mass	VAA10Bt-Bt vs. VAA10Bt-NBt	1.042	0.301	1.011
	VAA10Bt-NBt vs. BRO10Con-NBt	-7.255	0.000	2.235
	VAA10Bt-Bt vs. BRO10Con-NBt	-6.449	0.000	2.261
Male pupal mass	VAA10Bt-Bt vs. VAA10Bt-NBt	1.815	0.074	1.550
	VAA10Bt-NBt vs. BRO10Con-NBt	-5.893	0.000	1.782
	VAA10Bt-Bt vs. BRO10Con-NBt	-4.814	0.000	2.762
Longevity of male moths	VAA10Bt-Bt vs. VAA10Bt-NBt	-0.320	0.749	1.090
	VAA10Bt-NBt vs. BRO10Con-NBt	0.056	0.955	1.703
	VAA10Bt-Bt vs. BRO10Con-NBt	-0.221	0.825	1.562
Longevity of female moths	VAA10Bt-Bt vs. VAA10Bt-NBt	-0.743	0.460	1.079
	VAA10Bt-NBt vs. BRO10Con-NBt	2.712	0.008	1.399
	VAA10Bt-Bt vs. BRO10Con-NBt	2.078	0.042	1.151
Number of eggs laid per female	VAA10Bt-Bt vs. VAA10Bt-NBt	-0.096	0.923	0.029
	VAA10Bt-NBt vs. BRO10Con-NBt	-1.411	0.163	1.336
	VAA10Bt-Bt vs. BRO10Con-NBt	-1.508	0.136	1.375
Number of egg batches laid per female	VAA10Bt-Bt vs. VAA10Bt-NBt	1.810	0.075	2.234
	VAA10Bt-NBt vs. BRO10Con-NBt	-2.384	0.020	1.986
	VAA10Bt-Bt vs. BRO10Con-NBt	-0.421	0.675	1.124
Percentage unfertilised eggs per female	VAA10Bt-Bt vs. VAA10Bt-NBt*	0.984	0.328	1.842
	VAA10Bt-NBt vs. BRO10Con-NBt	-1.475	0.145	9.907
	VAA10Bt-Bt vs. BRO10Con-NBt*	-0.920	0.360	5.378
Percentage eggs that did not hatch	VAA10Bt-Bt vs. VAA10Bt-NBt	-0.071	0.942	1.290
	VAA10Bt-NBt vs. BRO10Con-NBt	-1.646	0.105	1.769
	VAA10Bt-Bt vs. BRO10Con-NBt	-1.628	0.108	1.371

* df=57

VAA10Bt-NBt - Vaalharts Bt-resistant population on non-Bt maize

VAA10Bt-Bt - Vaalharts Bt-resistant population on Bt maize

BRO10Con-NBt - Bronkhorstspuit control population on non-Bt maize

Table 6.2 Life history parameters of field-collected Bt-resistant and susceptible F₀-generations of *Busseola fusca* reared on Bt and non Bt maize under laboratory conditions (Mean ± S.E).

Parameter	Population		
	VAA10Bt-Bt	VAA10Bt-NBt	BRO10Con-NBt
Female pupal mass (mg)	24.13 ± 0.71a	23.28 ± 0.71a	32.49 ± 1.00 b
Male pupal mass (mg)	20.99 ± 0.72a	18.90 ± 0.89a	27.81 ± 1.18 b
Longevity female (days)	8.80 ± 0.43 ab	9.26 ± 0.45 b	7.36 ± 0.53 a
Longevity male (days)	9.03 ± 0.37	9.20 ± 0.36	9.16 ± 0.46
Number of egg batches	7.70 ± 0.97	5.56 ± 0.65	7.83 ± 0.94
Number of eggs / female	283.96 ± 34.83	288.73 ± 34.18	342.46 ± 41.16
Percentage unfertilised eggs	8.84 ± 2.78	7.15 ± 3.08	7.19 ± 3.18
Percentage of eggs not hatching	14.62 ± 5.16	25.94 ± 5.94	55.88 ± 29.79

* Means within rows followed by the same letter do not differ significantly according to the Tukey test-HSD (F = 23.09; df = 87; P = 0.0001).

** Means within rows followed by the same letter do not differ significantly according to the Tukey test-HSD (F = 36.29; df = 87; P = 0.0001).

*** Means within rows followed by the same letter do not differ significantly according to the Tukey test-HSD (F = 4.32; df = 87; P = 0.0016).

VAA10Bt-NBt - Vaalharts Bt-resistant population on non-Bt maize

VAA10Bt-Bt - Vaalharts Bt-resistant population on Bt maize

BRO10Con-NBt - Bronkhorstspuit control population on non-Bt maize

Table 6.3 Larval mass, percentage survival and LT50 of F₁ *Busseola fusca* populations after 66 days of feeding on Bt and non-Bt maize under laboratory conditions ($\pm$ SE).

<i>Busseola fusca</i> population	Percentage survival on day 66 $\pm$ SE*	Mean mass (mg) on day 31 $\pm$ SE**	LT50 (days) (95% Fiducial limits)
VAA10Bt-NBt	36.33 a $\pm$ 3.66	29.48 c $\pm$ 2.70	25.42 (23.55 – 27.39)
VAA10Bt-Bt	33.66 a $\pm$ 3.33	25.49 bc $\pm$ 0.88	35.64 (33.68 – 37.69)
BRO10Con-NBt	12.33 b $\pm$ 1.81	17.38 a $\pm$ 1.86	0.55 (0.16 – 1.16)
BRO10Con-Bt	6.00 b $\pm$ 1.89	22.71 ab $\pm$ 1.10	10.31 (9.17 – 11.42)

* Means within columns followed by the same letter do not differ significantly according to the Tukey test-HSD (F = 28.98; df = 116; P = 0.0001).

** Means within columns followed by the same letter do not differ significantly according to the Tukey test-HSD (F = 5.69; df = 116; P = 0.0012).

VAA10Bt-NBt - Vaalharts Bt-resistant population on non-Bt maize

VAA10Bt-Bt - Vaalharts Bt-resistant population on Bt maize

BRO10Con-NBt - Bronkhorstspuit control population on non-Bt maize

BRO10Con-Bt - Bronkhorstspuit control population on Bt maize

Table 6.4 Mean mass of F₁-generation of *Busseola fusca* pupae originating from Vaalharts and Bronkhorstspuit field-collected (F₀) larvae which were reared on Bt- and non-Bt maize.

	<i>Busseola fusca</i> population	Mean mass (mg) ($\pm$ SE)	df	t-value	P-value	F-ratio
Male pupae	VAA10Bt-Bt	21.31 $\pm$ 0.66	69	1.388	0.619	1.372
	VAA10Bt-NBt	18.53 $\pm$ 0.85				
Female pupae	VAA10Bt-Bt	22.44 $\pm$ 0.68	104	-0.467	0.641	1.109
	VAA10Bt-NBt	22.99 $\pm$ 0.69				
Male pupae	BRO10Con-Bt	18.82 $\pm$ 1.16	20	-1.258	0.222	1.381
	BRO10Con-NBt	20.87 $\pm$ 1.10				
Female pupae	BRO10Con-Bt	17.74 $\pm$ 1.84	11	-0.619	0.133	2.616
	BRO10Con-NBt	23.17 $\pm$ 2.36				

VAA10Bt-NBt - Vaalharts Bt-resistant population on non-Bt maize

VAA10Bt-Bt - Vaalharts Bt-resistant population on Bt maize

BRO10Con-NBt - Bronkhorstspuit control population on non-Bt maize

BRO10Con-Bt - Bronkhorstspuit control population on Bt maize

Table 6.5 Sex ratio of field collected (F_0 -generation) and laboratory reared (F_1 -generation) of Bt-susceptible and Bt- resistant populations of *Busseola fusca*.

<i>Busseola fusca</i> population	Sex ratio Male : Female	Observations (n)	Chi-square	p-value
F_0-generation				
VAA10Bt-NBt	1 : 0.86	136	0.544	0.478
VAA10Bt-Bt	1 : 0.73	64	0.375	0.627
BRO10Con-NBt	1 : 0.83	189	0.382	0.738
F_1-generation				
VAA10Bt-NBt	1 : 1.21	97	0.518	0.616
VAA10Bt-Bt	1 : 1.74	96	0.583	0.804
BRO10Con-NBt	1 : 1.61	67	0.666	0.797
BRO10Con-Bt	1 : 1.11	19	0.872	0.865

VAA10Bt-NBt - Vaalharts Bt-resistant population on non-Bt maize

VAA10Bt-Bt - Vaalharts Bt-resistant population on Bt maize

BRO10Con-NBt - Bronkhorstspuit control population on non-Bt maize

BRO10Con-Bt - Bronkhorstspuit control population on Bt maize



Fig. 6.1 Oviposition chambers in which longevity and fecundity of moths were determined.



Fig. 6.2 Test tubes used to rear stem borer larvae in the laboratory.

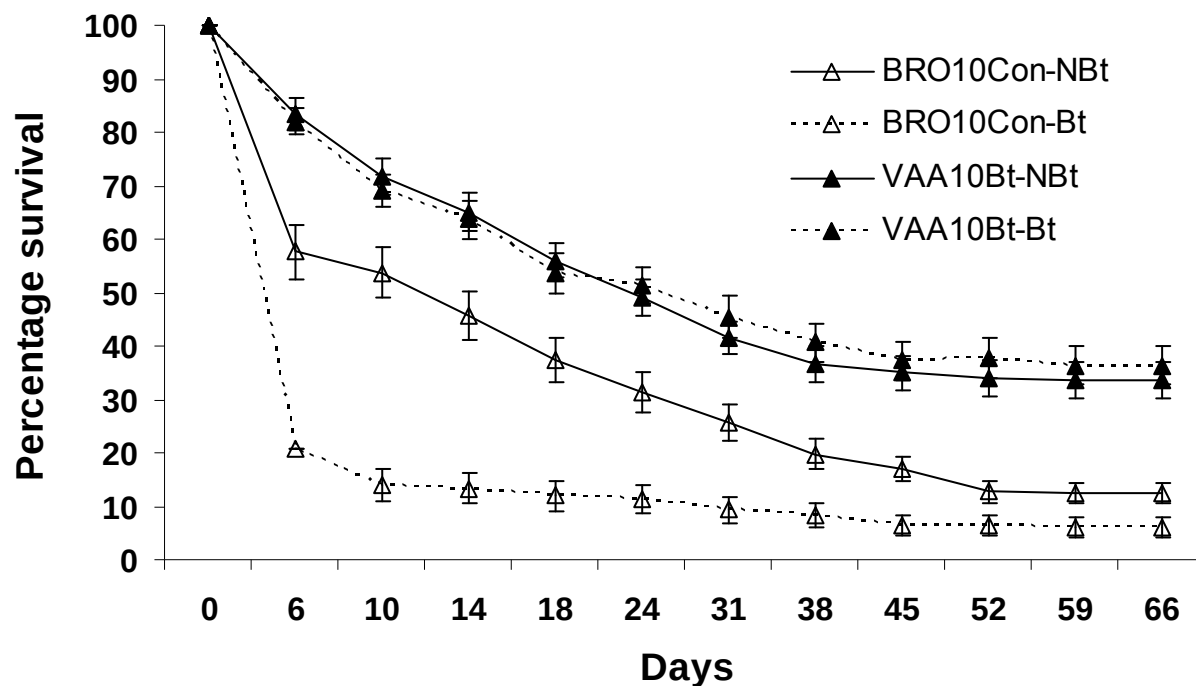


Fig. 6.3 Larval survival of different *Busseola fusca* populations on Bt and non-Bt maize under laboratory conditions (Bars indicate Standard errors). Solid lines represent Bt maize and dotted lines represent non-Bt maize. Bt-resistant population collected at Vaalharts. Control population collected at Bronkhorstspuit.

VAA10Bt-NBt - Vaalharts Bt-resistant population on non-Bt maize
VAA10Bt-Bt - Vaalharts Bt-resistant population on Bt maize
BRO10Con-NBt - Bronkhorstspuit control population on non-Bt maize
BRO10Con-Bt - Bronkhorstspuit control population on Bt maize

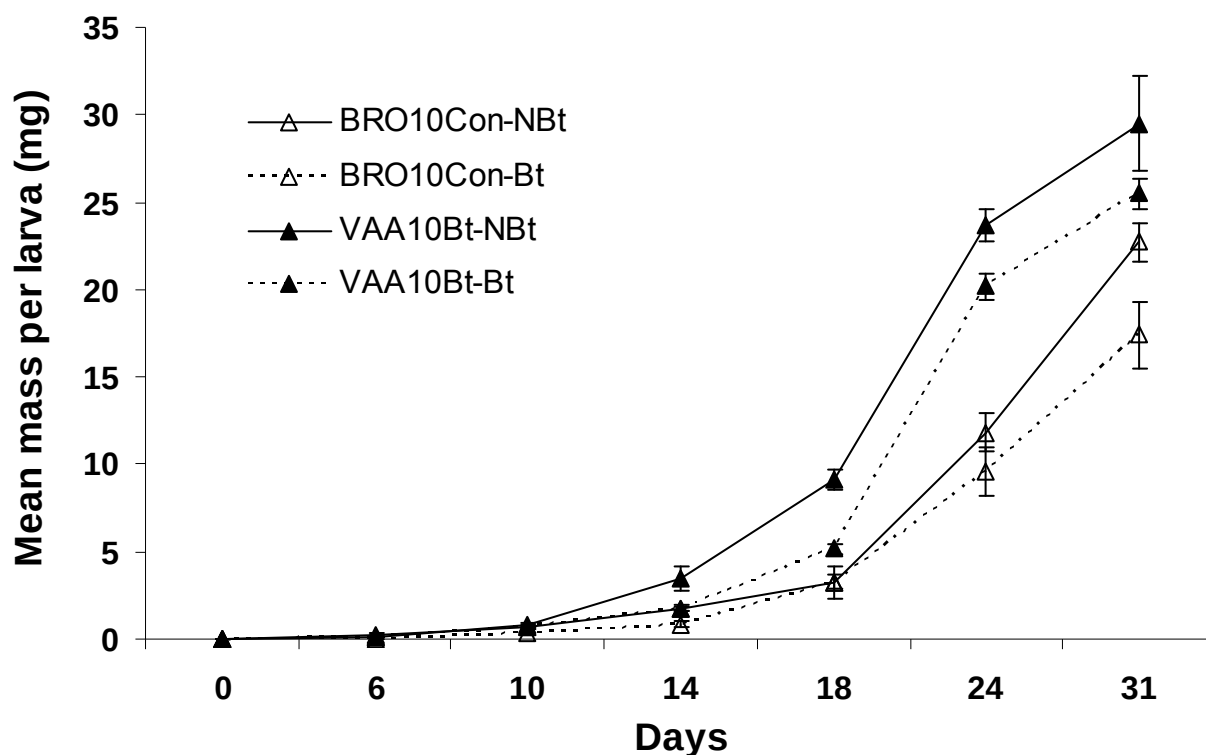


Fig. 6.4 Mean mass of larvae of different *Busseola fusca* populations reared on Bt and non-Bt maize under laboratory conditions (Bars indicate Standard errors). Solid lines represent Bt maize and dotted lines represent non-Bt maize. Bt-resistant population collected at Vaalharts. Control population collected at Bronkhorstspuit.

VAA10Bt-NBt - Vaalharts Bt-resistant population on non-Bt maize
VAA10Bt-Bt - Vaalharts Bt-resistant population on Bt maize
BRO10Con-NBt - Bronkhorstspuit control population on non-Bt maize
BRO10Con-Bt - Bronkhorstspuit control population on Bt maize

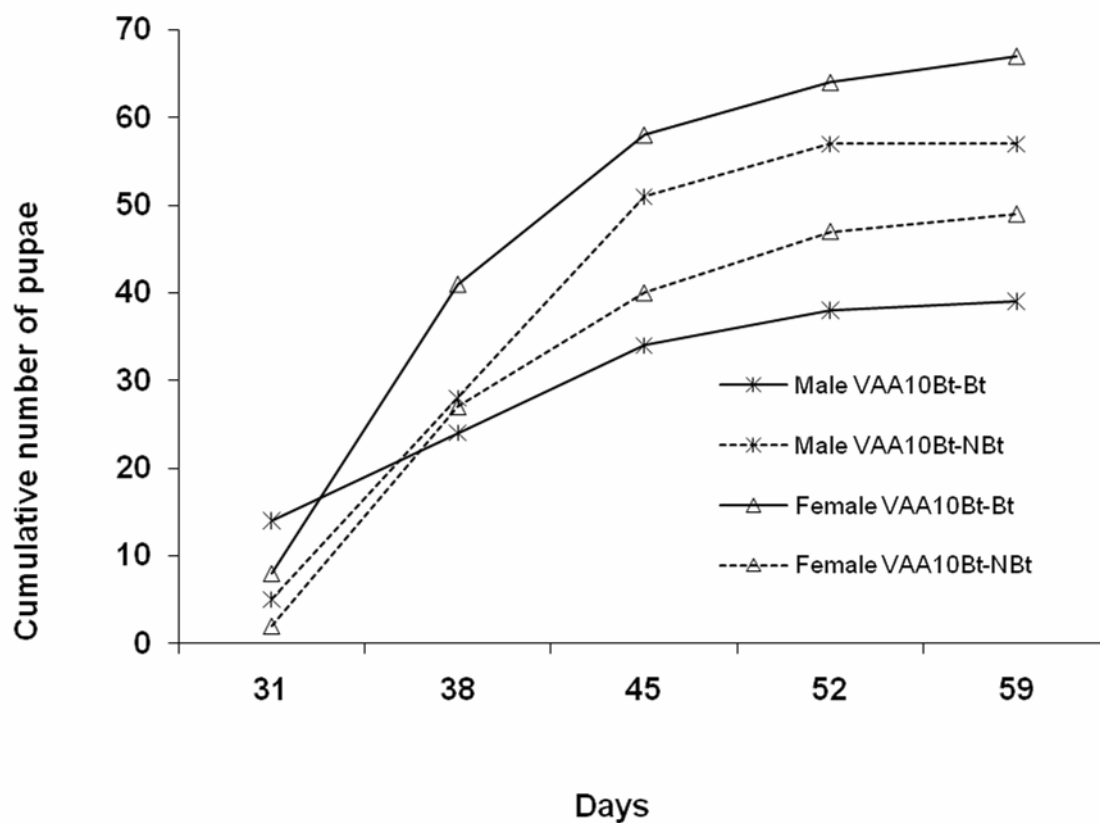


Fig. 6.5 Cumulative number of pupae developing from *Busseola fusca* larvae collected at Vaalharts on Bt and non-Bt maize under laboratory conditions. Solid lines represent Bt maize and dotted lines represent non-Bt maize.

VAA10Bt-NBt - Vaalharts Bt-resistant population on non-Bt maize
VAA10Bt-Bt - Vaalharts Bt-resistant population on Bt maize

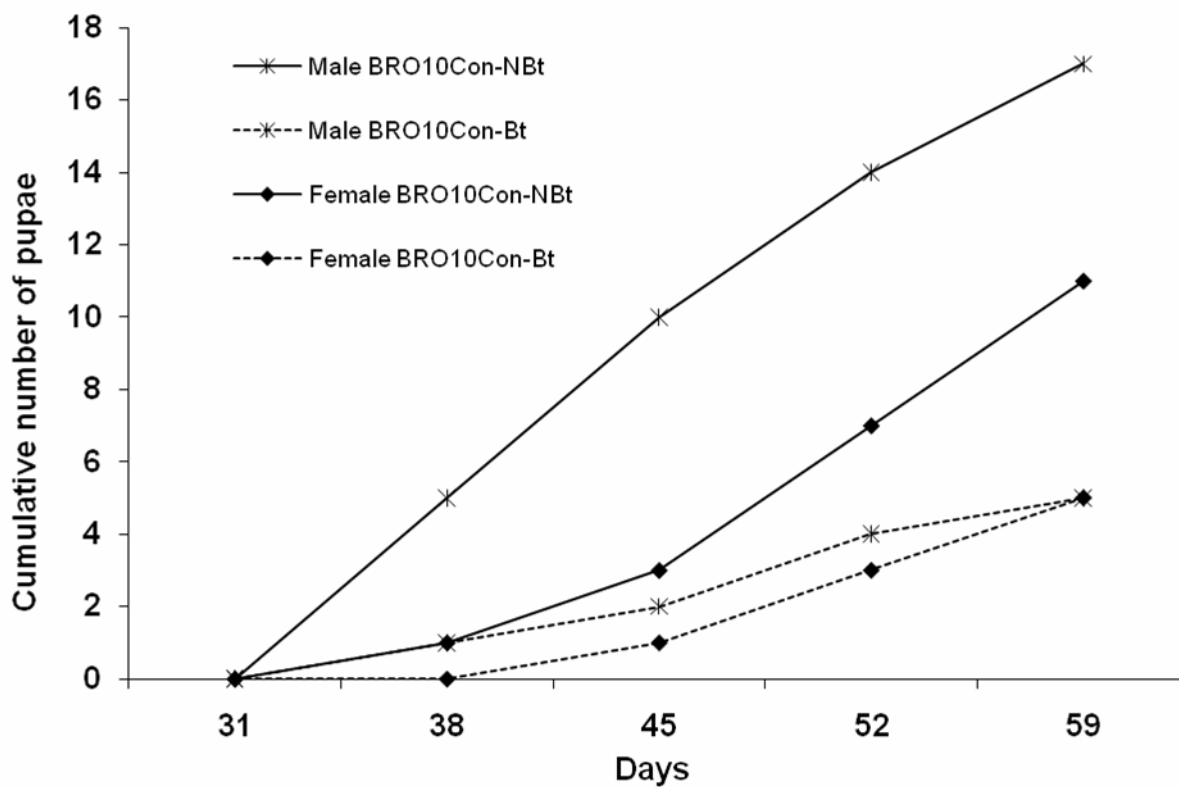


Fig. 6.6 Cumulative number of pupae developing from *Busseola fusca* larvae collected at Bronkhorstspuit on Bt and non- Bt maize under laboratory conditions. Solid lines represent non-Bt maize and dotted lines represent Bt maize.

BRO10Con-NBt - Bronkhorstspuit control population on non-Bt maize
 BRO10Con-Bt - Bronkhorstspuit control population on Bt maize

Chapter 7

Conclusions

Bt maize (MON810) has been grown to control lepidopterous stem borers in South Africa since its first release during 1998. The first report of field resistance of *Busseola fusca* (Lepidoptera: Noctuidae) to Bt maize was made during the 2006 cropping season in South Africa (Van Rensburg, 2007). Research into various aspects of resistance evolution and possible contributing factors was stimulated by this first report of resistance evolution to an insecticidal genetically modified (GM) crop in the world.

7.1 High dose/refuge strategy

In order to develop an insect resistance management (IRM) strategy that will delay the evolution as well as the spread of stem borer resistance to Bt maize in South Africa, it is important to evaluate the current refuge strategy and make improvements if necessary. The current refuge requirements are either a 20 % refuge planted to conventional maize which may be sprayed with insecticides, or a 5 % refuge area of conventional maize that may not be sprayed. The high dose/refuge strategy, employed to limit resistance evolution, comprises planting of Bt maize plants producing high doses of toxin in close proximity to non-Bt plants. The purpose of the high dose of toxin is to kill as many individuals of the target pest as possible, whereas the purpose of the refuge is to produce pest individuals that survive on that particular crop (Renner, 1999; Gould, 2000). The aim is to ensure that those rare, Bt-resistant individuals that survive on the Bt crop do not produce completely resistant offspring by mating with other toxin-resistant individuals and that the crop will express 25 times the toxin required to kill 99 % of the susceptible individuals (Glaser & Matten, 2003).

This study indicated that the level of compliance to refuge requirements was low (Chapters 3 & 4) and that it could have contributed to resistance evolution. However, while it is relatively easy to monitor compliance to refuge requirements, it is impossible to monitor whether the high-dose component of the high dose/refuge strategy is complied to. Low levels of expression of Bt-protein in plants could also

result in a high rate of resistance evolution (Tabashnik *et al.*, 2008) and the contribution of this factor towards resistance development in South Africa should not be excluded.

Currently no effective management strategy exists to combat the spread of resistant populations, or to delay resistance development in South Africa. Prior to the study no investigation had been done to quantify the status of resistance, attempt an explanation for resistance development or develop strategies to manage the resistant populations. It seemed that the prescribed high dose/refuge strategy may not have been very effective and the level of compliance was unknown in the main maize production areas of the country.

The possibility existed that non-compliance to refuge requirements in the Highveld region could have played a role in evolution of resistance. A unique opportunity existed after 12 years of cultivation of Bt maize in South Africa to gain insight from farmers on the use of Bt maize as well as on regulatory aspects pertaining to the planting thereof. The opportunity was also used to determine levels of compliance to regulatory requirements such as planting of refugia and to highlight possible areas where resistance development may be a risk. For Bt maize in South Africa, as for any other GM crop, it is necessary to understand the resistance risk and which IRM strategies to delay or prevent resistance occurring may be feasible. This is done by collecting information on the agricultural system, the biology of the target pest and the behaviour of growers and through generating local information on product performance (Head, 2010). This information contributes to develop and define the IRM tactics that can and should be used (Head, 2010).

According to Head (2010) who considers the risk of resistance evolution from the viewpoint of the private industry, the following factors should be considered in order to develop and manage an effective IRM strategy: the cropping system, biology of target pests, grower behaviour and attitudes as well as product performance. An attempt is made below to integrate findings of this study into relevant aspects of IRM as highlighted by Head (2010).

7.2 The cropping system

In order to develop an IRM strategy, Head (2010) recommended investigation into the following:

- Is the crop grown in monoculture or polyculture?
- What is the approximate farm size?
- What pest control methods are used for the target pests and what level of product adoption is expected?
- Do other transgenic products already exist in this crop or in other crops in the same agricultural system?

In this study Bt and non-Bt maize were grown in monoculture fields and farmers in the Vaalharts irrigation scheme were restricted to 25 ha plots. In the semi-arid areas of South Africa the farm size did not have an influence on the number of hectares planted to Bt maize, which was determined by the farmers' preference (Chapters 3 & 4). The adoption rate of Bt maize was high and farmers in Vaalharts irrigation scheme started adopting Bt maize during the 1998/99 season when approximately 47 % of them planted Bt maize for the first time. Since 1999, the number of farmers planting Bt maize for the first time increased with an average of 5.9 % per year until 2007 when all farmers planted Bt maize (Chapter 3). During the past nine years Bt maize production was perceived to provide effective control of stem borers at the Vaalharts irrigation scheme and Highveld regions in South Africa (Chapter 3 & 4). Farmers in Vaalharts indicated that stem borers were effectively controlled with Bt maize but that they already observed an increase in damage levels during the 2005/06 season. These levels of borer infestation on Bt maize increased towards the 2007/08 season, when nearly 50 % of farmers observed stem borer damage on Bt maize for the first time. During the 2007/08 growing season farmers experienced high stem borer infestation levels on Bt maize and were compelled to use insecticides to prevent economic crop losses (Chapter 3).

The importance of knowledge on grower behaviour and the possible role that it could play in pest management was illustrated in this study. As indicated by Head (2010) it is important to know current management practices employed for the target pests as well as non-target pests since that could impact on the efficacy of IRM strategies.

Chemical control was the norm for control of the target stem borer species on maize before the advent of Bt maize. Chemical control was not supposed to be done on Bt maize for target pests and this should be discouraged and indicated in farmer training with introduction of GM technology. In this study it was shown that an alarmingly high number of farmers in the Highveld region applied insecticides as preventative sprays on Bt maize and refugia. A large proportion of these farmers reported significant borer infestation levels on Bt maize and between 5 – 93 % farmers in some districts applied insecticides to Bt maize to limit borer damage, indicating that the occurrence of resistance is more wide-spread in the country than previously thought. This study showed irresponsible management of GM crop technology by farmers, chemical- and seed companies (Chapter 4).

The two greatest perceived advantages associated with Bt maize in Vaalharts were convenient management (88 %) and increased productivity (61.3 %) while 42.5 % of farmers indicated that they perceived Bt technology to be environmentally friendly (Chapter 3). Farmers preferred to plant the refuge option where 5 % of the field area is planted to conventional maize which is not sprayed with insecticide instead of the 20 % refuge area on which insecticide application against the target pest is allowed (Chapters 3 & 4).

Although much information on adoption rates of GM technology and compliance to regulatory requirements such as planting of refugia is available for the commercial farming sector in South Africa, no information is available about these factors in developing agriculture in South Africa or the rest of the continent. It is of critical importance that this target group of farmers and farming systems be clearly understood and that farmers should understand management requirements prior to release of GM crops since incorrect management of this GM technology could result in rapid evolution of resistance or development of secondary pests of which farmers may be unable to control.

7.3 Biology of the target pest

For an IRM strategy to function effectively, the following information is needed regarding target pest biology (Head, 2010):

- For each target pest, how many generations per year are there and how many of these are on the crop of interest?
- Does the insect use wild or cultivated hosts other than the crop of interest as hosts?
- If so, what are they and how common are they in the same agricultural systems?
- How much do the adult insects move before and after mating?
- Where do the insects mate?
- Do they have a history of developing resistance to insecticides and Bt crops?
- Are transgenic products used to control this species in this crop or other crops in the same agricultural system?

It is essential to assess the environmental risk (including resistance development) that Bt maize may hold and to study its effect on species assemblages that fulfil a variety of ecosystem functions. Environmental risk assessment can be improved through the use of an ecological model and by applying this model to specific environments, local species can be classified functionally and prioritized to identify potential test species (Van Wyk *et al.*, 2007). Although the stem borers *B. fusca* and *Chilo partellus* (Lepidoptera: Pyralidae) are the target species of Bt maize in South Africa various other Lepidoptera species are also directly exposed to Bt toxin (Van Wyk *et al.*, 2007). Van Wyk *et al.* (2007) followed an ecological approach for selection of non-target Lepidoptera species for ecological risk assessment of Bt maize, using data collected on Lepidoptera biodiversity on maize over a 2-year period. Nine primary non-target lepidopterous consumers were identified. A selection matrix was developed in which each species was ranked for its maximum potential exposure to Bt toxin by assessing its occurrence, abundance, presence and linkage to other host plants in the maize ecosystem. Non-target Lepidoptera which are most likely to be affected were identified and prioritized for future testing and inclusion in risk assessments. Several non-target species were prioritized for their close association with maize, general occurrence in the maize growing regions and their

potential for economic damage if they become secondary pests (Van Wyk *et al.*, 2007). Through use of the selection matrix, knowledge gaps were identified for future research and guidance of the design of ecologically realistic experiments.

Wild host plants are often referred to as a potential component of an IRM strategy since the wild host could function as a non-structured refuge which sustains susceptible individuals of the target species. Wild host plants are regarded as alternative hosts of the pest species during the non-cropping season and could play an important role in their ecology in maize agro-ecosystems. However, large gaps in information exist about the diversity and abundance of stem borers in wild host plants in Africa (Le Rü *et al.*, 2006). Lepidopteran stem borer species vary in their host exploitation strategies, which range from monophagous and oligophagous to polyphagous. These stem borer larvae feed inside stems of many species of monocotyledonous plants which belongs to the Poaceae, Cyperaceae and Typhaceae families (Le Rü *et al.*, 2006). In the recent past surveys have been conducted to determine the distribution and diversity of lepidopteran stem borers on the African continent. Most of these studies showed that the diversity of lepidopteran species and host plant range are much higher than earlier surveys showned (Le Rü *et al.*, 2006). Only a few studies to determine lepidopteran stem borer distribution have been done in South Africa, even though this group of insects is important as pests of crops in the country (Harris & Nwanze, 1992; Van den Berg & Rebe, 2001).

The extensive survey of the diversity of lepidopteran stem borers on monocotyledonous plants in eastern Africa, conducted by Le Rü *et al.* (2006), was the first of its kind. Le Rü *et al.* (2006) found that the majority of the stem borer larvae collected was on host plants with thick and robust stems growing in the wetter parts of the surveyed areas. What was notable about the study of Le Rü *et al.* (2006) and current research (Van den Berg, pers. comm.) is that the primary target pests of Bt maize, *B. fusca* and *C. partellus*, occurred in wild hosts species at negligible levels that could not explain the high infestation levels in crop fields. Current information therefore indicates that wild host plants will not contribute towards the refuge component of the IRM strategy.

In this study information regarding the target and its fitness on Bt maize was generated. It also contributed to information regarding the biology of *B. fusca* on Bt maize, the status of resistance to Bt maize, life history parameters, fitness and fitness costs associated with resistance development. It was shown that larvae of the susceptible Christiana population on Bt-maize and the susceptible Bethal population on Bt-maize did not survive on Bt-maize for longer than 12 days and that these populations were still highly susceptible to Bt maize (Chapter 2). However, populations collected from both Bt maize and non-Bt maize refuges at Vaalharts were resistant and the subsequent generation of larvae also completed their life cycle on Bt-maize. Similar results were observed in laboratory experiments. This study confirmed resistance of *B. fusca* to the Cry1Ab toxin (MON810) (Chapter 2).

The study on the fitness of *B. fusca* larvae on Bt and non-Bt maize showed that there was a tendency that the sex ratio was biased towards male and female pupae (Chapter 5). There were significant differences between the mass of male and female pupae for both the resistant and susceptible populations collected during spring as well as the summer populations. Larval survival of different *B. fusca* populations on Bt and non-Bt maize under laboratory conditions showed the Vaalharts Bt-resistant populations had the highest level of survival and mean mass per lava and that there were no differences between the resistant *B. fusca* larvae reared on non-Bt maize and tested on Bt and non-Bt maize. It seemed that Bt maize had an influence on the fitness of the Bt-resistant population (Chapter 5).

Further studies were conducted on the fitness costs of Bt-resistant populations on non-Bt maize and their development under laboratory and field conditions on Bt and non-Bt maize. This study indicated that there were minimal differences between life history parameters of the different field-collected *B. fusca* populations and that there were no fitness costs were associated with resistance evolution (Chapter 6). Larval survival of different *B. fusca* populations on Bt and non-Bt maize under laboratory conditions showed that the Vaalharts Bt-resistant population had the highest level of survival and mean mass per lava. There were also no significant differences between the resistant *B. fusca* larvae reared on non-Bt maize and tested for fitness costs differences on Bt and non-Bt maize (Chapter 6). The LT50 indicated some

degree of fitness cost but did not translate into observable costs in terms of fecundity, larval mass and survival (Chapter 6).

The quick development and spread of resistant populations, only eight years after the release of Bt maize in South Africa is difficult to explain. This highlights the knowledge gap in the biology of *B. fusca* on how far the adult insects move before and after mating and also on where they mate and whether they mate before or after a significant first flight away from the site where eclosion occurred. This data could provide valuable information that could assist in improved IRM strategies. These aspects and knowledge gaps were also highlighted in the ecological risk assessment model described by Van Wyk *et al.* (2007). It is a pity that these questions which form part of risk assessments are often asked but not addressed by companies that develop the technology.

In South Africa there is no history of resistance development of stem borers against insecticides, even after decades of use (Van den Berg, pers. comm.). Transgenic maize was considered a favourable alternative control method of stem borers and was developed with confidence that resistance would not develop rapidly, or at all.

The above mentioned aspects indicated by Head (2010) remain important questions regarding IRM, a more formalized manner of questioning would be of greater benefit when risks such as resistance development are anticipated. Andow and Hilbeck (2004) developed an ecological model for risk assessments of GM crops, prior to their release into the environment. Use of the ecological model resulted in the accurate prediction of resistance development of *B. fusca* to Bt maize by Andow and Hilbeck (2004) and Van Wyk *et al.* (2007).

7.4 Grower behaviour and attitudes

With regard to the IRM strategy Head (2010) indicated several aspects pertaining to grower behaviour that could be important in IRM:

- Will growers be willing to practice integrated pest management (IPM) and IRM?
- Do they have a history of doing so?
- What is their knowledge level?
- Have growers been sensitized to the concept of resistance through the loss of other pest control tools?

In South Africa stewardship programs instituted by private industry during the 2008/09 growing season, involve grower education programs as well as the compulsory signing of grower-agreements (contracts) between companies and farmers. These contracts bind farmers to comply with refuge requirements accompanied by on-farm inspections (Chapter 3). This study showed that farmers were reluctant to employ the prescribed IRM strategies and that companies did not emphasize and enforce compliance to strategies. Since compliance to refuge requirements only started to increase significantly after the reports of resistance, it can be assumed that farmers were becoming more aware of the importance of IRM at that time (Chapters 3 & 4).

Observations that larvae collected from refugia at Vaalharts were resistant show that the efficacy of the refuge strategy is compromised in this area because the contribution of refugia did not produce large enough numbers of susceptible individuals to mate with moths of which larvae survived inside Bt maize fields (Chapter 2). Farmers need to be educated with regard to management of transgenic maize and regulatory requirements in order to prevent further spread of stem borer resistance. If farmers are not educated in this regard, it may compromise other GM insecticidal maize events that may be introduced into the country in future. Farmers must be informed of the role natural enemies and non-target organisms have in maize fields and the influence the irresponsible use of insecticides could have on the agro-ecosystem.

7.5 Product performance

In order to compile an IRM strategy, Head (2010) suggested the following:

- For each target pest species, what is the level of control (% mortality) of the Bt crop for each life stage infesting the crop?
- Will the use of insecticides be part of the overall IPM system in the Bt crop?
- If so, will insecticide applications still be needed on the Bt crop for this target pest?

During the past nine years Bt maize production was perceived to provide effective control of stem borers at the Vaalharts irrigation scheme and in the Highveld region in South Africa before resistance became a problem in several areas. Since the level of resistance is high and resistant populations are present throughout the maize production region, the use of an alternative Bt-resistance management strategy, such as a multi-gene strategy (stack genes) is currently the only viable option.

Because of local differences in cropping systems and farmer behaviour, IRM strategies must vary with respect to the nature of the refuge strategy (whether a structured refuge is needed and, if so, its size and placement); and the means by which farmers are educated with regard to IRM. It is important that seed-, chemical companies and farmers work together to make the IRM strategy work so that farmers can experience the economical advantages of Bt maize and to protect the benefits provided to them in the form of biotechnology.

7.6 References

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