The refuge concept in insect resistance management: its history and future application in South Africa

M. Gouws

Dissertation submitted in fulfilment of the requirements for

the degree Masters of Environmental Science

at the North-West University

(Potchefstroom Campus)

Supervisor: Prof. J. van den Berg

November 2011

Potchefstroom

Abstract

Genetically modified (GM) crops developed for insect control express *cry* genes from the bacterium *Bacillus thuringiensis* (Bt). These genes produce target specific insecticidal proteins that protect the plant against insect pest attacks throughout the growing season. The largest threat to the continued success of these insecticidal GM crops is the potential development of target pest resistance. Models and theories suggested several possible strategies to delay the development of resistance. Of these strategies the high-dose/refuge strategy was selected as the optimal insect resistance management (IRM) option and is currently implemented throughout the world. The high-dose/refuge strategy comprises planting Bt maize plants that produce high doses of the toxin and non-Bt plants (refugia) in close proximity to one another. The theory behind this strategy is that the high dose of toxin kills nearly all the individuals of the target pest while the refuge area sustains susceptible pest individuals that survive on the crop and mate with survivors on the Bt crop. Recent reports of resistance development to Bt crops has raised questions about the refuge concept. In the cases where resistance has developed it was largely ascribed to non-compliance to the prescribed refuge requirements or non-functionality of the refuge approach. The Bt crops used throughout the world were developed in North America against the insect pests that occur there. Since there are differences in the lifecycles and behaviour of insect species targeted by Bt crops the refuge areas also needs to be specified for different target pests. For example, the currently used IRM strategies do not differentiate between polyphagous and monophagous pests and also do not take into account differences that exist in biology and behaviour of different pest species. These IRM strategies have also been developed mainly with large scale commercial farming systems in mind and do not take into account farming systems in developing countries. Current IRM strategies need to be revised and adapted for use by small-scale African farmers.

Key words: Africa, GM crops, Insect resistance management, refugia

Opsomming

Geneties-gemodifiseerde (GM) gewasse wat gebruik word vir plaagbeheer druk *cry* gene uit wat afkomstig is vanaf die bakterium *Bacillus thuringiensis* (Bt). Hierdie gene produseer ‘n teiken-spesifieke insekdodende proteïen wat die plant regdeur die seisoen teen sekere plaaginsekte beskerm. Die grootste bedreiging vir die sukses van die GM gewasse is die ontwikkeling van weerstand deur die teikenorganismes. Deur middel van modelle en teorieë is verskeie strategieë voorgestel om die ontwikkeling van weerstand te vertraag. Van hierdie word die hoë-dosis/toevlug-strategie as die optimum strategie beskou en dit word wêreldwyd toegepas. Die hoë-dosis/toevlug strategie behels dat die Bt plante ‘n baie hoë dosis toksien produseer en dat nie-Bt plante naby daaraan geplant word. Die onderliggende teorie agter hierdie strategie is dat die hoë dosis byna alle teikenorganismes dood terwyl die toevlugsarea ‘n vatbare plaagpopulasie onderhou waarvan die individue dan met die oorlewendes vanaf die Bt gewas paar. Die ontwikkeling van weerstandbiedendheid in verskeie plaagspesies het vrae laat ontstaan oor die effektiwiteit van bogenoemde weerstandbestuurstrategieë. Die ontwikkeling van weerstand kan moontlik toegeskryf word aan die feit dat bestuurstrategieë nie toegepas word nie of dat strategieë in wese oneffektief is. Omdat die Bt plante wat regoor die wêreld aangeplant word hoofsaaklik in Noord Amerika ontwikkel is, is hierdie gewasse grootliks gemik teen Noord-Amerikaanse insekplae. Byvoorbeeld word daar nie gedifferensieer tussen bestuurstrategieë vir polifage en monofage plaagspesies nie en word daar nie voorsiening gemaak vir verskille in biologie en gedrag van verskillende plaagspesies nie. Aangesien die lewensiklus en gedrag van insek tot insek verskil, moet die toevlugsareas van die verskillende plae ook vir verskillende plae gespesifiseer word. Huidige weerstandbestuurstrategieë is grootliks ontwikkel met grootskaalse kommersiële boere in gedagte en neem nie kleinskaalse boerderystelsels in ontwikkelende lande in ag nie. Huidige strategieë om weerstandontwikkeling te vertraag moet hersien en aangepas word vir kleinboere in Afrika.

Sleutelwoorde: Afrika, GM gewasse, Insekweerstandbeheer, vlugoorde

Table of Contents

Chapter 1

Introduction and literature review

1.1. Development of pesticide resistance in insects

The development of resistance is a process of natural selection where, due to a specific selection pressure, susceptible genotypes of a particular population are effectively removed from the population over time (Comins, 1977). Whalon and McGaughey (1998) defined resistance as the micro-evolutionary process whereby genetic adaptation through pesticide selection results in populations of arthropods which present unique and more often more difficult management challenges. Various factors may contribute to the development of arthropod resistance to pesticides and stressors such as insecticidal proteins. These factors include arthropod host and reproduction ecology, pesticide use intensity, frequency of exposure, pre-existing resistance alleles in treated populations, dose-toxicity relationships and cross- and multiple-resistance phenomena (Whalon *et al.*, 2008).

The number of insecticide resistant insect species has increased exceedingly over the past decade. The diamondback moth (*Plutella xylostella* (Lepidoptera: Plutellidae)) for example, has evolved resistance to 81 different insecticides in 23 countries (Whalon *et al.*, 2011). The first documented case of an insect that evolved resistance to pesticides was the San Jose scale, *Quadrispiotus perniciousus* (Hemiptera: Diaspididae) in 1914 which became resistant to lime sulphur in deciduous fruit orchids in the USA (cited by Whalon *et al.*, 2008). By 2007, 553 species of arthropods were reported to be resistant to some type of pesticide according to the Arthropod Pesticide Resistance Database (APRD) (Fig 1.1) (Whalon *et al.*, 2008; 2011). The arthropod order with the highest number of resistant species is the Diptera with 33.8 %. Other important orders are Lepidoptera (15.4 %), Acari (13.7 %), Coleoptera (13.4 %), Homoptera (10.5 %) and Hemiptera (4.0 %) (Whalon *et al.*, 2008).

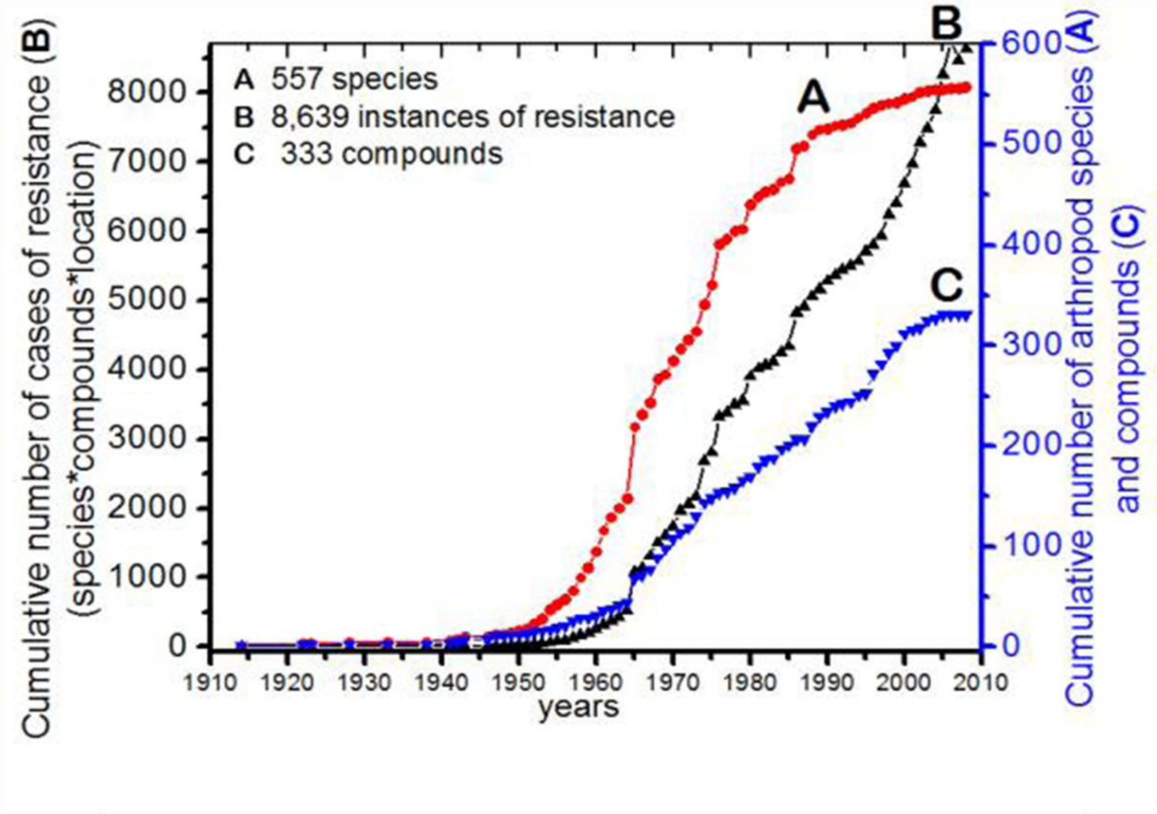

Figure 1.1. Reports of arthropod resistance to insecticides between 1914 and 2010 (species compounds and total number of instances) (IRAC *et al.*, 2010).

Resistance cases and species reported to the APRD prior to 2008 can be divided into groups according to their importance. Data on resistance cases in the fields of agriculture, medical and other fields are provided in table 1.1. It is interesting to note that 62.9 % of the reported cases and 55.3 % of the resistant species are of agricultural importance. The other 37.1 % and 44.7 % of the resistant species are of medical importance or parasitoids/pollinators. Several arthropods have become resistant to more than one pesticide or unique compound. The top 20 resistant species listed according to their resistance to unique compounds are provided in table 1.2. Seventy-five percent of the listed species are of agricultural importance, while the other 25 % are relevant for humans or cattle, as well as in urban ecosystems. Resistance management in the agricultural sector is therefore of absolute importance.

Table 1.1. Numbers, cases and species of insecticide resistant arthropods (Whalon *et al.*, 2008).

	Cases						**Species**					
Arthropod group	Total	Agricultural	Medical	Parasitoid	Other	Pollinator	Total	Agricultural	Medical	Parasitoid	Other	Pollinator
Acari	1025	708	248	69			76	41	23	12		
Araneae	1		1		3		1		1			
Coleoptera	884	860	13	8			74	68	1	3	2	
Copepoda	1		1				1		1			
Dermaptera	222		220		2		4		3		1	
Diptera	2265	291	1937	4	33		187	26	149	2	10	
Ephemeroptera	2				2		2				2	
Hemiptera	162	85	77				22	17	5			
Homoptera	992	989	3				58	57	1			
Hymenoptera	37	6	2	26		3	16	3	1	11		1
Lepidoptera	1799	1799					85	85				
Neuroptera	21			21			1			1		
Phthiraptera	114	4	110				9	1	8			
Siphonaptera	89		89				9		9			
Thysanoptera	133	133					8	8				
Total	7747	4875	2701	128	40	3	553	306	202	29	15	1
%	100	62.9	34.9	1.7	<1	<1	100	55.3	36.5	5.2	2.7	<1

Table 1.2. Top 20 insecticide resistant arthropods in the world, ranked by number of unique compounds they are resistant to (Whalon *et al.*, 2008).

Rank	Species	Order: Family	Number of compounds resistant to	Number of references in the APRD*	Year of first reported case	Relevant crops/organisms
1	*Teteranychus urticae*	Acari: Tetranychidae	80	112	1943	Cotton, flowers, fruits, vegetables
2	*Plutella xylostella*	Lepidoptera: Pluttelidae	76	72	1953	Crucifers
3	*Myzus persicae*	Hemiptera: Aphididae	68	78	1955	Vegetables, trees, grains, tobacco
4	*Leptinotarsa decemlineata*	Coleoptera: Chrysomelidae	48	43	1955	Aubergine, pepper, potato, tomato
5	*Musca domestica*	Diptera: Muscidae	44	35	1947	Urban
6	*Boophilus microplus*	Acari: Ixodidae	43	32	1947	Cattle
7	*Blatella germanica*	Blattidea: Blattellidae	42	65	1956	Urban
8	*Bemisia tabaci*	Homoptera: Aleyrodidae	39	34	1981	Greenhouse, cotton
9	*Panonychus ulmi*	Acari: Tetranychidae	38	68	1951	Fruit trees
10	*Aphis gossypii*	Hemiptera: Aphididae	37	24	1965	Cotton, vegetables
11	*Culex pipiens pipiens*	Diptera: Culicidae	34	27	1961	Human
12	*Phodoron humuli*	Hemiptera: Aphididae	34	20	1965	Hops, plum
13	*Helicoverpa armigera*	Lepidoptera: Noctuidae	33	49	1969	Cotton, maize, tomato
14	*Heliothis virescens*	Lepidoptera: Noctuidae	33	41	1961	Chickpea, maize, cotton, tomato
15	*Culex quinquefasciatus*	Diptera: Culicidae	31	42	1952	Human
16	*Spodoptera littoralis*	Lepidoptera: Noctuidae	30	21	1962	Lucerne, cotton, vegetables
17	*Tribolium castaneum*	Coleoptera: Tenebrionidae	30	31	1962	Stored grain, groundnuts
18	*Lucilla cuprina*	Diptera: Calliphoridae	25	13	1958	Cattle, sheep
19	*Rhizoglyphus robini*	Acari: Acaridae	22	2	1986	Ornamental plants, stored onions
20	*Anopheles albimanus*	Diptera: Culicidae	21	12	1964	Human

*APRD – Arthropod Pesticide Resistance Database

Compared to insecticides, there are only a few cases of resistance development to genetically modified (GM) crops with insecticidal properties. Although this dissertation largely deals with GM crops and insect resistance management (IRM), most of the examples referred to deal with insecticide resistance. The principles of IRM, however, remain the same and due to the novelty of GM crops, and especially resistance development, much can be learned from the history of insecticide resistance development.

1.2. Insect resistance management (IRM) to insecticides

Insect resistance management attempts to conserve susceptibility of insects to pesticides, irrespective whether these insecticidal products are sprayed or produced inside plants (e.g. the Cry1Ab insecticidal protein produced by Bt crops). These strategies are aimed at either overcoming resistance to currently used compounds or preventing the development of resistance to existing or new pesticides (Denholm & Rowland, 1992). It is, however, important to realize that resistance development (evolution) cannot be prevented, but only delayed.

The efficacy of a chemical control treatment is determined by complex interactions between insects and insecticides. Most of these interactions are beyond our control and are concerned with the ecology, genetics, behaviour and physiology of the pest. Only the operational factors, such as the insecticide used, the area coverage and the timing, rate and method of application, are within our control. In order to use chemicals in such a way that it delays the development of resistance it is essential to understand the parameters influencing the process of selection of resistant individuals (Georghiou & Taylor, 1977a). These factors can then be managed to reduce the ecological pressure selecting for resistance (Denholm & Rowland, 1992). The same applies to Bt crops.

Since resistance development can only be delayed by ensuring that the majority of a pest population consists of susceptible individuals, it is important that the particular control strategy selects for susceptibility. Techniques for manipulating selection pressure in IRM strategies can be divided into two main strategies (Roush & Daly, 1990). Firstly, a strategy can either reduce the fitness of resistant individuals when the insecticide is applied, or secondly they can reduce the total selection pressure. A summary of these two different strategies, which could also be used in a complimentary way, is provided in table 1.3.

Table 1.3. Strategies used for manipulating selection pressure in integrated resistance management (Roush & Daly, 1990).

A. Reduce the fitness of resistant individuals when insecticide is applied.

1. Increase the dose to kill heterozygotes or resistant homozygotes.
2. Use compounds that confer lower levels of resistance.
3. Treat the most vulnerable life stage.
4. Use synergists to suppress detoxification mechanisms.
5. Mix pesticides of different modes of action and metabolism.

B. Reduce the total amount of selection pressure applied.

6. Decrease the concentration of insecticide so that some susceptible individuals exposed to the pesticide can survive.
7. Reduce the number of pesticide applications.
8. Use pesticides with short residual activity and avoid slow release formulations.
9. Do not spray all habitats of the pest, use spot treatments.
10. Rotate pesticides so that not all generations are exposed to the same one, but avoid spatial mosaic treatments.

Current IRM strategies still rely on the approaches listed in table 1.3. Several of these approaches are relevant to managing pests that have become resistant to insecticidal proteins, such as Cry1Ab. The abovementioned strategies are briefly discussed below.

Firstly, the dose of the insecticide can be increased to also kill heterozygotes (RS) or resistant homozygotes (RR), thereby rendering resistance recessive. If the occurrence of resistance is sufficiently low that very few RR individuals can be found in the population and if relatively high frequencies of susceptible (SS) individuals escape insecticide exposure and mate with surviving RR genotypes and produce RS offspring, increases in the frequency of resistance may be very slow (Roush & Daly, 1990). If the pesticide concentration is high enough to kill all RR this strategy might be effective even if the gene frequency is high and the whole population is treated. Practical problems unfortunately make this strategy useful only in situations where resistance is not yet a problem, where the doses can be controlled and applied uniformly and where resistance levels in heterozygotes are low (Roush & Daly, 1990; Tabashnik & Croft, 1982).

Secondly, chemical compounds can be used that confer low levels of resistance. Where it might be difficult to increase the dose of the insecticide, as indicated above, another insecticide which does not confer cross-resistance may be effective (Roush & Daly, 1990). Thirdly, the focus should be on treating the most vulnerable life stages of target pests. Relative fitness varies between life stages because some resistance mechanisms are more poorly expressed during some life stages than others. Insecticide treatment against the life stage that shows the lowest level of resistance would thus be most effective (Roush & Daly, 1990). Careful field scouting in order to detect pest infestation and pressure of different life stages is necessary for this strategy to be implemented properly.

Fourthly, synergists can be used to suppress detoxification mechanisms. Synergists applied with insecticides increase the potency of these insecticide by blocking the action of the resistance mechanism, thereby reducing the effect of the mechanism. Insects may, however, still have the ability to develop resistance to a particular insecticide if another metabolic pathway is used (Raffa & Priester, 1985). It is very important to select the proper synergists to apply with an insecticide if resistance against the insecticide is to be prevented.

Synergists can thus only be used if there is a single mechanism of resistance, if resistance doesn’t develop for the combination of synergist and insecticide, if the synergist is stable under field conditions, has low mammalian toxicity and is cost-effective (Roush & Daly, 1990). Chen *et al.* (2007) identified a peptide (CR 12-MPED) that can act as a synergist with the Bt toxin Cry1Ab. It was found that when this peptide was provided together with Cry1Ab to *Manduca sexta* (Lepidoptera: Sphingidae) toxin-induced insect mortality increased.

Fifthly, mixtures of pesticides can be applied against a resistant target pest. According to Tabashnik (1989) two pesticides with different modes of action can be used in different ways to delay the development of resistance. When sequences of pesticides are used a pesticide is applied repeatedly until resistance occurs after which the particular pesticide is replaced by a different pesticide. This is, however, an out dated strategy and may speed up the development of resistance (Onstad, 2008). The continued use of a single protein such as Cry1Ab over wide areas such as was done in South Africa between 1998-2011 could be considered as a sequence "insecticide" since the target pest was exposed to only this one "insecticide" for a period of 12 years.

Mixtures of pesticides can also be used. A mixture of pesticides is applied and individuals are exposed to two compounds simultaneously. This strategy is based on the idea that if resistance to each of the compounds is initially rare, then resistance to both compounds would be extremely rare (Tabashnik, 1989). Each treatment is expected to kill individuals resistant to the other treatment (Onstad, 2008). Resistance can be substantially delayed if both resistance genes are effectively recessive. In terms of GM crops this approach of mixtures would be the use of pyramid genes with different modes of action.

In the sixth place the dose can be decreased to that which kills most but not all of the susceptible insects. This is not a very viable strategy since the dose that allows some susceptible individuals to survive will allow an even greater survival of heterozygotes which increases the frequency of resistant alleles in the population. For this strategy to work the number of susceptible individuals that survive may be so high that it may not be economically viable (Roush & Daly, 1990). This strategy also does not fit into the "high-dose" strategy proposed for IRM in GM crops.

Insecticides can also be applied less frequently to give susceptible insects an opportunity to reproduce and to dilute resistance by inter-breeding with resistant individuals. This can be done as part of an integrated pest management strategy by using a higher pest population threshold for insecticide application (Roush & Daly, 1990). Chemicals with a short residual period should be used while slow-release formulations should be avoided. This is because persistent pesticides are functionally equivalent to frequent applications of less persistent insecticides. This may allow the survival of resistant individuals. By using less persistent insecticides only the target pest is killed and subsequent colonizers are not affected (Roush & Daly, 1990).

Refuges of crops on which insecticides are not applied, can be used to allow for the escape of susceptible individuals and a subsequent sustained susceptible population. The recruitment of susceptible individuals into a pest population is important in the delay of resistance development. This can be done by only treating the parts of the field that have damaging populations, or by leaving a certain part of the field untreated (Roush & Daly, 1990). In many instances the maintenance of refuge areas may be difficult as little is known about the size of the population outside the treated area and the degree of migration between areas (Roush & Daly, 1990). For example, with Bt insecticide sprays, no resistance other than *P. xylostella* (Tabashnik *et al.*, 1990) have been reported in spite of these sprays being used by farmers since the 1950's. The reason advanced for the successful use and no development of resistance was that organic farmers that are the main users of Bt sprays largely apply sprays only when needed and because farmers are more inclined to make use of "spot-treatments" than general sprays or covering large areas of land.

Finally, pesticides can be alternated over time so that not all pest generations are exposed to the same insecticide all the time (Roush & Daly, 1990). Pesticides should be alternated temporally across generations of the target pest. This strategy is based on the idea that the frequency of individuals resistant to one pesticide will decline during the application of different pesticide (Tabashnik, 1989). Individuals resistant to one treatment will be killed by the next treatment in the rotation (Onstad, 2008). Using pesticides in mosaics is another option to delay resistance development. In this strategy adjacent areas should be treated at the same time with different pesticides (Tabashnik, 1989). The latter is not a good IRM tactic because no refuge is provided for susceptible individuals for either treatment and there is simultaneous selection for resistance to both toxins in the population (Onstad, 2008). Although alteration of GM insecticidal crop cultivars have been suggested (Roush, 1997) this is not provided due to the infrastructure and constraints associated with producing a large number of cultivars with different traits.

Theoretical studies indicate that, under certain conditions, combinations of pesticides are the most effective strategy at delaying resistance development (Tabashnik, 1989). These studies, however, focused on the effects of the control tactic on the target pest only. However, in agro-ecosystems the effects on non-target pests and beneficial organisms should also be considered (Tabashnik, 1989). The importance of non-target organisms, such as natural enemies should always be kept in mind as they are also an important component of any IPM strategy.

1.2.1. Patterns of resistance development

Differences in patterns of migration, fitness and frequency of resistance alleles may result in different patterns of resistance development. Tabashnik and Croft (1982) described three general patterns of resistance development:

a) *Steadily increasing resistance*: When there is no immigration of susceptible (S) individuals and no reduction in fitness associated with the resistance gene (R), resistance increases steadily as the pesticide kills SS and RS insects. The frequency of R genes increases to a point and then stabilizes despite fluctuation in population size (Fig 1.2a).

b) *Stable cycle resistance*: When there is immigration of susceptible insects, it is possible to achieve a stable cycle of increasing and decreasing resistance under some circumstances. As the population size increases the frequency of the R gene decreases. However, when the population size decreases (after pesticide application) the frequency of the R-gene increases again (Fig 1.2b).

c) *Increasing cycle resistance*: This pattern of resistance development is intermediate between steadily increasing resistance and a stable cycle. Immigration of susceptible individuals causes short term decreases in the R gene frequency, but there is a long term increase in resistance (Fig 1.2c).

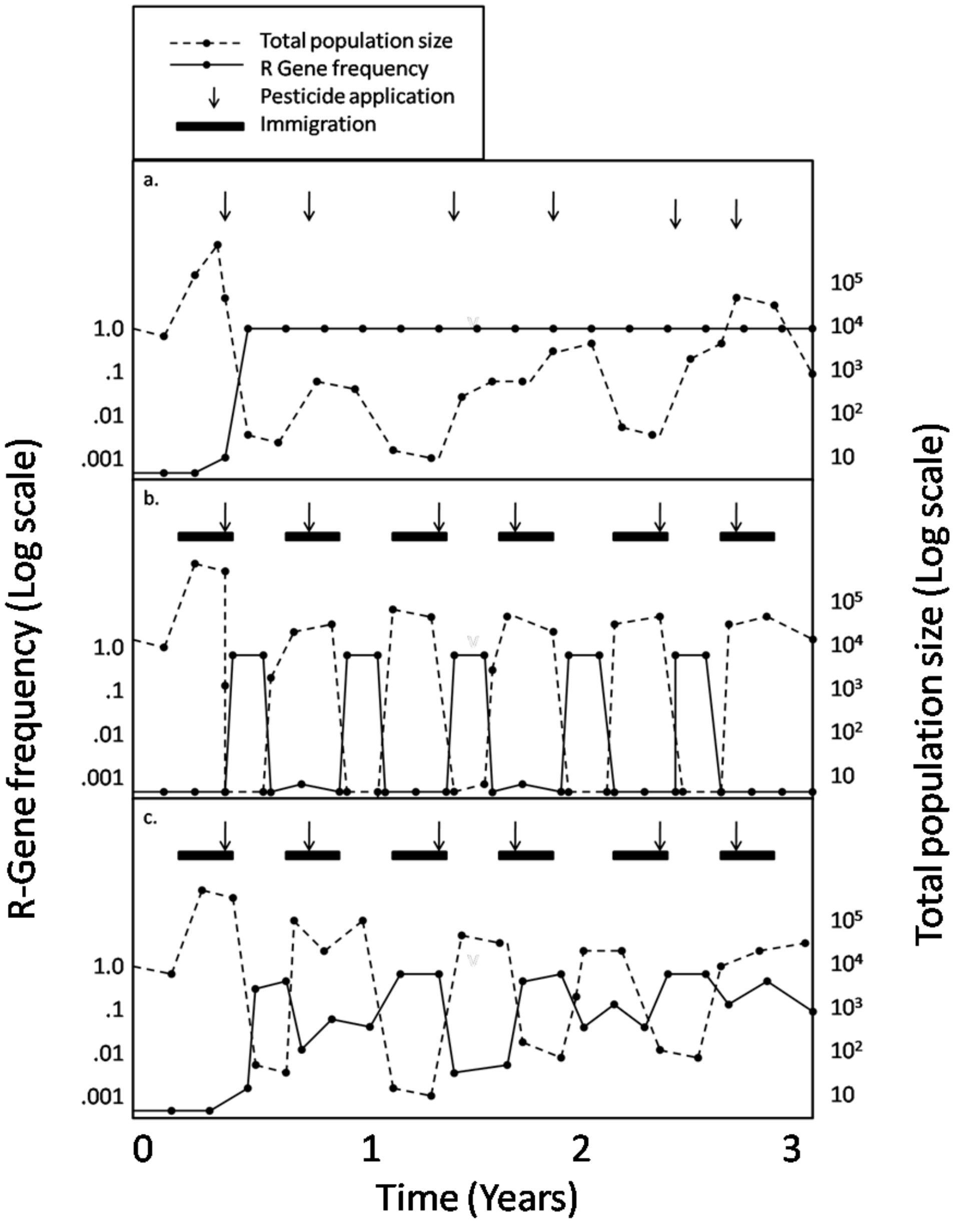

Figure 1.2. Patterns of resistance development to an insecticide: a) Steadily increasing resistance, b) Stable cycle resistance, c) Increasing cycle resistance (Tabashnik & Croft, 1982).

Various factors influence the rate of resistance development. These are for example operational factors such as insecticide dose, spray frequency and life stages exposed as well as biological factors such as fecundity, survivorship, initial population size, initial R-gene frequency, reproductive disadvantage and number of generations per year. By studying these factors Tabashnik and Croft (1982) developed a decision scheme for devising strategies to slow down the rate of resistance development (Fig. 1.3). Although these strategies were suggested almost three decades ago, they are highly relevant and applicable to the high-dose/refuge strategy, which is currently used to manage resistance development to Bt crops.

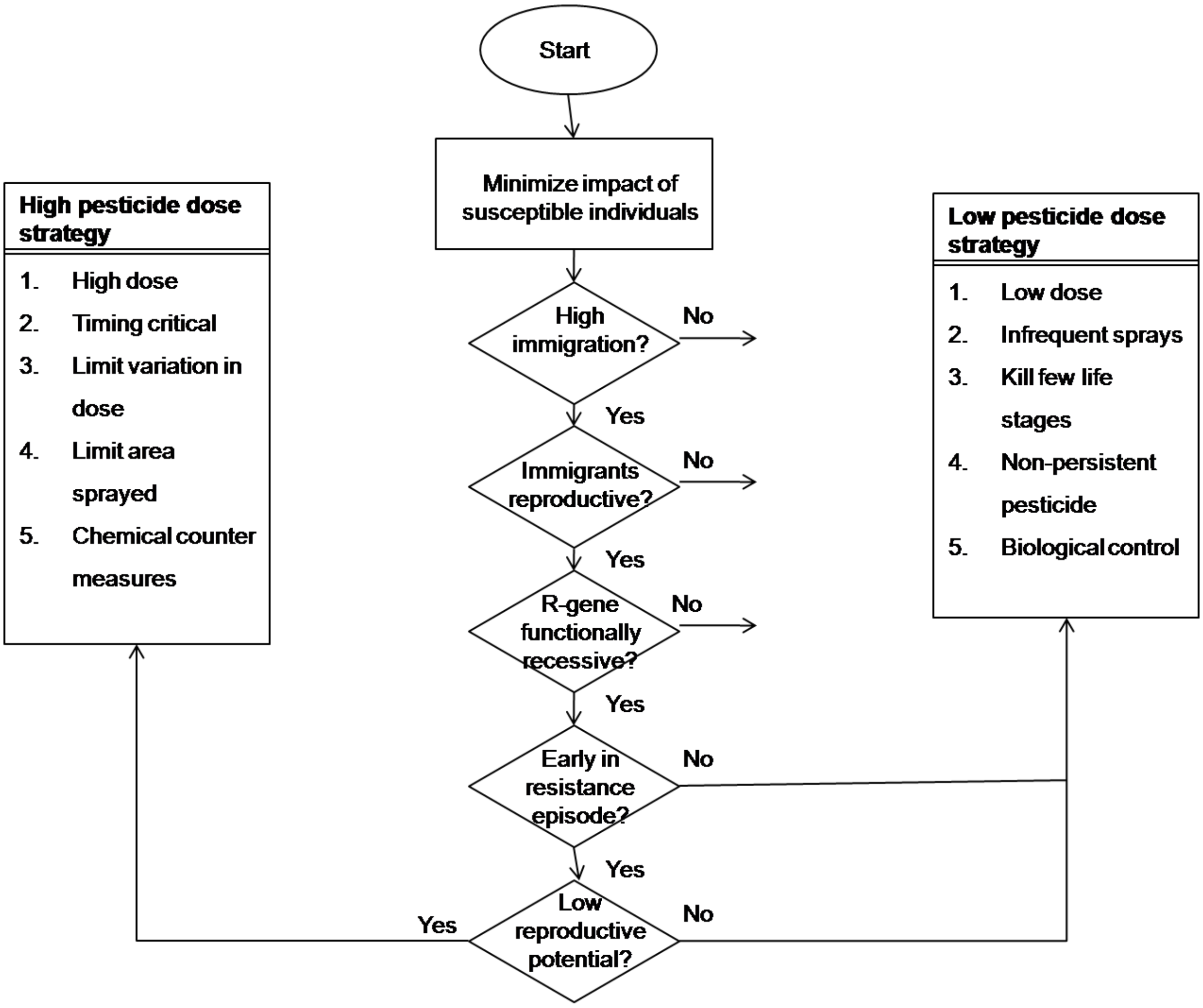

Figure 1.3. Flow chart decision scheme for delaying pesticide resistance development in pests. If the answer to any of the questions in the diamonds are “no”, the best tactic would be the low pesticide strategy summarized on the right. If the answers to all of the questions are “yes”, the best tactic would be the high dose tactic summarized on the left (Tabashnik & Croft, 1982).

The low-dose pesticide use strategy as indicated in figure 1.3 involves as little pesticide use as possible to reduce the rate at which susceptible insects are removed from the population, thereby reducing the rate of resistance development. This strategy also promotes the conservation of natural enemies and therefore maximises the potential for biological control. This can be achieved by lowering the pesticide dose, spray frequency, the number of life stages killed and pesticide half life (Tabashnik & Croft, 1982).

The high-dose strategy is based on the concept of using a high enough pesticide dose to kill the RS heterozygotes, rendering the resistance gene effectively recessive. This strategy requires that the immigration of susceptible individuals ‘swamp out’ the resistant individuals. The dose required to kill the RS heterozygotes has, however, to be identified. Tabashnik and Croft (1982) highlighted several concerns regarding the high-dose strategy. These concerns were that if too many life stages are killed, the immigration rate of susceptible insects may be reduced. Furthermore, spatial and temporal variation in dosage may result in intermediate doses which promote resistance development, as well as disruption of natural enemies which could result in secondary pest outbreaks. Although, these are valid concerns when spraying pesticides, they are not really applicable when using GM crops, unless high doses of plant produced insecticidal proteins are detrimental to beneficial organisms.

Using computer models Georghiou and Taylor (1977a; 1977b) found that, as expected, pesticide doses that produce only slight or no kill had no effect on the evolution of resistance. A higher dose that killed 99.99 % of the target insects caused an immediate increase in the R-gene frequency, since all heterozygotes and susceptible homozygotes were killed. Resistance can therefore easily be attained through intense selection pressure, such as high insecticide doses, or GM crops in which insecticidal toxins such as Cry proteins are constitutively expressed.

Despite all the efforts and strategies that are used, several pest species still managed to develop resistance to a large number of insecticides. The importance of IRM strategies were realised even before the development and commercialization of GM crops (Tabashnik & Croft, 1982; Georghiou & Taylor, 1977a; 1977b). Many of the strategies discussed above are relevant to IRM in cropping systems where GM crops that produce insecticidal Cry proteins are cultivated.

1.3. Managing resistance development in genetically modified crops

Gould (1998) highlighted five mechanisms in the toxicity pathway of Bt sprays where genetic change in the insect could result in resistance against the Bt-toxin. These were: 1) decreased solubilisation of the insecticidal crystal, 2) decreased cleavage of the protein into the active fragment, 3) increased proteolytic digestion of the active fragment, 4) decreased binding of the active fragment to the midgut epithelial cells, and 5) decreased pore formation in the midgut epithelial cells.

While all of the five mentioned mechanisms are relevant to resistance development to insecticides or Bt spray formulations, only three of these are relevant to development of resistance to Bt crops. These are: increased proteolytic digestion of the active fragment, decreased binding of the active fragment to the midgut epithelial cells and decreased pore formation. Since these genetic changes toward a resistant genotype can happen in different ways it is important to manage Bt crops in a way that minimizes selection for rapid adaptation.

Before the commercial release of genetically modified Bt crops several tactics to delay target pest resistance development were proposed. A summary of these tactics are listed in table 1.4.

Table 1.4. Potential strategies and tactics for deploying insecticidal genes in plants to effectively delay the development of resistance (Whalon & McGaughey, 1998).

Gene tactics			
Gene strategies	**Promoter**	**Expression**	**Operational tactic**
Single gene	Constitutive	High dose	Uniform seed
Multigene Pyramid Stacked	Tissue specific	Low dose	Seed mixtures Multi-lines
Chimeric genes	Induction Wound Phenology Elicitor Combinations above	Mixtures Combinations above	Refugia Seed rotation: Sequential release Seed mosaic: Spatial and temporal Combinations above within IPM

The tactics listed below are those that have been studied or considered as good resistance management options in GM crops.

- **Moderate toxin dosage to ensure survival of a proportion of the population of susceptible insects** (Roush, 1997). Models indicated that lower doses resulted in a delay in resistance development, but also that the delay was small compared to other tactics. A moderate toxin dosage is not the optimum strategy to use since the efficacy of the Bt toxin at low expression levels is variable and vulnerable to environmental conditions (Bates *et al.*, 2005).

- **High toxin dosage to kill insects heterozygous for resistance** (Roush, 1997). The initial occurrence of individuals that are homozygous for resistance is likely to be so low that it can be ignored. A dose high enough to kill the heterozygous resistant individuals in a population should thus be acceptable. The high dose will also ensure that crop damage remains below the economic threshold level (Bates *et al.*, 2005).

- **The deployment of Bt-crop varieties expressing different toxins in mosaic, in rotation, sequentially or by incorporating both toxins in a single variety** (Roush, 1997; Gould, 1988). Of the above mentioned strategies, the stacking/pyramiding of two or more genes that express different toxins (with different binding sites to reduce the likelihood of cross-resistance) is the optimum way of delaying resistance development. This is because if resistant individuals are rare where one toxin is involved then resistant individuals where two toxins are involved would be extremely rare (Bates *et al.*, 2005). This strategy requires that the initial frequency of resistance genes is low and that cross resistance does not occur (Ferré *et al.*, 2008).
- **Temporal or tissue-specific toxin expression** (Roush, 1997; Gould, 1988). In this case Bt toxins are only expressed at specific times or in specific plant parts. Insects may move between toxic and non-toxic parts which may counteract the resistance related advantages of selective Bt expression (Bates *et al.*, 2005).
- **The provision of non-toxic plants to sustain susceptible insects within the field with seed mixtures or an external refuge** (Roush, 1997; Gould, 1988). Seed mixtures previously seemed like a good option, but inter-plant movement of immature stages of pests actively reduces the size of the refuge. The larvae can either move from a non-toxic plant to a toxic plant and die, or larvae may avoid eating Bt plants (Tabashnik, 1994a). When all individuals, resistant, heterozygote or susceptible, have equal reproductive potentials then development of resistance can be delayed (Georghiou & Taylor, 1977a). As can be expected the larger the refuge area, the slower the rate of resistance development. External refuges are more effective in conserving susceptible alleles, but managing these insects from an economic and IRM standpoint is a challenge (Bates *et al.*, 2005).

The strategy that is currently implemented for Bt cotton and Bt maize resistance management in South Africa and throughout most of the world, is the high-dose/refuge strategy. This strategy is based on numerous models and theories developed from years of experimentation (Georghiou & Taylor, 1977a; 1977b; Tabashnik & Croft, 1982; Roush, 1994; Gould, 1998). These strategies will be discussed in the following chapters.

1.4. Conclusions

The development of field resistance of target pests to GM Bt crops highlighted the necessity for effective IRM strategies. It is therefore necessary to investigate the development of the refuge concept and its application in IRM, to document new developments in this field and to record current insect resistance management strategies suggested for crops/pests relevant to Africa. It is also important to identify knowledge gaps that may lead to incorrect assumptions regarding the refuge strategy and consider factors that may contribute to resistance development. This information will not only facilitate appropriate risk assessment for insecticidal GM crops but it will also be assessed for its relevance and applicability to both commercial and small-scale farming systems in Africa.

1.5. Aim of the study

To review IRM strategies recommended GM insecticidal crops in South Africa.

1.5.1. Specific objectives

- To review insect resistance management strategies.
- To review the high-dose/refuge strategy currently used in IRM.
- To review refuge requirements of currently available GM crops.
- To review the possible impact of insect behaviour on the refuge approach.
- To identify knowledge gaps and discuss the relevance of current insect resistance management strategies to African farming systems.

1.5.2. Chapter outlines

- Chapter 2 – The high-dose/refuge strategy and refuge requirements of currently available GM crops.
- Chapter 3 – Case studies: the possible impact of insect behaviour on the refuge approach.

- Chapter 4 – Knowledge gaps and relevance of current insect resistance management strategies to African farming systems.
- Chapter 5 – Recommendations and conclusions.

Chapter 2

Insect resistance management strategies and refuge requirements of currently available genetically modified crops

2.1. Introduction

To reduce the reliance on insecticide sprays for pest control crops have been genetically modified to express genes from the bacterium *Bacillus thuringiensis* (Bt) that produce target specific insecticidal proteins (Tabashnik, 2008). The result is plants that constitutively produce insecticidal proteins throughout the growing season. Before the commercial release of Bt transgenic plants it was expected that pests would rapidly evolve resistance. The persistent tendency of the development of resistance against pesticides as well as laboratory selected resistance to Bt toxins in some insects and field evolved resistance in others are reasons for concern (Tabashnik *et al.*, 2003; Tabashnik, 1994b; Ferré & Van Rie, 2002).

Resistance development is the micro-evolutionary process whereby genetic adaptation through pesticide selection results in populations of arthropods which present unique and difficult management challenges (Whalon & McCaughey, 1998). The Insecticide Resistance Action Committee (IRAC) (2010) defined field evolved resistance as the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species.

The number of insects resistant to pesticides has increased rapidly over the past decade. Attempts to kill tolerant individuals lead to ever increasing doses and eventually resistant populations (Casida & Quistad, 1998). The diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), for example has evolved resistance to 81 different insecticides in 23 countries (Whalon *et al.*, 2011). By 2007, 553 arthropod species have been reported to be resistant to some insecticide (Whalon *et al.*, 2011).

To date only five lepidopteran and one coleopteran species have developed resistance to Bt crops. They are *Heliothis zea* (Lepidoptera: Noctuidae) to Bt cotton in the south-eastern United States (Luttrell *et al.*, 2004), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Bt maize in Puerto Rico (Matten *et al.*, 2008), *Busseola fusca* (Lepidoptera: Noctuidae) to Bt maize in South Africa (Van Rensburg, 2007), *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) to Bt cotton in India (Monsanto, 2010), *Helicoverpa armigera* in South Africa (J. van den Berg, pers comm.) and more recently *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to Bt maize in the USA (Gassmann *et al.*, 2011).

A summary of the insects species resistant to Bt crop varieties, formulations or crystals (Table 2.1) as well as Bt proteins (Table 2.2) was provided in the Arthropod Resistance Database (APRD) (Whalon *et al.*, 2011). This database shows a large number of examples of insecticide resistance and includes organisms from two orders of the Arthropoda. It is therefore important for efficient resistance management strategies to be developed and implemented to prevent/delay resistance development in other economically important pest species.

According to Huang *et al.* (2011) Bt crops in North America remain effective against their major pest species, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), *Diatraea grandiosella* (Lepidoptera: Crambidae), *Heliothis virescens* (Lepidoptera: Noctuidae) and *P. gossypiella*, even after 15 years of intensive use of Bt maize and Bt cotton. Huang *et al.* (2011) claim no evidence of resistance in these species. The tables below, from Whalon *et al.* (2011), however, show reports of resistance to Bt toxins and/or crops in some of the species mentioned.

Table 2.1. Insects resistant to *Bacillus thuringiensis* varieties, formulations or crystals (Adapted from Whalon *et al.*, 2011).

Species	Order: Family	Host
***Bacillus thuringiensis* (variety unspecified)**		
Helicoverpa armigera	Lepidoptera: Noctuidae	Cotton, maize, sorghum, tomato
Heliothis virescens	Lepidoptera: Noctuidae	Chickpea, maize, cotton, tobacco
Ostrinia nubilalis	Lepidoptera: Pyralidae	Maize
Pectinophora gossypiella	Lepidoptera: Gelechiidae	Cotton
Plodia interpunctella	Lepidoptera: Pyralidae	Cereals, groundnut
Plutella xylostella	Lepidoptera: Plutellidae	Crucifers, nasturtium
Spodoptera exigua	Lepidoptera: Noctuidae	Cotton, tomato, celery, lettuce, cabbage and alfalfa
Spodoptera littoralis	Lepidoptera: Noctuidae	Alfalfa, cotton, potato, vegetables
Trichoplusia ni	Lepidoptera: Noctuidae	Crucifers
***Bacillus thuringiensis* HD73 spore/crystal**		
Helicoverpa armigera	Lepidoptera: Noctuidae	Cotton, maize, sorghum, tomato
Plutella xylostella	Lepidoptera: Plutellidae	Crucifers, nasturtium
***Bacillus thuringiensis* MVPII**		
Culex pipiens pipiens	Diptera: Culicidae	House Mosquito
Helicoverpa armigera	Lepidoptera: Noctuidae	Cotton, maize, sorghum, tomato
Pectinophora gossypiella	Lepidoptera: Gelechiidae	Cotton
***Bacillus thuringiensis* var. *kurstaki* HD1**		
Plodia interpunctella	Lepidoptera Pyralidae	Cereals, groundnut
Plutella xylostella	Lepidoptera Plutellidae	Crucifers, nasturtium
Bacillus thuringiensis* var. *tenebrionensis		
Leptinotarsa decemlineata	Coleoptera: Chrysomelidae	Eggplant, pepper, potato, tomato
Bacillus thuringiensis* var. *aizewai		
Plodia interpunctella	Lepidoptera: Pyralidae	Cereals, groundnut
Plutella xylostella	Lepidoptera: Plutellidae	Crucifers, nasturtium
Spodoptera littoralis	Lepidoptera: Noctuidae	Alfalfa, cotton, potato, vegetables
Bacillus thuringiensis* var. *israelensis		
Culex pipiens	Diptera: Culicidae	Mammals
Culex quinquefasciatus	Diptera: Culicidae	Mammals
Bacillus thuringiensis* var. *kurstaki		
Helicoverpa armigera	Lepidoptera: Noctuidae	Cotton, maize, sorghum, tomato
Heliothis virescens	Lepidoptera: Noctuidae	Chickpea, maize, cotton, tobacco
Plodia interpunctella	Lepidoptera: Pyralidae	Cereals, groundnut
Plutella xylostella	Lepidoptera: Plutellidae	Crucifers, nasturtium
Pseudoplusia includens	Lepidoptera: Noctuidae	Soybean
Spodoptera exigua	Lepidoptera: Noctuidae	Cotton, tomato, celery, lettuce, cabbage and alfalfa
Trichoplusia ni	Lepidoptera: Noctuidae	Crucifers
***Bacillus thuringiensis* var. *kurstaki* (Dipel)**		
Ostrinia nubilalis	Lepidoptera: Pyralidae	Maize
***Bacillus thuringiensis* var. *kurstaki* (Javelin)**		
Plutella xylostella	Lepidoptera: Plutellidae	Crucifers, nasturtium

Table 2.2. Insects resistant to *Bacillus thuringiensis* proteins (Adapted from Whalon *et al.*, 2011). This includes laboratory studies only using the active protein or plant produced insecticidal proteins.

Species	Order: Family	Host
	***Bacillus thuringiensis* Cry1Da**	
Plutella xylostella	Lepidoptera: Plutellidae	Crucifers, nasturtium
	***Bacillus thuringiensis* Cry1F**	
Ostrinia nubilalis	Lepidoptera: Pyralidae	Maize
	***Bacillus thuringiensis* Cry1Aa**	
Ostrinia nubilalis	Lepidoptera: Pyralidae	Maize
Pectinophora gossypiella	Lepidoptera: Gelechiidae	Cotton
Plutella xylostella	Lepidoptera: Plutellidae	Crucifers, nasturtium
	***Bacillus thuringiensis* Cry1C**	
Plutella xylostella	Lepidoptera: Plutellidae	Crucifers, nasturtium
	Cry1Ba	
Ostrinia nubilalis	Lepidoptera: Pyralidae	Maize
	Cry1Ca	
Plutella xylostella	Lepidoptera: Plutellidae	Crucifers, nasturtium
Spodoptera exigua	Lepidoptera: Noctuidae	Cotton, tomato, celery, lettuce, cabbage and alfalfa
	Cry2Aa	
Ostrinia nubilalis	Lepidoptera: Pyralidae	Maize
	Cry2B	
Culex quinquefasciatus	Diptera: Culicidae	Mammals
	Cyt1Aa	
Plutella xylostella	Lepidoptera: Plutellidae	Crucifers, nasturtium
	Cry1Ac	
Heliothis zea	Lepidoptera: Noctuidae	Maize, tomato, cereals, vegetables
	Cry3Bb1	
Diabrotica virgifera virgifera	Coleoptera: Chrysomelidae	Maize

The rate of resistance development to Bt crops since its release in 1996 is alarming. The number reports of insect species resistant to plant produced Bt proteins since the commercial release of transgenic crops is provided in figure 2.1. Compared to figure 1.1 the trend of resistance development to transgenic crops is very similar to the trend observed in the case of insecticides. If this trend continues, there will soon be many more insect species that are resistant to Bt crops. This observed tendency, even though the number of resistance cases is still comparatively low, further highlights the importance of IRM programs.

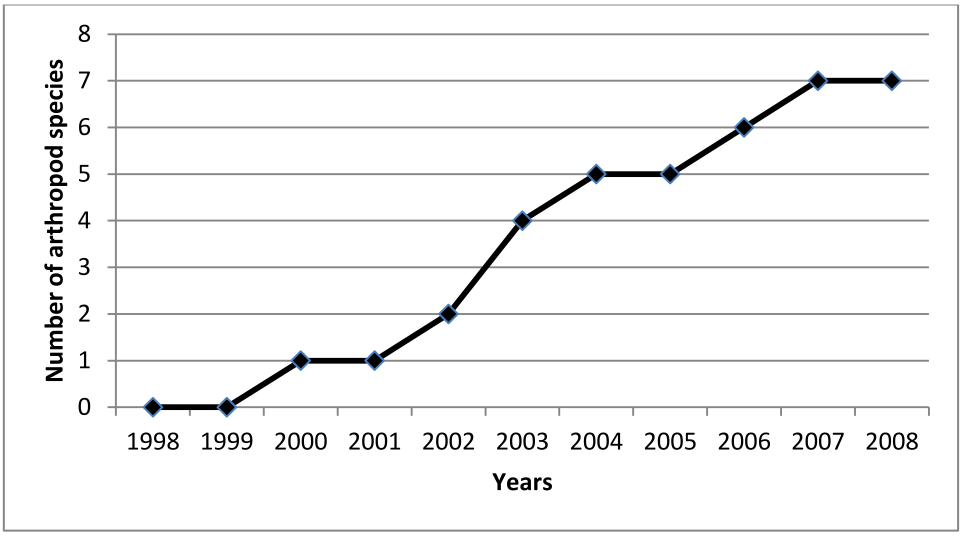

Figure 2.1. The number of insect pest species reported to be resistant to Bt crops since its release in 1998.

Insect resistance management attempts to conserve susceptibility of the insects to pesticides or GM crops with insecticidal properties. In the case of insecticides these strategies are aimed at either overcoming resistance or preventing the development of resistance (Denholm & Rowland, 1992). In the case of Bt crops the resistance management strategy currently mandated is the high-dose/refuge strategy. This strategy is based on evolutionary theory developed before the advent of GM crops (Georghiou & Taylor, 1977a; 1977b; Tabashnik & Croft, 1982; Roush, 1994; Gould, 1998). The theory underlying the strategy is to reduce heritability of resistance by providing refuges of non-Bt host plants (not producing insecticidal protein) that allow for survival of susceptible adults, thereby promoting mating between resistant and susceptible adults which decreases the dominance of resistance (Tabashnik *et al.*, 2004).

In the United States the Environmental Protection Agency (USEPA) requires specific IRM tactics for Bt crops and that these are based on the high-dose/refuge strategy. Proper IRM and adherence to the strategy is to the advantage of growers, producers, researchers and ultimately the public (Glaser & Matten, 2003). The current strategy designed to manage insect resistance to plant produced Bt Cry proteins is firstly to alleviate the potential for pest resistance development in the field by instituting IRM measures and secondly to develop a better understanding of the mechanisms behind pest resistance (USEPA, 2001).

2.2. The high-dose/refuge strategy for insect resistance management

The high-dose/refuge strategy relies on a very high dose insecticide applied. This dose should be high enough to kill close to 100% of susceptible pest individuals. This strategy also requires a segment of the crop area where no insecticide is applied the purpose of which is to sustain a susceptible pest population. The principle of this strategy relies on the assumption that the rare homozygous resistant (RR) individuals will only mate with the susceptible (SS) individuals producing heterozygotes (RS) and that resistance is recessive. The insecticide dose should then be high enough to kill at least 95% of the RS insects (Fig. 2.1) (Gould, 1998). The high-dose component of this strategy differs between insecticides and Bt crops. In the case of insecticides, the high dose is in the form of an insecticide whereas in the case of Bt crops, the plant expresses a high dose. This high dose should in theory be 25 times the dose to kill 99 % of the susceptible individuals (Gould, 1998; Roush, 1994; USEPA, 1998; 2001; Glaser & Matten, 2003).

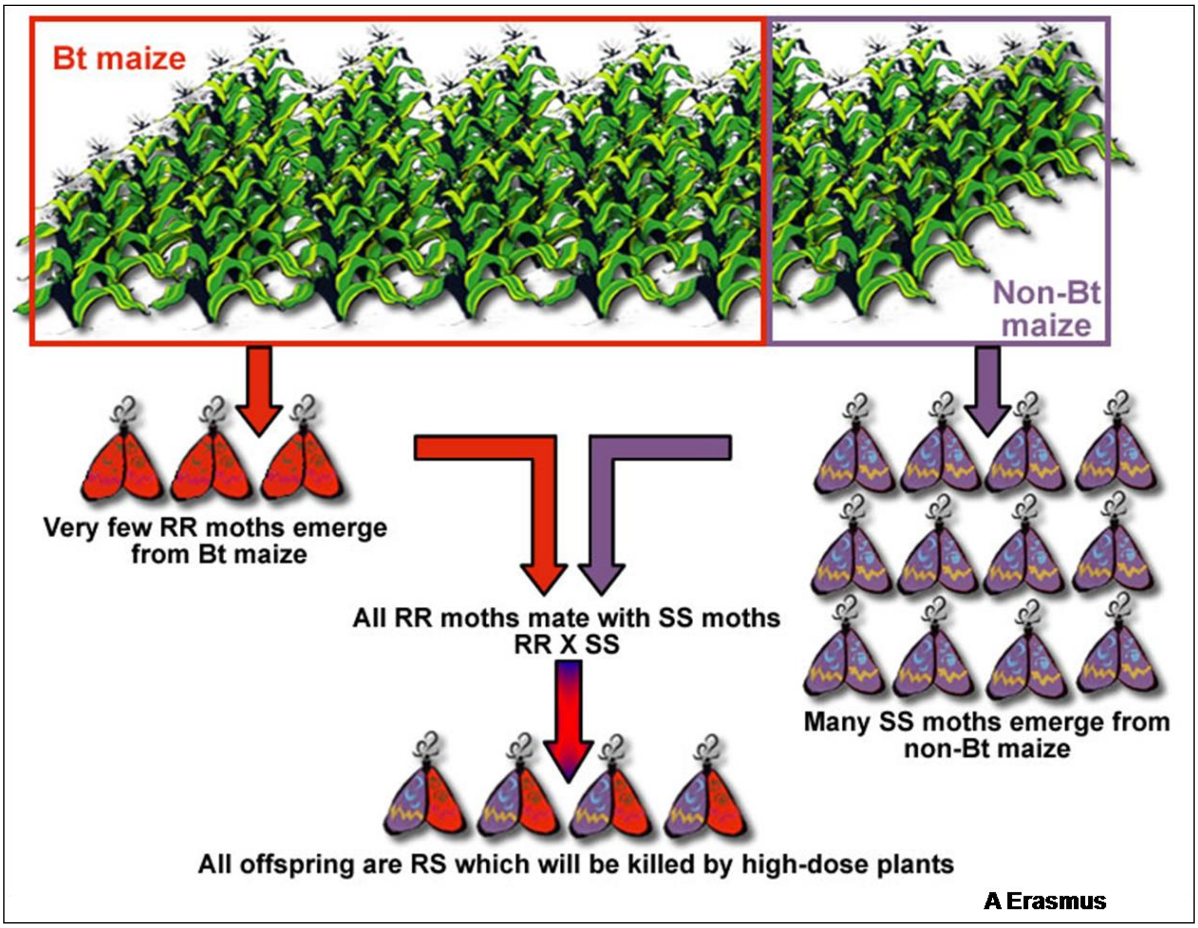

Figure 2.2. Illustration of the high dose/refuge strategy for insect resistance management.

The effectiveness of a refuge area and success of the strategy depend on its size and spatial arrangement relative to the Bt crop, the behavioural characteristics of the target pest and the additional management requirements of the refuge (Fitt, 2008). For example, the maximum distance between the Bt crop planting and the refuge planting depends on the frequency of movement and distances that target pest adults disperse (Fitt, 2008). High male dispersal is favourable for resistance management, as this can increase the probability that RR individuals will mate with SS individuals and vice versa. On the other hand high female dispersal could be detrimental to resistance management (Fitt, 2008).

Some practical considerations may limit the success of the high-dose/refuge strategy. Delivery of the high toxin dose and the separation of toxic and non-toxic plants may be compromised by contamination of Bt seed with non-expressing ‘off-types’. These ‘off-types’ do not express toxins at the correct concentrations or maybe not at all. The proportion of "off-types" may include up to 3 % of the crop (Gould, 1998). This in effect creates a mixture of Bt and non-Bt plants within a single field (Bates *et al.*, 2005) and could accelerate the development of resistance.

2.2.1. Assumptions and requirements of the high-dose refuge strategy

There are several key assumptions on which the high-dose/refuge strategy relies. These assumptions are that resistance is recessive and conferred by a single locus, that resistance genes are initially rare, that there is random mating between resistant and susceptible adults, that heterozygote survival is low, that non-transgenic refugia sustains a susceptible pest population and that the crop expresses a high toxin concentration. These key assumptions are further discussed below:

2.2.1.1. Resistance is recessive and conferred by a single locus

The key assumption on which the high-dose/refuge strategy is based is that resistance is recessive and is conferred by a single locus with two alleles in three insect genotypes of the target pest (RR, SS and RS) (Roush, 1994; Gould, 1998; USEPA, 1998; 2001; Glaser & Matten, 2003). This means that the dominant allele (in this case susceptibility) determines the response of the heterozygote to its environment (Onstad & Guse, 2008). Dominance is often described as a constant genetic property, but it depends on the environment experienced by insects, including the dose of the toxin (Roush & Daly, 1990). The effect that toxin dose can have on the survival of heterozygotes in a population is illustrated in figure 2.3 (Onstad & Guse, 2008). Toxin concentration on different plant parts, in pesticide residues and even in transgenic crops can vary over time. Selection pressure on the target pest is therefore dynamic and may vary under different conditions.

Studies done on insects that are resistant to Bt spray formulations (such as Dipel) showed that Bt resistance in *P. xylostella* is recessive and most likely controlled by a single major gene (Tabashnik *et al.*, 1994). In *Heliothis virescens* (Lepidoptera: Noctuidae) on the other hand, resistance is partially recessive (incompletely dominant), but probably controlled by several genetic factors (cited by Whalon & McGaughey, 1998). It is therefore inaccurate to assume that resistance is always recessive, even within a particular population.

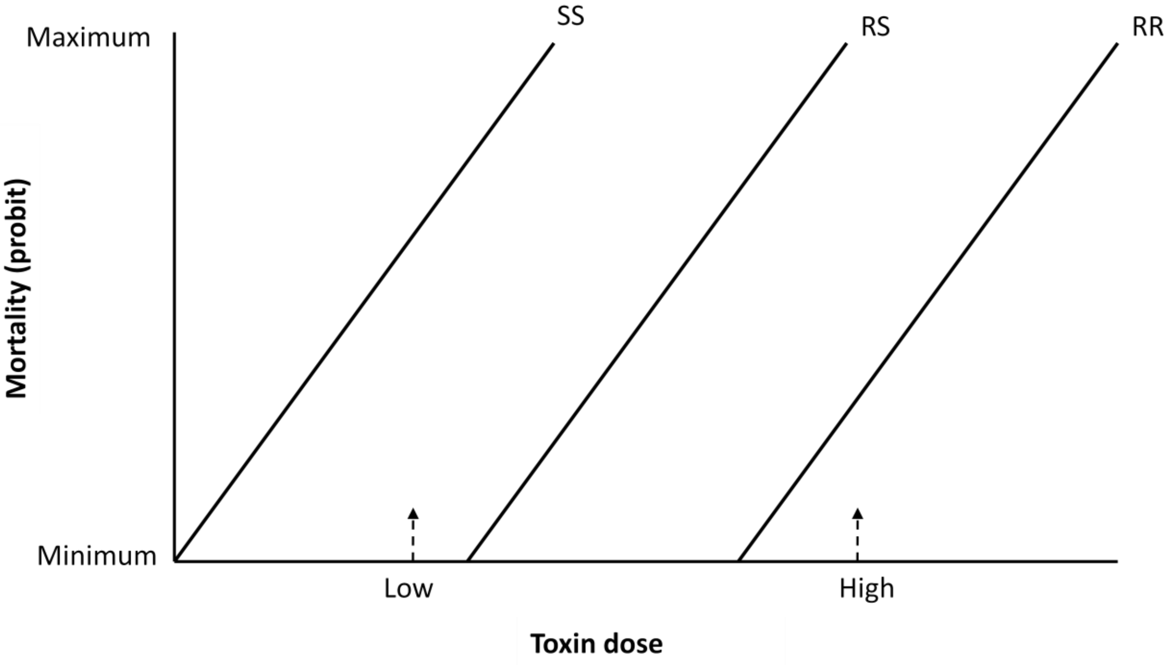

Figure 2.3. The effect of toxin concentration encountered by pests on mortality of three genotypes. Most of the susceptibles (SS) and none of the heterozygotes (RS) are killed by a low dose. Resistance is thus dominant. On the other hand, at a high dose all SS and RS and some RR individuals are killed and resistance is thus recessive (Onstad & Guse, 2008).

2.2.1.2. Resistance genes are initially rare

Resistance genes in a particular population are initially rare (Roush, 1994; Gould, 1998; USEPA, 1998; 2001; Glaser & Matten, 2003). The selection of insect strains resistant to Bt in the laboratory can be used to estimate the initial frequency of resistance genes (Gould, 1998). Since the number of insects used in laboratory studies is usually low (between 100 and 1000) the frequency of resistance alleles must be at least 0.005-0.0005. The initial frequency is therefore likely to be higher than the conventional expectation of 10^{-6} (Gould, 1998). Tabashnik *et al.* (1997) for example found that a Bt susceptible laboratory colony of *P. xylostella* had a resistance frequency of 0.12. The initial frequency of a resistance allele affects the number of generations until resistance evolves and becomes evident in the population, but regardless of the initial frequency it eventually reaches 1 (Onstad & Guse, 2008). If the initial resistance frequency is low, the time it takes for resistance to develop will be longer. Knowledge of this initial R-gene frequency will contribute to the effective use and development of resistance management tactics (Onstad & Guse, 2008).

2.2.1.3. Random mating between resistant and susceptible adults

Random mating between resistant and susceptible adults is another assumption of the high-dose/refuge strategy (Roush, 1994; Gould, 1998; USEPA, 1998; 2001; Glaser & Matten, 2003). A few factors are critical to this assumption. For instance, if the refuge area is too far away from the toxin producing plants an RR individual may not find an SS individual to mate with, compromising this assumption (Gould, 1998). The possible differential rate of development of the target species on Bt and non-Bt plants may also result in temporal isolation between resistant and susceptible individuals. This has been observed for *Ostrinia nubilalis* in Europe (Bourguet *et al.*, 2003) as well as *Busseola fusca* in South Africa (Kruger *et al.*, in review). Liu *et al.* (1999) also found that a resistant strain of *P. gossypiella* takes longer to develop on Bt cotton than a susceptible strain on non-Bt cotton. This asynchrony would result in assortative mating which would generate a disproportionately high number of homozygous resistant (RR) insects which will accelerate the evolution of resistance (Liu *et al.*, 1999; Tabashnik, 1994c). The biology of each target pest species should thus be studied and understood before random mating can be assumed and for this strategy to be correctly implemented.

2.2.1.4. Heterozygote survival is low

Heterozygote (RS) survival should be lower than 5 % of resistant (RR) survival (Roush, 1994; Gould, 1998; USEPA, 1998; 2001; Glaser & Matten, 2003). According to Taylor and Georghiou (1979) if the LD_{95} of

SS individuals are 0.001 ppm of toxin then the LD_{95} of RS individuals is 0.09 ppm. Toxin induced mortality of RS individuals is therefore likely to occur when there is 99 % mortality of SS individuals (Taylor & Georghiou, 1979). If the toxin dose is thus increased to cause 95 % mortality in the RS proportion of a pest population, then SS mortality should be close to 100 % (Gould, 1998).

2.2.1.5. The non-transgenic refuge will sustain susceptible population

A common assumption of the high-dose/refuge strategy is that the non-transgenic refugia will sustain susceptible pest populations (Roush, 1994; Gould, 1998; USEPA, 1998; 2001; Glaser & Matten, 2003). The dispersal behaviour during the adult phase of the target pest is an important aspect when assuming the statement mentioned above. If for instance the females of the target pest disperse greatly, it cannot be assumed that all of the individuals found in the refuge area will be susceptible. As with some of the other above mentioned assumptions, knowledge of the target pests' biology is of utmost importance.

Once resistance has developed interesting scenarios regarding this basic assumption of the refuge strategy can develop. For example, studies conducted by Kruger *et al.* (2011a) reported that *Busseola fusca* larvae occurring in the refuge areas were also resistant to Bt maize and that individuals completed their lifecycles. Once such a high frequency of the resistance allele is reached in a population, the refuge does not contribute further to delaying the rate of resistance development. In situations where the frequency of resistance alleles is increasing (indicating failure of the refuge strategy if it was implemented correctly) the only contribution of the refuge could be by means of its possible indirect negative effect on fitness of the resistant alleles (fitness costs). The possible effect of fitness cost in IRM has not previously been studied under field conditions and no data exist on this topic. 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 resistance management value that the presence of fitness costs may hold (Gassmann *et al.*, 2009) 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. Kruger *et al* (In review) reported that no fitness costs were associated with resistance in a highly resistant *B. fusca* population. The percentage survival and mean larval and pupal mass of individuals reared on Bt maize indicated a high level of resistance to Bt-toxin and general fitness of the resistant population. Kruger *et al.* (In review) also reported no measurable fitness costs in terms of pupal and moth parameters in a field-collected Bt-resistant population.

2.2.1.6. The crop expresses a high toxin concentration

The high dose requirement is that the crop expresses 25 times the toxin concentration required to kill 99 % of the susceptible pest population (Roush, 1994; Gould, 1998; USEPA, 1998; 2001; Glaser & Matten, 2003). There is no way to be certain of the concentration of toxin that the insects ingest. For instance if toxic and non-toxic plants are planted too close to one another, individuals of the target pest could move between plants and ingest varying portions of the toxin (Gould, 1998). The concentration of toxin expressed by the plant also has to be maintained throughout the period of pest attack. Toxin concentrations declines at the reproductive stage in a maize line expressing the *cry1Ab* gene (cited by Cohen, 2000). This was also observed in cotton at later stages of plant development and under some environmental conditions (cited by Cohen, 2000).

The toxin dose expressed by the plant can be verified using methods indicated by the Scientific Advisory Panel (SAP) of the USEPA (1998). These methods are: a) serial dilution larval bioassay with artificial diet containing lyophilized tissue of Bt plants with tissue from non-Bt plants as control; b) determination of toxin expression using plant lines having expression levels 25-fold lower than a non-plant incorporated protectant cultivar as quantified by ELISA or technique of comparable reliability; c) sufficient plants must be sampled in any survey of field plants to ensure that the cultivar is expressing a dose at the $LD_{99.9}$ or higher to assure that 95 % of heterozygotes would be killed; d) the use of controlled infestation, using the conditions specification (c) with a laboratory strain of the pest having an LD_{50} value similar to field strains; or e) determination of a later-instar survivability for the targeted pest found with an LD_{50} value that was 25-fold higher than that of the neonate larvae (USEPA-SAP, 1998).

A high-dose Bt plant for one pest species is not necessarily a high dose against another target pest. According to Huang *et al.* (2011) published data have shown that all the commercial Bt crop cultivars are very effective against their major pest species, *O. nubilalis* and *D. grandiosella* for Bt maize and *H. virescens* and *P. gossypiella* for Bt cotton. The available cultivars are presumed to meet the high-dose requirement of the IRM strategy (Huang *et al.*, 2011), but there is no way in which this can easily be confirmed under field conditions.

A summary of the fundamental requirements of the high-dose/refuge strategy and its possible effects on the development of actual cases of resistance is provided in table 2.3. In cases where resistance has been confirmed or suspected, the status of some of these fundamental requirements and assumptions as described above is unknown. The opposite is also true, where resistance is recessive, the initial resistance allele frequency is known (and low) and where refuges are planted the development of resistance has been efficiently delayed (Huang *et al.*, 2011). The data summarized in table 2.3 therefore suggest that where the high-dose/refuge strategy is properly implemented the development of resistance is delayed.

Table 2.3. Summary of the available information regarding three of the fundamental requirements of the high-dose/refuge strategy for Cry1Ab maize and Cry1Ac cotton. Data indicate Bt crops and localities with documented (dark grey boxes) or suspected (light grey boxes) field resistance (Huang *et al.*, 2011).

Bt crop and locality	Pest	Recessive resistance (high dose)[1]	Resistance allele <0.001[2]	Refuge[1]
Cry1Ab maize in USA	*Ostrinia nubilalis*	+	<0.0004	+
Cry1Ab maize in USA	*Diatraea grandiosella*	+	<0.0035	+
Cry1Ab maize in South Africa	*Busseola fusca*	-	?	-
Cry1F maize in Puerto Rico	*Spodoptera frugiperda*	-	?	-?
Cry 1Ac cotton in USA	*Heliothis virescens*	+	0.0004	+
Cry 1Ac cotton in USA	*Pectinophora gossypiella*	+	<0.0003	+
Cry 1Ac cotton in USA	*Heliothis zea*	-	?	+
Cry 1Ac cotton in India	*Pectinophora gossypiella*	+	?	-?
Cry 1Ac cotton in China	*Helicoverpa armigera*	+/-	?	-?
Cry3Bb1 maize in USA	*Diabrotica virgifera virgifera*	+/-	?	+

[1] ”+” = meets the requirement of the high-dose/refuge strategy; “-“ = does not meet the requirement; “+/-“ = meets the requirement in the vegetative plant stages but not for the reproductive stages; “?” = information is unavailable.

[2] Bt resistance allele frequencies are based on data from Bourguet *et al.* (2003) for *O. nubilalis*, Huang *et al.* (2007) for *D. grandiosella*, Blanco *et al.* (2009) for *H. virescens* and Tabashnik *et al.* (2006) for *P. gossypiella*. Inequalities provide upper range of the 95 % credibility interval for *O. nubilalis* and *D. grandiosella* and a 95 % confidence interval for *P. gossypiella*. The value for *H. virescens* is an expected frequency.

2.3. Spatial configuration of refuge areas

Vacher *et al.* (2003) used modelling to determine the optimum percentage and configuration of refuge fields. They found that there is an optimal proportion of refuge beyond which resistance does not evolve and for which pest densities are minimal. This percentage is 26 %. It is best to use a percentage that is equal to or slightly higher and aggregated into strips of non-Bt plants (Vacher *et al.*, 2003). They concluded that refuge areas should be specific for specific pest species, aggregated for pests with large dispersal distances like *O. nubilalis* and less clumped for insects with restricted movement such as *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae).

The currently accepted refuge requirements comprise of a 20 % insecticide sprayed or 5 % unsprayed refuge which can be planted in different specified ways. The different spatial arrangements of refuges are illustrated in figure 2.4. A block of non-Bt plants can be planted adjacent to or within the Bt maize field (Fig 2.4a and b). A perimeter of non-Bt plants can also be planted around the Bt maize field if this is more practical (Fig 2.4c). Another possibility is to plant strips of non-Bt plants within Bt maize fields, i.e. at least four rows of non-Bt plants with at least six rows of Bt plants in between (Fig 2.4d). If circumstances are such that the Bt field and non-Bt refuge is planted separately the field of non-Bt plants should be planted within at least 800 metres (preferably 400 metres) of the Bt maize field (Fig 2.4e). In the case of centre pivot irrigation systems an option is to plant the non-Bt plant refuge in the corners of the pivot with Bt maize plants (Fig 2.4f).

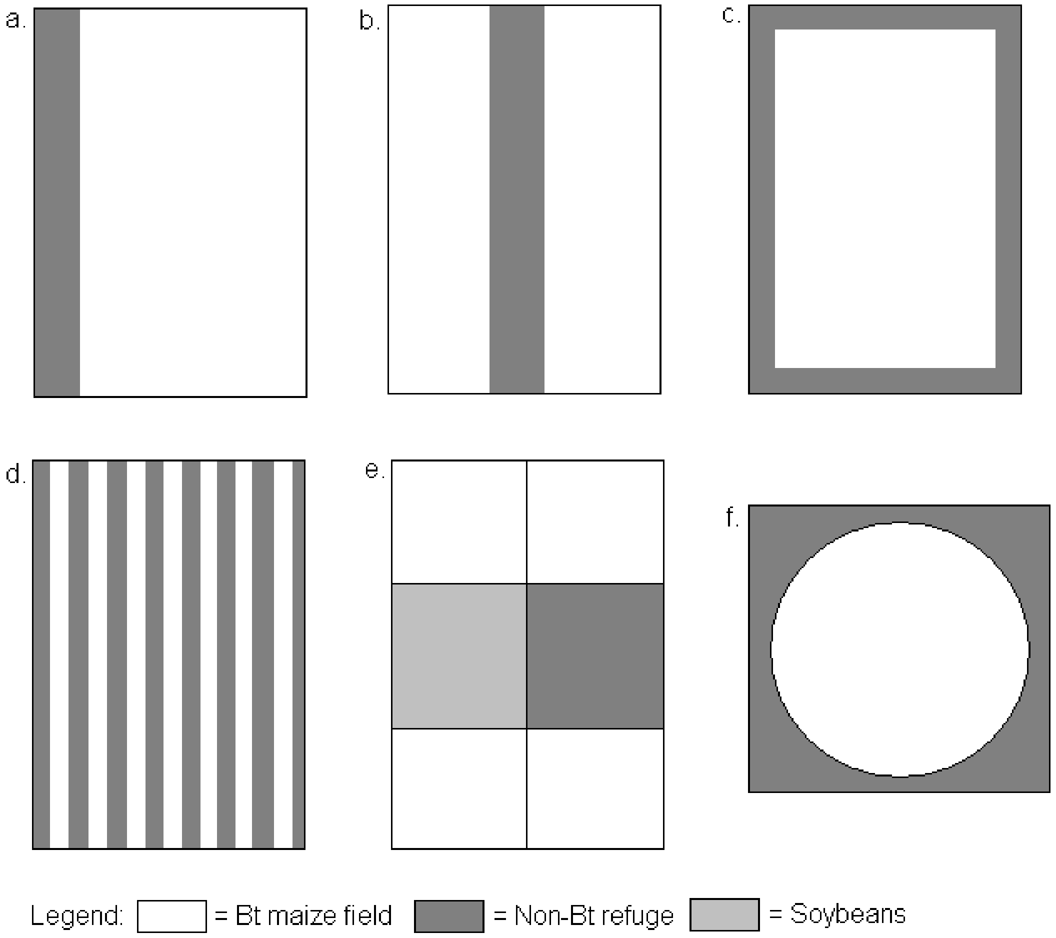

Figure 2.4. Different spatial arrangements in which refuges can be planted: a) Block refuge (adjacent), a block of non-Bt maize adjacent to the Bt maize field; b) Block refuge (within), a block of non-Bt maize within the Bt maize field; c) Refuge perimeter, non-Bt maize surrounding the Bt maize field; d) Split planter refuge, strips of non-Bt maize at least four rows wide within the Bt maize field (6 rows preferred); e) Separate field refuge, a separate field of non-Bt maize within 0.8 km of the Bt maize field (0.4 km preferred); f) Pivot corners refuge, non-Bt maize in pivot corners adjacent to the Bt maize field (Adapted from Lauer, 2003).

Figure 2.5. An example of a 5 % refuge planted adjacent to the Bt maize plants.

2.4. Seed mixtures as insect resistance management strategy

Recently, despite research proving that it may not be effective, Pioneer Hybrid International released a seed mixture of Bt and non-Bt maize as IRM strategy (Pioneer, 2011). This seed mixture approach is termed the "refuge-in-a-bag" strategy. According to the source this strategy provides the farmer with *D. v. virgifera* protection without the challenges associated with planting a normal refuge. Seed mixtures are also under consideration for management of lepidopterous stem borers, of which larvae are known to migrate extensively between plants (J. Van den Berg, pers. comm.).

The "refuge-in-a-bag" strategy mentioned above does not take into account the possible effects that insect behavioural characteristics could have on IRM and the sustainable use of Bt crop technology. The "refuge-in-a-bag" strategy does not critically consider the assumption that dispersal and random mating occurs in pest populations. Block refuges have the advantage of minimising differential selection due to larval movement from toxic to non-toxic plants, but have disadvantages relative to mating. Susceptible individuals may not readily find resistant or heterozygous individuals to mate with. The “refuge-in-a-bag” strategy may allow very good mixing of genotypes of the adult stage of the pest, but are riskier with regard to selection during the larval stage (Onstad & Gould, 1998). This highlights the importance of timeous identification of knowledge gaps in development of IRM strategies.

Various aspects regarding pre- and post-mating adult migration behaviour as well as larval behaviour is still unclear. For example, it is uncertain whether larvae of *Diabrotica* spp., which is managed by Bt maize in the USA, move between host plants after initial establishment. Hibbard *et al.* (2003) studied the movement of *D. v. virgifera* larvae in a maize field and observed that the extent of larval movement that occurs between plants or between rows after initial establishment was likely to be affected by a number of factors. These factors include the proximity of roots of neighbouring plants, the amount of competition for the available food source, and possibly soil structure and the quantity of favourable food sources in neighbouring plants (Hibbard *et al.*, 2003). Post-establishment larval movement was recorded in only two of eight experiments over two seasons in which experiments were conducted under artificial infestations of *D. v. virgifera*. It is therefore still not clear whether or not larger larvae will disperse between damaged plants, and in the case of seed mixtures of Bt maize, whether they are alternately exposed to Bt and non-Bt plants (Hibbard *et al.*, 2003).

Root structure and other factors that could affect attractiveness of plants to larvae of soil dwelling pests such as *D. v. virgifera* may also have an influence on pest behaviour. An undamaged transgenic plant in a mixed crop stand of Bt and non-Bt plants may attract *Diabrotica* spp. larvae because of the mass of roots available compared to those of non-Bt plants that were initially attractive but damaged by the pest (Hibbard *et al.*, 2003). The same may occur in block or strip plantings of narrow-row maize. If these larvae develop initially on susceptible plants, they would likely be larger and more tolerant of the transgenic toxin. This could result in increased survival and a possible increase in the development rate of resistance. On the other hand, if a low-dose product results in the survival of some susceptible larvae, movement of larger larvae from less suitable alternate hosts or highly damaged maize in a seed mix, to roots of Bt plants could actually increase product durability by producing additional susceptible insects from within the Bt field (Hibbard *et al.*, 2003).

However, other research also showed that if larvae are able to move between plants these seed mixtures may hasten the development of resistance (Mallet & Porter, 1992; Tabashnik, 1994c). Results from Mallet and Porter (1992) as well as Tabashnik (1994c) showed that a refuge will delay the development of resistance as long as, or longer than a seed mixture, because refugia reduces selection without altering dominance.

A recent review by Onstad *et al.* (2011) compared the use of blocks of refuges and seed mixtures as IRM strategies within IPM. A summary of IPM aspects that play a role in the selection of seed mixtures or refuge blocks as an IRM strategy is provided in table 2.3. Pests in Bt crop fields are controlled throughout the season, regardless of infestation levels. This season-long protection provided by Bt crops disregards many aspects of IPM such as the monitoring of pest levels before control measures are implemented and concentrating treatments when or where appropriate (Onstad *et al.*, 2011). The benefit of block refuges therefore is that the onset of resistance can be detected early. Seed mixtures on the other hand may contribute in a decline of pest monitoring by farmers due to increased difficulty in monitoring. Since non-Bt (refuge) plants cannot be phenotypically distinguished from the Bt plants without the use of protein specific test strips (Onstad *et al.*, 2011), monitoring fields for damage to Bt plants will be impossible. Seed mixtures will therefore the detection of resistance at field level.

Table 2.3. Summary of IPM aspects pertaining to choice of deployment of IRM strategies as seed mixtures or blocks.

Aspect	**Seed mixture**	**Block refuge**
Pest monitoring	Difficult	Typical
Control of secondary pests	New approaches	Traditional
Biological control	New approaches	Traditional
Insecticide use	Less	More
Quality of refuge relative to Bt crop	Similar	Probably different
Effects on IRM of larval behaviour	More risk	Less risk
Effects on IRM of adult behaviour	Less risk	More risk
Adoption of plant produced insecticide technology	Higher	Lower
Compliance with IRM rules	Higher	Lower

Bt crops used are designed for specific target pests and they may are usually not effective against secondary pests. These secondary pests may also move more or less than the target pests which can expose them to different concentrations of the toxin in different plant parts (Onstad *et al.*, 2011). Resistance can thus develop in secondary pests which are initially only suppressed, giving them increased pest status. Minor secondary pests which are suppressed by specific Bt proteins may become resistant to these proteins and become important secondary pests. For example the African bollworm (*Helicoverpa armigera*) which is not the target pest of Cry1Ab Bt maize in South Africa is only suppressed by this toxin. Farmers have experienced a decline in *H. armigera* pest status during the past decade, but increased bollworm levels are anticipated if this pest becomes resistant to the Bt toxin (Erasmus *et al.*, in review).

While several disadvantages and adverse effects regarding primary and secondary pests are foreseen with the use of seed mixtures, beneficial effects may arise for certain natural enemies. Pest density and dispersion affect the foraging efficacy of natural enemies and the behaviour and abundance of natural enemies that specialize on specific target and non-target pests. Refuge areas planted in blocks provide relatively dense patches of target pests for specialist natural enemies to persist in the field. Seed mixtures on the other may result in pests being more evenly distributed throughout the field which may facilitate the persistence of specialized natural enemies (Onstad *et al.*, 2011).

The abovementioned plant-insect interactions and their possible effects on the success of IRM strategies indicate that the understanding of pest behaviour is essential in developing IRM and IPM strategies. There are only minor differences between the structured refuge and seed mixtures and it may come down to practical constraints of growing different varieties at various spatial scales, plant compensatory responses and other factors that affect the economic and political feasibility of implementation to determine the best option (Tabashnik, 1994c).

2.5. Target pests and USEPA refuge requirements

A list of Bt crops currently registered by the USEPA, their target pests and refuge requirements is provided in table 2.4. Most of the refuge requirements are the same for different crops and even the diverse range of target pests. The specific target pests for specific Bt crops also differ between countries. The information available in the literature is mostly based on insect pests that occur in the USA as is illustrated in figure 2.6 and 2.7. These figures are general distribution maps of the pests listed as target pests (Fig 2.6) and other pests (Fig 2.7). As expected the target pests of a Bt crop differs between geographic areas. Although the development of Bt crop cultivars that produce specific Cry proteins is largely done with North American pest species in mind, these cultivars are also deployed to other regions of the world if found effective against similar pests of these crops. It is clear from fig 2.6 and 2.7 that few of the target pest species for which Bt products are developed occur in Africa.

Table 2.4. Genetically modified Bt crops currently available, their target pests, other pests controlled and refuge requirements (adapted from USEPA, 2010; Cullen, 2010).

Bt Protein	**Products**	**Target pests**	**Other pests controlled**	**Refuge requirements**
Maize				
Cry1Ab	YieldGard® Corn Borer, Agrisure® CB/LL, Agrisure GT/CB/LL	*Ostrinia nubilalis* (Lepidoptera: Pyralidae)	*Heliothis zea* (Lepidoptera: Noctuidae) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *Papaipema nebris* (Lepidoptera: Noctuidae) *Diatraea grandiosella* (Lepidoptera: Crambidae) *Diatraea saccharalis* (Lepidoptera: Crambidae)	20 % non-Bt maize within 800 m
Cry1F	Herculex® 1	*Ostrinia nubilalis* (Lepidoptera: Pyralidae) *Diatraea grandiosella* (Lepidoptera: Crambidae) *Diatraea saccharalis* (Lepidoptera: Crambidae)	*Heliothis zea* (Lepidoptera: Noctuidae) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *Richia albicosta*(Lepidoptera: Noctuidae) *Agrotis ipsilon* (Lepidoptera: Noctuidae)	20 % non-Bt maize within 800 m
Cry3Bb1	YieldGard® Rootworm, YieldGard VT Rootworm	*Diabrotica virgifera* (Coleoptera: Chrysomelidae) *Diabrotica barberi* (Coleoptera: Chrysomelidae)		20 % non-Bt maize adjacent
Cry34/35Ab1	Herculex® Rootworm	*Diabrotica virgifera* (Coleoptera: Chrysomelidae) *Diabrotica barberi* (Coleoptera: Chrysomelidae)		20 % non-Bt maize adjacent
mCry3A	Agrisure® Rootworm, Agrisure GT/RW	*Diabrotica virgifera* (Coleoptera: Chrysomelidae) *Diabrotica barberi* (Coleoptera: Chrysomelidae)		20 % non-Bt maize adjacent
Cry1Ab + Cry3Bb1	YieldGard® Plus, YieldGard Plus RR2, YieldGard VT Triple	*Ostrinia nubilalis* (Lepidoptera: Pyralidae) *Diabrotica virgifera* (Coleoptera: Chrysomelidae) *Diabrotica barberi* (Coleoptera: Chrysomelidae)	*Heliothis zea* (Lepidoptera: Noctuidae) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *Papaipema nebris* (Lepidoptera: Noctuidae) *Diatraea grandiosella* (Lepidoptera: Crambidae) *Diatraea saccharalis* (Lepidoptera: Crambidae)	20 % non-Bt maize adjacent
Cry1F + Cry34/35Ab1	Herculex® XTRA	*Ostrinia nubilalis* (Lepidoptera: Pyralidae) *Diatraea grandiosella* (Lepidoptera: Crambidae) *Diatraea saccharalis* (Lepidoptera: Crambidae) *Diabrotica virgifera* (Coleoptera: Chrysomelidae) *Diabrotica barberi* (Coleoptera: Chrysomelidae)	*Heliothis zea* (Lepidoptera: Noctuidae) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *Richia albicosta*(Lepidoptera: Noctuidae) *Agrotis ipsilon* (Lepidoptera: Noctuidae)	20 % non-Bt maize adjacent
Cry1Ab + mCry3A	Agrisure® CB/LL/RW, Agrisure 3000GT	*Ostrinia nubilalis* (Lepidoptera: Pyralidae) *Diatreae grandiosella* (Lepidoptera: Crambidae) *Diatreae saccharalis* (Lepidoptera: Crambidae) *Diabrotica virgifera* (Coleoptera: Chrysomelidae) *Diabrotica barberi* (Coleoptera: Chrysomelidae)	*Heliothis zea* (Lepidoptera: Noctuidae) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *Papaipema nebris* (Lepidoptera: Noctuidae)	20 % non-Bt maize adjacent

Cry1A.105 + Cry2Ab2	Genuity® VT Double Pro (VT2P)	*Ostrinia nubilalis* (Lepidoptera: Pyralidae) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *Heliothis zea* (Lepidoptera: Noctuidae)		5 % non-Bt maize within 800 m
Cry1A.105 + Cry2Ab2 + Cry3Bb1	Genuity® VT Triple Pro (VT3P)	*Ostrinia nubilalis* (Lepidoptera: Pyralidae) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *Heliothis zea* (Lepidoptera: Noctuidae) *Diabrotica virgifera* (Coleoptera: Chrysomelidae) *Diabrotica barberi* (Coleoptera: Chrysomelidae)		20 % non-Bt maize adjacent
Cry1A.105 + Cry2Ab2 + Cry1F + Cry3Bb1	Genuity® SmartStax™ (GENSS)	*Ostrinia nubilalis* (Lepidoptera: Pyralidae) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *Diabrotica virgifera* (Coleoptera: Chrysomelidae) *Diabrotica barberi* (Coleoptera: Chrysomelidae) *Richia albicosta*(Lepidoptera: Noctuidae) *Agrotis ipsilon* (Lepidoptera: Noctuidae)		5 % non-Bt maize adjacent
Vip3A + Cry1Ab + mCry3A	Agrisure® Viptera™ 3111	*Ostrinia nubilalis*(Coleoptera: Chrysomelidae) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *Helicoverpa* zea (Lepidoptera: Noctuidae) *Diabrotica virgifera* (Coleoptera: Chrysomelidae) *Diabrotica barberi* (Coleoptera: Chrysomelidae) *Richia albicosta*(Lepidoptera: Noctuidae) *Agrotis ipsilon* (Lepidoptera: Noctuidae)		20 % non-Bt maize adjacent
		Cotton		
Cry1Ac + Cry2Ab	Bollgard® II	*Heliothis zea* (Lepidoptera: Noctuidae) *Heliothis virescens* (Lepidoptera: Noctuidae) *Pectinophora gossypiella* (Lepidoptera: Gelechiidae)	*Helicoverpa armigera* (Lepidoptera: Noctuidae) *Diparopsis castanea* (Lepidoptera: Noctuidae) *Earias* spp. (Lepidoptera: Noctuidae)	20% non-Bt cotton, sprayed; or 5% non-Bt cotton, unsprayed
Cry1F + Cry1Ac	WideStrike®	*Heliothis virescens* (Lepidoptera: Noctuidae) *Heliothis zea* (Lepidoptera: Noctuidae) *Pectinophora gossypiella* (Lepidoptera: Gelechiidae)	*Ostrinia nubilalis* (Lepidoptera: Pyralidae) *Helicoverpa armigera*(Lepidoptera: Noctuidae) *Estigmene acrea* (Lepidoptera: Arctiidae) *Bucculatrix thurberiella* (Lepidoptera: Lyonetiidae) *Pseudoplusia includens* (Lepidoptera: Noctuidae) *Spodoptera exigua* (Lepidoptera: Noctuidae) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *Spodoptera ornithogalli* (Lepidoptera: Noctuidae)	20% non-Bt cotton, sprayed; or 5% non-Bt cotton, unsprayed

		Soybean		
Cry1Ac	MON87710	*Anticarsia gemmatalis* (Lepidoptera: Erebidae) *Pseudoplusia includens* (Lepidoptera: Noctuidae)	*Epinotia aporema* (Lepidoptera: Tortricidae) *Rachiplusia nu* (Lepidoptera: Noctuidae)	Use on a limited basis, relies on natural refuge
		Potato		
Cry3A	NewLeaf™	*Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)		20 % non-Bt refuge
		Brinjal		
Cry1A	EC-11	*Leucinodes orbonalis* (Lepidoptera: Crambidae)		20 % non-Bt refuge

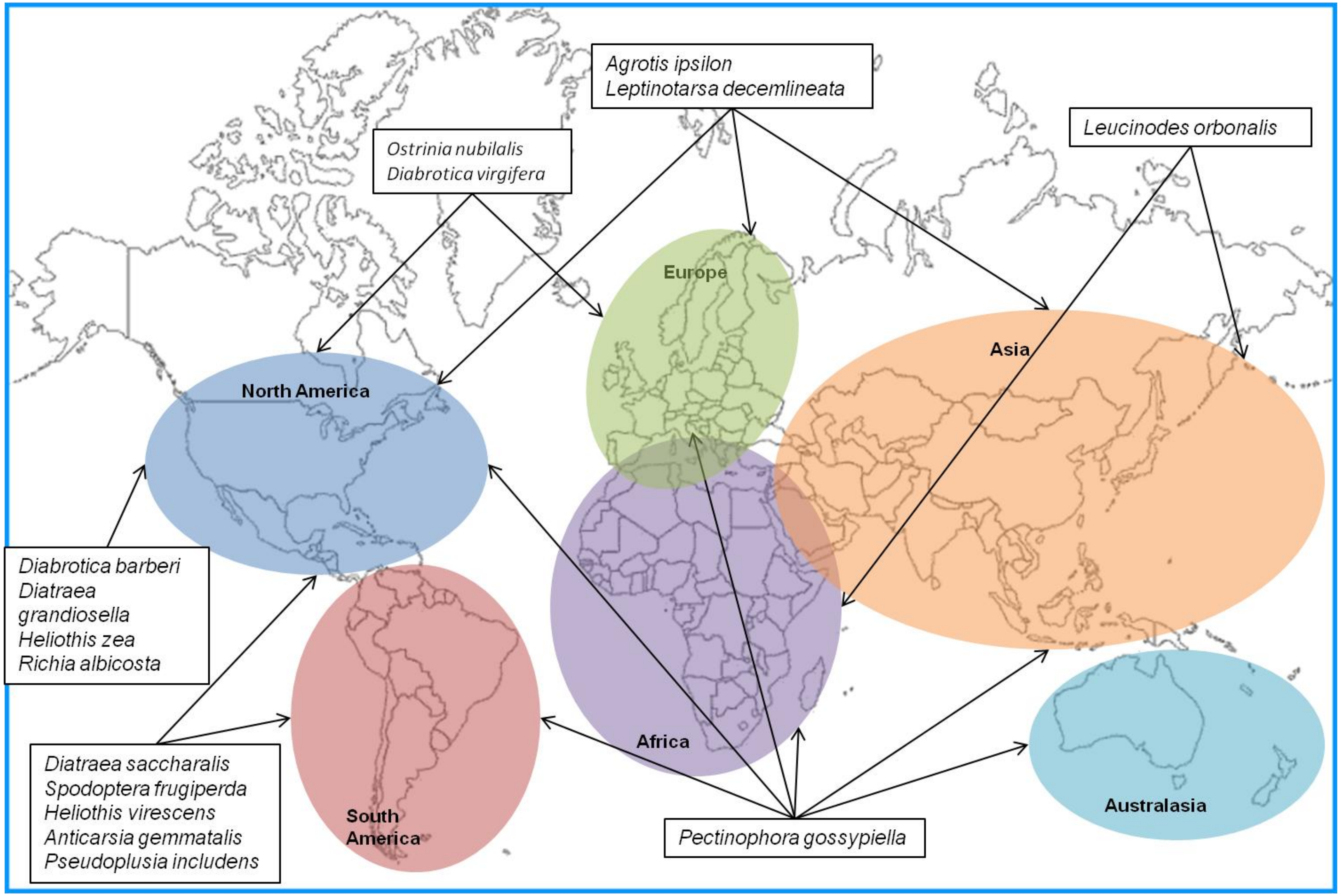

Figure 2.6. A general distribution map of the target pest species controlled by Bt crops as listed in table 2.4.

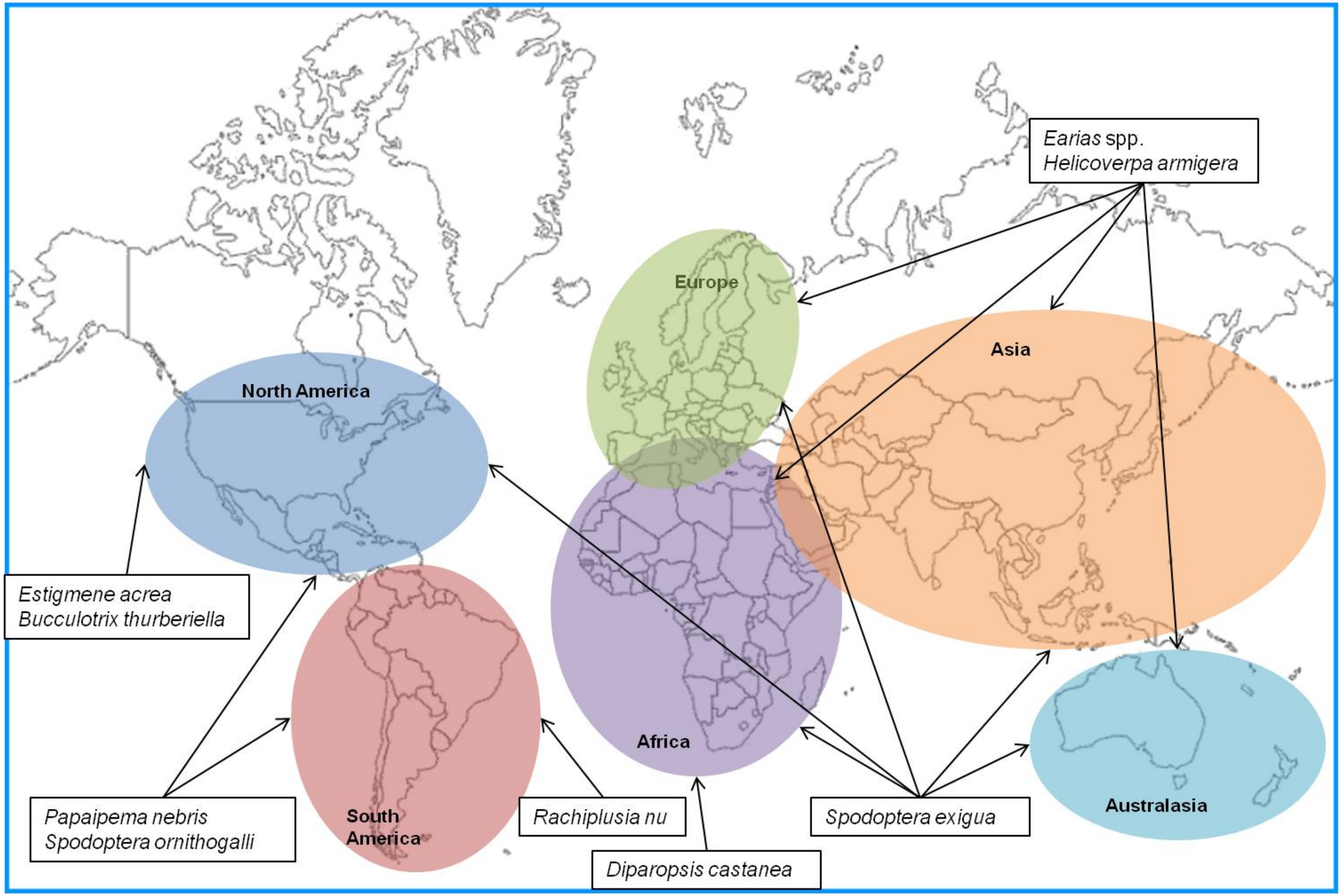

Figure 2.7. A general distribution map of other pests controlled by Bt crops as listed in table 2.4.

In South Africa the target pests of Bt maize are the stem borers *B. fusca* and *Chilo partellus* (Lepidoptera: Noctuidae) (Monsanto, 2011a). Bt cotton is protected against the noctuid bollworm complex, *Helicoverpa armigera, Diparopsis castanea*, *Erias insulana* and *Erias biplaga* (Lepidoptera: Noctuidae) (Monsanto, 2011b). For all of these pests and in some cases on different crops, refuge requirements are mostly uniform. The huge differences in life history strategies of different pest species as well as the fact that even different life stages of pests are subject to the identical IRM strategies, leads to the realization that one strategy cannot serve all and that more pest-specific management strategies should be developed.

2.6. Conclusion

As with conventional insecticides the best way to prolong the efficacy of insecticidal proteins produced by transgenic plants is to use them in conjunction with alternative control methods through an IPM approach. This will minimize exposure of pests to toxins. Insect resistance management would be most effective and also sustainable when it is regarded as part of an integrated pest management program (Onstad, 2008; McGaughey & Whalon, 1992; Glaser & Matten, 2003).

Chapter 3

Case studies: the possible impact of insect behaviour on the efficiency of the refuge approach to insect resistance management

3.1. Introduction

Herbivorous insects, like all consumer species, can be classified as monophagous, oligophagous or polyphagous. Monophagous insects only feed on one plant species, oligophagous insects feed on a few plant species and polyphagous insects feed on a wide range of plant species (Andow, 1991; Begon *et al.*, 2006). Polyphagous species can also be divided into sequential polyphages that alternate hosts between generations in a temporal sequence, and simultaneous polyphages that alternate hosts within a generation with individuals moving from host to host (Andow, 1991). The different host utilization strategies of herbivorous species will influence the efficacy of the refuge approach to insect resistance management (IRM). The number of host plant species utilized by an insect pest species, and their presence in a particular geographic area can also influence the configuration and percentage of refuge used (Andow, 1991).

Refugia are expected to promote mating between susceptible and resistant individuals. Random mating between moths emerging from refugia and Bt crop fields is a prerequisite for effective IRM. It has also been highlighted that non-recessive inheritance would drastically reduce the efficiency of the refuge strategy (Tabashnik *et al.*, 2008). Tabashnik *et al*. (2009) showed that a refuge should account for approximately 55 % of the total surface area to delay the resistance for ten years. A similar approach to refuge design was implemented in Australia against *Helicoverpa armigera* (Lepidoptera: Noctuidae) on Bt cotton with non-Bt cotton refugia accounting for 70 % of the surface area planted to Bt cotton (Tabashnik *et al*., 2009).

Vacher *et al.* (2003) used a model to determine the optimum percentage and configuration of refuge fields. They concluded that refuge area requirements should be specific for specific pest species, aggregated for pests with large dispersal distances and less clumped for insects with restricted movement.

In this chapter, a range of pests with different life histories are discussed with reference to the possible influences that pest behaviour could have on the efficacy of the refuge approach to IRM.

3.2. Monophagous insect pests

3.2.1. *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae): biology, ecology and control with Bt crops

Diabrotica virgifera virgifera is the most serious insect pest of maize in the USA (Spencer *et al.*, 2005; Spencer *et al.*, 2009). Larvae feed mainly on maize roots, which interferes with water and nutrient uptake, facilitates pathogen entry and reduces the ability of roots to hold the plants upright (Spencer *et al.*, 2009). This pest has only one generation per year. The cycle commences with the female laying eggs in the soil near the base of the host plant (Fig 3.1(a)). These eggs pass through diapause during winter and hatch in late spring (late May, early June in the Northern hemisphere) (Fig 3.1(b)). The larvae feed on the roots of the maize plans for 2-3 months and then pupate in the soil close to the plant (Fig 3.1(c)). Five to ten days later the adult beetles emerge and start feeding on leaves and silks of maize plants (Fig 3.1(d)). Adults mate within the first few days after emergence and females start to lay eggs about three weeks after mating (Hodgson, 2008).

Figure 3.1. The life cycle of *Diabrotica virgifera virgifera*. (a) Eggs; (b) larva; (c) pupa and (d) adult beetle.

Although maize is the most suitable host plant, larvae are also able to use other grasses as hosts. *Diabrotica virgifera virgifera* has been effectively controlled through crop rotation, with soybeans in particular, for the past 100 years in the USA. This is a very effective control method for this pest as they have a strong ovipositional fidelity to maize fields and an inability to complete development on most other crops (Spencer *et al.*, 2009). These insects have, however, become resistant to crop rotation. Resistant females now show a tendency to exit maize fields to oviposit in soybean fields (or other locations in addition to maize fields) (Spencer *et al.*, 2005).

In areas where rotation-resistant *D. v. virgifera* populations are present, season-long inter-field movement between maize fields and other crops leads to widely distributed populations (Spencer *et al.*, 2009). Adults show three basic types of movement, intra-field activity, inter-field activity and long distance movement.

Flight activity of *D. v. virgifera* adults is influenced by environmental factors such as temperature, wind speed, solar radiation and precipitation (Spencer *et al.*, 2005). Intra-field movement of *D. v. virgifera* adults usually have to do with feeding, oviposition, mating or sheltering. After emergence females only move short distances before being intercepted by mate-seeking males (Ball, 1957). After mating the females usually remain in their natal maize field for a few days before dispersing to other fields (Spencer *et al.*, 2009). A small percentage of mated females will also engage in sustained migratory flights shortly after emergence, which facilitates long-distance dispersal from their natal field (Spencer *et al.*, 2009). Males are less likely to initiate sustained long distance flights, they mostly move extensively within fields to locate mates (Spencer *et al.*, 2009). Rotation-resistant *D. v. virgifera* populations have a tendency to move between fields more than rotation-susceptible populations (Spencer *et al.*, 2009).

Information on the potential dispersal of *D. v. virgifera* larvae is limited. However, it is known that dispersal is affected by density, moisture and macropores in the soil (Hibbard *et al.*, 2003). Hibbard *et al.* (2003) studied the post-establishment movement of these larvae. The proximity of roots from neighbouring plants, the amount of competition for the food source and possibly the soil structure and the quantity of favourable food sources in neighbouring plants are factors that influence the extent of *D. v. virgifera* larval movement (Hibbard *et al.*, 2003). Observations indicated that larvae moved from one plant to another, some larvae moved up to three plants along a maize row and that immigration across a 0.46 m row-spacing also occurred (Hibbard *et al.*, 2003). This information on behaviour is highly applicable in resistance management strategies where Bt maize with specific traits is used to control this pest.

Resistance of *D. v. virgifera* to insecticides and crop rotation led to the development of other control methods against these pests. One of these new methods was the development of insect resistant transgenic maize expressing *Bacillus thuringiensis* endotoxins (Chege *et al.*, 2009). Three products, each containing a different gene are currently available: the *Cry3Bb1* gene, from Monsanto, the *Cry34Ab1* + *Cry35Ab1* genes from collaboration between Dow Agro Sciences and Pioneer, and the modified *Cry3Aa* gene from Syngenta (Chege *et al.*, 2009). Companies that develop these transgenic products are required to develop and implement an IRM plan, such as the high-dose/refuge strategy (Chapter 2).

The importance of biological and ecological studies when developing an IRM plan, and considerations of unique aspects in the biology of a target pest is described below for *D. v. virgifera*. Larval movement of *D. v. virgifera* could influence the development of resistance. For example if *D. v. virgifera* larvae initially develop on Bt maize roots and later move to a nearby non-Bt plant, like in seed mixtures (Bt and non-Bt), they would be larger and therefore more tolerant to the toxin. This could result in an increased rate of development of resistance if heterozygotes survived exposure to the toxin (Hibbard *et al.*, 2003).

An undamaged transgenic plant in a mixed crop stand of Bt and non-Bt plants may attract *D. v. virgifera* larvae because of the comparatively higher mass of roots available compared to those of non-Bt plants that were initially attractive and damaged (Hibbard *et al.*, 2003). The same may occur in block or narrow-row strip plantings of Bt maize. If these larvae develop initially on non-Bt plants, they would likely be larger and more tolerant of the transgenic toxin. However, if a low-dose product results in the survival of susceptible larvae, movement of larger larvae onto transgenic roots from less suitable alternate hosts or highly damaged maize in a seed mix could actually increase product durability by producing additional susceptible insects from within the Bt field (Hibbard *et al.*, 2003).

Chege *et al.* (2009) as well as Meihls *et al.* (2008) observed that *D. v. virgifera* larvae that feed on alternate hosts before feeding on transgenic maize may have an enhanced ability to withstand the effects of the toxin and develop into viable beetles. For the same reasons the movement of larvae between weeds and transgenic maize could impact the IRM strategies currently used.

3.2.2. *Busseola fusca* (Lepidoptera: Noctuidae): biology, ecology and control with Bt crops

Busseola fusca is one of the most important pests of maize in southern Africa (Kfir, 1998). Larvae feed on the young leaves of the plant and later penetrate and tunnel into the stems (Kfir, 1998). This tunnelling weakens the stem and interferes with translocation of nutrients and metabolites in the plant. *Busseola fusca* can also utilize sorghum, pearl millet, sugarcane and other grasses as hosts (Harris & Nwanze, 1992). This stem borer has a number of indigenous African grasses as wild host plants. Haile and Hofsvang (2002) showed that although *B. fusca* prefer to oviposit on sorghum and its relatives, maize is more suitable for larval development than any of the other host plant. This can be ascribed to the higher nutritional value, less deleterious secondary metabolites and larger stalks that support the development of the insect (Haile & Hofsvang, 2002).

The life cycle of *B. fusca* is completed in seven to eight weeks under favourable conditions (Harris & Nwanze, 1992). During the maize growing season there can be up to three generations of *B. fusca*. Moths live for five to nine days and females lay their eggs during the three to four days after emergence (Fig 3.2(a)). Established larvae inside the stems of plants feed for three to five weeks before pupation (Fig 3.2(b)) (Harris & Nwanze, 1992). During winter months larvae enter diapause in the stubble where they spend approximately six months before pupating during the next favourable period (Fig 3.2(c)). The adult emerges 9-14 days later to complete the cycle (Fig 3.2(d)).

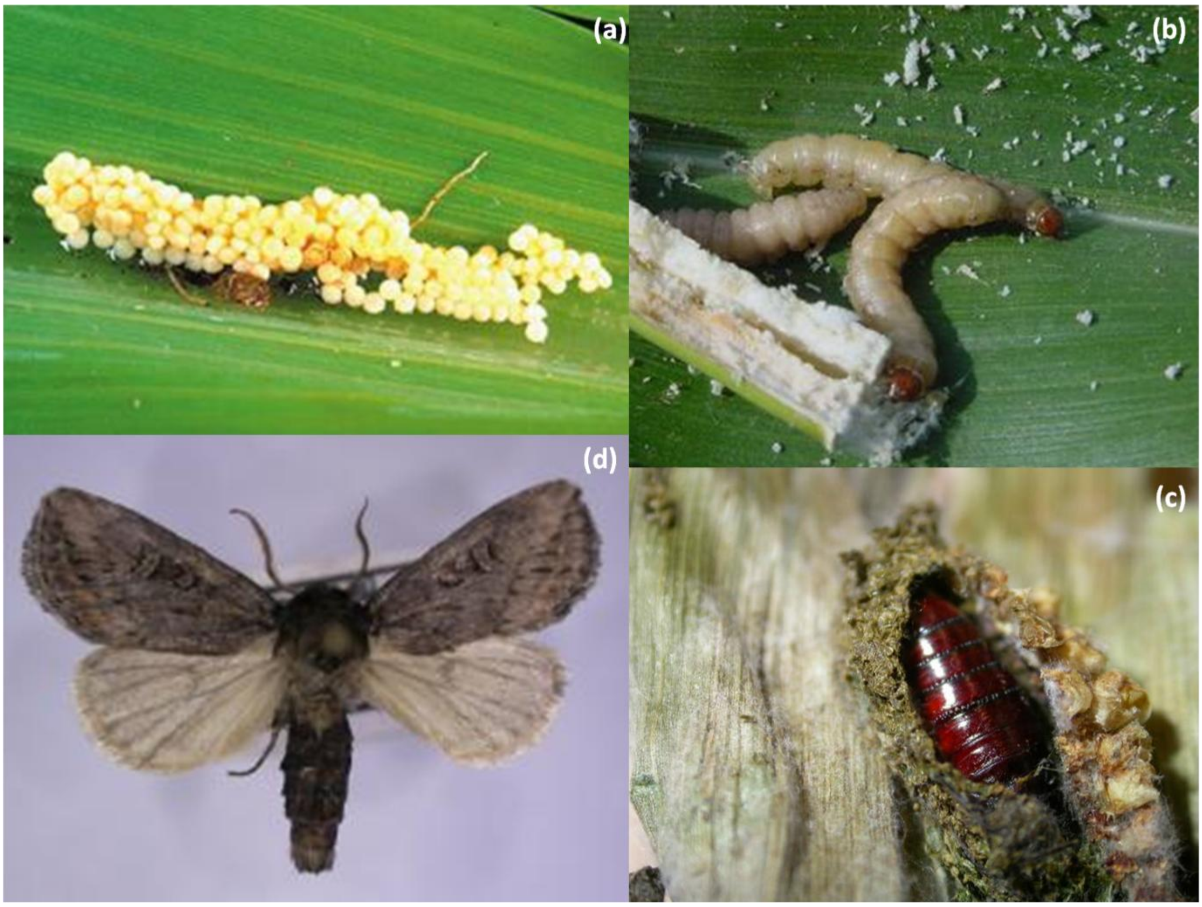

Figure 3.2. The life cycle of *Busseola fusca*. (a) Eggs; (b) larvae; (c) pupa and (d) adult moth.

Larvae may leave the stem that was initially attacked, especially if it was severely damaged (Harris & Nwanze, 1992). Adult movement of *B. fusca* has not been studied, and until recently it was assumed that their dispersal is thought to be mainly local. They have an area of dispersal of up to 1.6 km and occasionally further (Turner *et al.*, 2011). In a study by Mbugi (1999) it was found that *B. fusca* adults have a high dispersal rate and uniformly disperse their eggs.

Large-scale planting of Bt maize and cotton in South Africa began during 1998. The Bt maize containing the *cry1Ab* gene was released to specifically control the stem borers *B. fusca* and *Chilo partellus* (Lepidoptera: Crambidae). Field resistance of *B. fusca* to Bt crops was reported in 2006 by Van Rensburg (2007) in the Christiana area. A follow-up study during the 2008/09 season confirmed field resistance approximately 50 km from the original site (Kruger *et al.,* 2011a).

A study by Kruger *et al.* (2009) showed that poor refuge compliance in this area could have been the main reason for resistance development. The larval migration that occurs can also influence the efficacy of Bt maize. If Bt and non-Bt plants are planted too close to one another, for example in block refuges or in seed mixtures, larvae could ingest varying concentrations of the toxin reducing the efficacy thereof (Gould, 1998). The development of field resistance reinforces the need for Bt events that express a high dose of one or more toxins, that resistance management practices are carefully developed and that the implementation of these management practices be monitored.

3.3. Polyphagous insect pests

3.3.1. *Helicoverpa armigera* (Lepidoptera: Noctuidae): biology, ecology and control with Bt crops

Helicoverpa armigera is a major agricultural pest in South Africa and worldwide. This species have a wide distribution and even wider crop host range (Fitt, 1989). Consequently, the literature on its ecology, biology and management is vast. This species is highly polyphagous and attacks a wide range of crops including maize, sorghum, sunflower, cotton, tobacco, soybean, pulses, rapeseed and groundnut (Fitt, 1989). This can be ascribed to the larvae having a feeding preference for plant parts that are high in nitrogen (Hardwick, 1965). These are typically the reproductive structures and growing points of the plant.

On cotton, *H. armigera* eggs are laid on the leaves of the host plant, mostly on the top third of the plant (Fig 3.3(b)). On maize, eggs are laid on ears and the upper parts of plants after flowering. The eggs hatch about four days later. Larvae feed on tender young foliage for 1-2 days, and then move to feed on buds, flowers or young pods, bolls or fruits (Fig 3.3(c)). Two to three weeks after egg hatch the larvae migrate to the base of the plant, tunnel a few centimetres into the soil and pupate (Fig 3.3(d)). After 10-16 days the moths emerges, ready to mate, lay eggs and start the next cycle (Fig 3.3(a)) (Anon., 2006). One *H. armigera* generation is completed in 40-45 days. Four to five generations can thus occur within one growing season (Anon., 2007).

Figure 3.3. The life cycle of *Helicoverpa armigera*. (a) Adult moth; (b) eggs; (c) larva and (d) pupa.

Helicoverpa armigera have several physiological, behavioural and ecological characteristics that enable them to survive in unstable habitats and in turn to colonize and exploit agricultural systems successfully. These characteristics are polyphagy, high mobility, high fecundity and a facultative diapause (Fitt, 1989).

There are three ways in which polyphagy influences the population dynamics and pest status of *H. armigera*: a) populations may develop simultaneously on a number of hosts within a region; b) populations may develop continuously during suitable periods by exploiting a succession of different cultivated and wild hosts through the season; and c) populations can persist at low density in seemingly unsuitable areas, since females have a high probability of locating a host able to sustain larval development (Fitt, 1989).

Although *H. armigera* is polyphagous, it does show preferences for particular hosts. These preferences depend on the availability of these hosts at the preferred stage of development (Fitt, 1989). Firempong and Zalucki (1990) observed that moths from different populations preferred tobacco and sunflower to cotton. *Helicoverpa armigera* would therefore only attack cotton when the other hosts become unattractive and less suitable.

The ability of *H. armigera* to undertake extensive local and inter-regional movements is a major factor contributing to the success of these insects as pests (Fitt, 1989). Moths migrate in response to poor local conditions for reproduction (shortage of adult nectar sources or larval hosts) and the passage of weather systems conducive to such movements (Fitt, 1989). The movements of *H. armigera* can be divided into two phases, i.e. larval and adult movement, which both need to be taken into account in the development of IRM strategies.

Moths are nocturnal and three categories of movement were identified by Farrow and Daly (1987) namely short-range, long-range and migratory movements. Short-range movements involve physiological needs such as feeding, oviposition, mating and sheltering and usually takes place just above the host canopy (Fitt, 1989). Long-range migration occur higher above the host canopy and involve less frequent responses to external stimuli. These flights include movements between crops, movements between feeding and oviposition sites and between moth emergence and local oviposition sites (Fitt, 1989; Farrow & Daly, 1987).

Migration movement occurs high above the host plants/canopy, even up to 1-2 km. This makes downwind displacement of hundreds of kilometres of these insects possible. During these migrations the appetitive responses are suppressed that might otherwise arrest the insect (Fitt, 1989). There is little information available on: a) the proportion of emerging populations of *H. armigera* that may undertake migratory as opposed to local movements; b) how this proportion is affected by weather conditions or other factors; and c) how and when newly emerged adults assess local conditions prior to moving (Fitt, 1989).

Zhang *et al.* (2004) studied the feeding behaviour of *H. armigera* larvae on transgenic and non-transgenic cotton and reported that neonate larvae had the ability to detect and avoid transgenic cotton. This characteristic which could benefit larval survival is, however, not of much value since the moth chooses the host plant. Larval migration from an unsuitable host plant such as Bt maize or cotton will not be of much value if Bt crops are planted in a monoculture and no other host plants are available. However, if the “refuge-in-a-bag” strategy is used, the ability of larvae to avoid a transgenic plant may ensure its survival. *Helicoverpa armigera* larvae have a tendency to move around rather than remain on one location. Forty-seven to 58 % of the larvae in the study done by Zhang *et al*. (2004) moved to more than one cotton bud to feed. In the field, even with their preferred boll, *H. armigera* tend to damage more than one bud.

If Bt and non-Bt cotton plants are grown in close proximity to one another in for example seed mixtures, it is possible that a proportion of *H. armigera* larvae would move between these plants (Zhang *et al.*, 2004). Therefore where *H. armigera* is a target pest, separate refuge areas for resistance management is a better option than seed mixtures. In seed mixtures it is difficult to maintain pure susceptible and resistant genotypes, which could lead to increased numbers of heterozygotes and subsequent faster resistance development.

Liu *et al.* (2008) confirmed that resistance has developed in *H. armigera* to Bt cotton in China. They found that the resistance allele frequency increased significantly since the commercial release of Bt cotton in the area. Precautions need to be taken to ensure the success of Bt cotton. Resistance management strategies, such as the high-dose/refuge strategy (Chapter 2), are thus necessary.

3.3.2. *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae): biology, ecology and control with Bt crops

Leptinotarsa decemlineata, which does not occur in Africa, is the most important and destructive pest of potato (*Solanum tuberosum*) in North America (Hare, 1990). These insects feed on approximately 20 species in the plant family Solanaceae, including cultivated tomato (*Lycopersicon esculentum*), cultivated eggplant (*Solanum melongena*) and peppers (*Capsicum* sp.). Beetles emerge during spring after completing diapause in the soil (Fig 3.4(a)). After emergence they feed on nearby host plants where mating also takes place. Females lay eggs on the underside of host plant leaves (Fig 3.4(b)). The eggs hatch 4-10 days later and larvae immediately start feeding on host plant leaves (Fig 3.4(c)). Young larvae tend to remain near the area where the egg mass was deposited but begin to move to other parts of the plant as the leaf is consumed (Ragsdale & Radcliff, 2011). There is a lack of information on the movement of *L. decemlineata* larvae, although it seems that they do not move away from the plant where the eggs were laid. After three weeks of feeding, the larvae drop down from the host plant and burrow into the soil where they pupate (Fig 3.4(d)). About ten days later the adults emerge to complete the lifecycle. Depending on latitude these insects can complete one to three generations in a year (Jacques Jr. & Fasulo, 2000).

Figure 3.4. The life cycle of *Leptinotarsa decemlineata*. (a) Adult beetle; (b) eggs; (c) larva and (d) pupa.

Leptinotarsa decemlineata has a complicated and diverse life history, well suited to agricultural environments. These beetles employ a ‘bet-hedging’ reproductive strategy to distribute its offspring. The bet-hedging theory addresses how individuals optimize their fitness in a variable and unpredictable environment (Olofsson *et al.*, 2009). Beetles use migration together with diapause, feeding strategies and reproduction strategies to employ bet-hedging (Alyokhin & Ferro, 1999). The adult *L. decemlineata* beetles are very mobile. They start walking soon after eclosion, but need to feed for 5-10 days for flight muscle development to be completed where after they can easily fly several kilometres (Alyokhin & Ferro, 1999).

Three types of flight can be distinguished in *L. decemlineata* adults namely short-range, long-range and diapause flights. Short-range or local flight is a low altitude flight which occurs within the host habitat. The purpose of these flights is to find a mate or distribute eggs. Long-range or migratory flights are at a higher altitude and may be several hundred meters long. This type of flight is used to colonize new areas. The third type of flight, the diapause flight, is also a low altitude flight where the beetles fly to wooded sites or uncultivated fields where they burrow into the soil to diapause (Alyokhin, 2009).

These insects also have the ability to develop resistance to pesticides very quickly. There are over 30 active ingredients registered for use against this pest in the USA (Alyokhin, 2008). In North America the use of GM potatoes expressing the *Bacillus thuringiensis* Cry3A toxin is used to control the insect. However, increased selection pressure can lead to resistance development. The high-dose/refuge strategy is currently imposed to delay the development of resistance.

Alyokhin and Ferro (1999) studied the reproduction and dispersal of summer-generation beetles. They observed that some of the beetles moved away from the site of larval development before completing maturation of the reproductive system and flight muscles. A significant proportion of the beetle population, however, remains in the vicinity of emergence sites (Alyokhin & Ferro, 1999). After maturation and subsequent mating the beetles would then disperse to other areas. There is thus a high probability that resistant beetles will mate with each other before dispersing from the area of their larval development. This could compromise the IRM strategy since the beetles would most likely not mate with individuals originating from non-Bt crops.

Hoy (1999) suggested that the high-dose/refuge strategy for *L. decemlineata* on Bt-potato should also include that the Bt potatoes be vine-killed first, so that any surviving beetles will move to the non-Bt potatoes to mate with susceptible beetles. This practice of spraying potatoes with herbicide prior to harvesting therefore acts as a push-pull mechanism to manipulate beetle behaviour. Beetles are “pushed” from the Bt potato crop of which the foliage is dying while they are also attracted to the available foliage of the non-Bt potato refuge. This should ensure the survival of a susceptible beetle population and thus delaying the development of resistance. However, Nault (2001) showed that harvesting the non-Bt refuge potatoes at least two weeks after first-generation beetles emerge will not enhance selection of the population for resistance after immigration into Bt fields because adults are not likely to resume feeding, but they would have had sufficient time to ingest enough non-Bt foliage to enable them to overwinter successfully. If the Bt-susceptible insects mate before entering the Bt field the Bt-resistant individuals will most likely mate with each other producing homozygous resistant offspring (Nault, 2001).

3.4. Conclusion

All of the insects discussed above can be controlled by Bt crops. Bt maize is used as a control method for *D. v. virgifera* in the USA and *B. fusca* and *H. armigera* in South Africa, although *H. armigera* is not a target pest on maize. Bt cotton is also used to control *H. armigera* and Bt potato controls *L. decemlineata*. For all of these pests the refuge requirements are largely uniform (Chapter 2).

In table 3.1 a comparison is drawn up between the different pests mentioned. The differences in their behaviour are clear, as well as the similarity in IRM strategies currently imposed. In conjunction with that, figure 3.5 visually shows the differences in dispersal and specificity between these pests.

Table 3.1. A summary of the characteristics of the pests described above and the implications on IRM.

	Monophagous pests		Polyphagous pests	
	Diabrotica virgifera virgifera	*Busseola fusca*	*Helicoverpa armigera*	*Leptinotarsa decemlineata*
Behaviour and ecology				
• Larval migration	Less	Extensive	Extensive	Less
Feeding	Extensive, roots	Extensive, stalks	Extensive, reproductive structures	Extensive, leaves
• Adult migration	Short & long distance movement	Mainly local, up to 1,6 km	Extensive local & inter-regional movement	Extensive, several kilometres
Feeding	Less, leaves	None	None	Less, leaves
• Lifecycles per season	One	Up to three	Up to five	Up to three
• Distribution	North America, Europe	Southern Africa	Africa, Europe, Asia, Australasia	North America, Europe, Asia
Control methods	Crop rotation, insecticides, Bt maize	Trap cropping, insecticides, Bt crops	Insecticides, Bt crops	Insecticides, Bt crops
Resistance				
• Insecticides	11 active ingredients[1]	N.a.	43 active ingredients[1]	51 active ingredients[1]
• Bt crops/toxins	Confirmed[2]	Confirmed[3]	Confirmed[4]	None
IRM				
• Current	20 % non-Bt maize, adjacent	20 % non-Bt maize, sprayed; or 5 % non-Bt maize, unsprayed	20 % non-Bt cotton, sprayed; or 5 % non-Bt cotton, unsprayed	20 % non-Bt refuge
• Preferred	Separate field non-Bt refuge	Separate field non-Bt refuge	Separate field non-Bt refuge	Non-Bt refuge strips

[1]Whalon *et al*., 2011; [2]Gassmann *et al.*, 2011; [3]Van Rensburg, 2007; [4]Liu *et al.*, 2008.

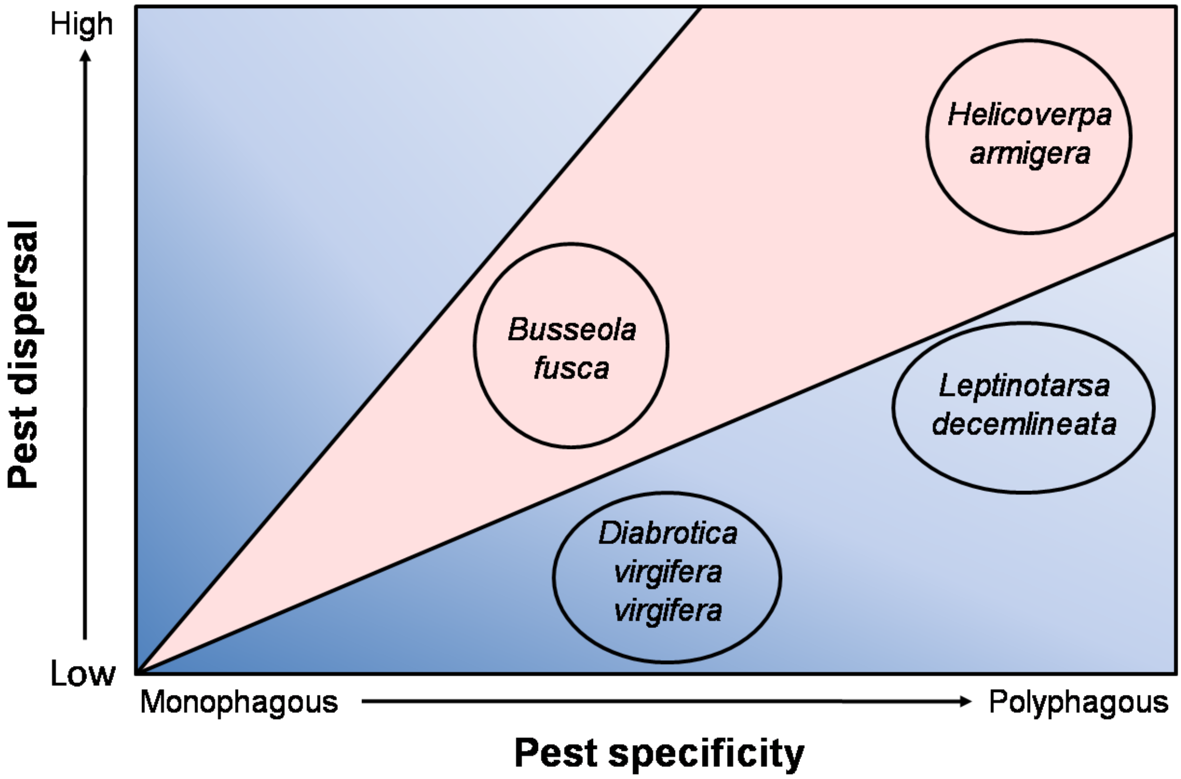

Figure 3.5. A graphical presentation to illustrate variation in the host specificity and dispersal capabilities of the pest species described above (Figure was adapted from Ratnadass *et al.*, 2010). Of the insects mentioned, *Helicoverpa armigera* is the species with the highest pest status at regional levels because of its ability to disperse greatly and extremely polyphagous host range. On the other hand *Diabrotica virgifera virgifera* has high pest status at more localized levels and IRM strategies should take into its narrow host range and low dispersal capabilities.

The spatial arrangement of the refuge area depends on the biology of the target pest. Within-field mixtures of Bt and non-Bt plants are not the best type of refuge for insects that move between plants during development, such as *B. fusca* and *H. armigera*. Separate refuge fields would thus better suit the biology of these target pests. These separate refuge fields may also not be too far from the Bt field, or else there might not be random mating between resistant and susceptible insects. In this case it is also important to know how far the adult insects will move before mating. The differences in life stage behaviour of these insects make a standard or "one size fits all" refuge requirements very unpractical. Not accounting for these differences in IRM may even contribute to the rate resistance development in some species.

Chapter 4

Knowledge gaps and relevance of current insect resistance management strategies to African farming systems

4.1. Introduction

Genetically modified (GM) crops have been grown since 1996 and can be herbicide tolerant, insect resistant or both. South Africa was the first African country to commercialize GM crops, followed by Burkina Faso and Egypt (James, 2010). The GM crops available for insect control express *cry* genes from the bacterium *Bacillus thuringiensis* (Bt). These genes produce target specific insecticidal proteins that protect the plant against target pest attacks throughout the growing season. The largest threat to the continued success of these insecticidal GM crops is the potential development of target pest resistance in the field (Ferré *et al.*, 2008). Several insect resistance management (IRM) strategies have been suggested to delay the development of resistance. The strategy currently mandated for all Bt crops is the high-dose/refuge strategy (USEPA, 1998; 2001). This strategy has been discussed in detail by Renner (1999), Gould (2000) and in Chapter 2 of this dissertation.

Increased selection for resistance against both insecticides and insecticidal GM crops necessitates IRM to be an essential component of product stewardship (Head & Savinelli, 2008). For an IRM strategy to be effective, the nature of the pest system as well as characteristics of the insecticides or GM crops needs to be considered to devise appropriate tactics. Local farming conditions and capabilities need to be analysed to understand the risk of resistance development in a particular system and how it may be mitigated (Head & Savinelli, 2008). The design of any IRM strategy should consider constraints imposed by the nature of different agricultural systems, human behaviour and economics (Forrester, 1990).

4.2. Developing effective IRM programs

The efficacy of an insecticide application is largely determined by complex interactions between insects and insecticides (Denholm & Rowland, 1992). Most of these interactions are beyond our control and concerns ecology, genetics, behaviour and physiology of the target pest. Only operational factors, such as the insecticide used, the area coverage and the timing, rate and method of application, are within our control. In order use chemicals in such a way that it delays the development of resistance it is essential to understand the parameters influencing the process of selection of resistant individuals. These parameters include the frequency and dominance of resistance alleles, the generation turnover and the offspring per generation (Georghiou & Taylor, 1977a). These factors can then be used in development of applicable strategies to reduce ecological pressure selecting for resistance (Denholm & Rowland, 1992).

The aim of an IRM program is to reduce selection pressure caused by an insecticide or GM crop so that the rate at which the target pest will evolve resistance is reduced. In order for IRM programmes to be successful, they have to be technically effective, feasible and economical (Head & Savinelli, 2008). However, even a carefully designed IRM program can fail if it is not properly implemented. In the case of *Diabrotica virgifera virgifera* insufficient refuge populations and the planting of Bt crops for up to three consecutive years was crucial in the development of resistance to Bt crops (Gassmann *et al.*, 2011). Farmer behaviour in executing of IRM programs is therefore key to successful implementation. It is especially in developing countries where challenges the implementation of IRM strategies. The greatest logistical challenges in practicing IRM arise in areas which are dominated by smallholders, such as Africa, Asia and Central America (Head & Savinelli, 2008).

The importance of farmer perceptions of new technologies and their attitude towards product stewardship play a significant role in IRM. Kruger *et al.* (2009, 2011b) studied farmers' perceptions and compliance to refuge requirements in South Africa and illustrated the impact that farmer behaviour has in IRM. They found that farmers in the Vaalharts area of South Africa benefited greatly from Bt maize which controlled the African stalk borer (*Busseola fusca*) (Lepidoptera: Noctuidae), and that farmers considered Bt maize to be an environmentally friendly and economically worthwhile pest control strategy. However, farmers also indicated that they did not comply to refuge requirements during the first eight years after the release of Bt maize (Kruger *et al.*, 2009). This low level of compliance most likely contributed to resistance evolution of the target pest in that area. In a follow-up survey which covered a large area of the maize production region of South Africa, similar low levels of compliance was observed (Kruger *et al*., 2011b).

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 with (Tabashnik *et al*, 2008). Low levels of expression of Bt-protein in plants could contribute to 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. Low expression levels have been mentioned in some cases of resistance development as a contributing factor (Gassmann *et al.*, 2011), but this has not been proven yet.

Information about the agricultural system, the biology of the target pest, the behaviour of growers and general local information about product performance is needed to design and implement region-specific IRM programs (Head, 2010). An attempt is made below to integrate information provided in this dissertation into relevant aspects of IRM.

4.2.1. The cropping system

Questions that need to be asked concerning the cropping system include: Is the crop grown in monoculture or polyculture? What is the approximate farm size? What pest control methods are used for the target pest? What level of product adoption is expected? Do transgenic crops already exist in this crop or in other crops in the same agricultural system?

As indicated by Head (2010) it is important to know current management practices employed for control of target pests as well as non-target pests since that could impact on the efficacy of IRM strategies. In South Africa chemical control was the norm for control of the target stem borer species on maize before the advent of Bt maize. Application of chemical control measures against target pests was not supposed to be done on Bt maize and this should be discouraged and emphasised during in farmer training with introduction of GM technology. An alarming number of farmers in South Africa still use conventional insecticides on Bt maize and refuge areas (Kruger *et al.*, 2011b). A large proportion of farmers reported that significant stem borer infestations on Bt maize started to occur approximately eight years after its release. Kruger *et al*. (2011b) reported that between 5 – 93 % of 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. Kruger *et al.* (2011b) concluded that farmers as well as chemical- and seed companies showed irresponsible management of GM crop technology especially during the first decade after the release of Bt maize in South Africa.

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 aspects of IRM in developing agriculture in South Africa. Since the introduction of GM crops into Burkina Faso and Egypt was only done during 2010, no data exist on compliance to refuge requirements in those countries. Research on farmer perception and behaviour as well as monitoring of compliance and GM crop adoption rates at local and regional levels is of critical importance in countries into which GM crops have been introduced (South Africa, Burkina Faso and Egypt). There is huge pressure on African governments to release GM crops, in spite of lack of knowledge regarding the abovementioned aspects. Care should be taken before introduction that farmers and all relevant stake holders understand management requirements prior to release of GM crops. Incorrect management of this GM technology could result in rapid evolution of resistance and/or development of secondary pests of which farmers may be unable to control.

4.2.2. The biology of the target pest

Some of the questions that need to be answered regarding the biology of the target pest are: How many generations per year and how many of these are on the crop of interest? What is the basic life cycle of the insect? Does the insect use wild or cultivated hosts other than the crop of interest as a host? 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 the insects have a history of developing resistance to insecticides and Bt formulations? Are transgenic crops used to control this species in this crop or other crops in the same agricultural system?

Theoretical studies indicate that, under certain conditions, combinations of pesticides are the most effective strategy at delaying resistance development (Tabashnik, 1989). These studies, however, focused on the effects of the control tactic on the target pest, but in agro-ecosystems the effects on non-target pests and beneficial organisms should also be considered (Tabashnik, 1989). The importance of non-target organisms, such as natural enemies should always be kept in mind as they are also an important component of any IPM strategy.

Information on the biology of target pests and the differential effects that exposure to Bt and non-Bt plants may have on a particular pest species is important in development of effective IRM strategies. For example, Liu *et al.* (1999) found that a resistant strain of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) takes longer to develop on Bt cotton than a susceptible strain on non-Bt cotton. This asynchrony would result in assortative mating which would generate a disproportionately high number of homozygous resistant insects accelerating the evolution of resistance (Liu *et al.*, 1999; Tabashnik, 1994b). Similar results were observed by Kruger *et al*. (in review) who reported a difference of approximately seven days in the development rate of male insects on Bt and non-Bt maize. These authors also indicated that this asynchrony would most likely result in increased incidence of mating between male and females originating from the Bt crop.

Insects may move between toxic and non-toxic parts which may counteract the resistance-related advantages of selective Bt expression (Bates *et al.*, 2005). There is no way to be certain of the concentration of toxin that the insects ingest. For instance if toxic and non-toxic plants are planted too close to one another, individuals of the target pest could move between plants and ingest plant tissues with containing sub-lethal concentrations of the toxin (Gould, 1998). Differences in protein expression levels in different plant parts may also contribute to increased survival of larvae on Bt plants, especially late in the season. Increased infestation levels of the African bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) was reported to be a regular occurrence in Bt cotton in the USA (Wu & Guo, 2005) and Australia (Fitt, 2004). In South Africa, where target pest resistance was first reported in 2007 (Van Rensburg, 2007), differences in survival of *B. fusca* on different parts of maize and survival under field conditions were already reported in 2001 (Van Rensburg, 2001).

Wild host plants are often referred to as a potential component of an IRM strategy since these wild hosts could function as a non-structured refuge which sustains susceptible individuals of the target species. Wild host plants are often regarded as alternative hosts of the pest species during the non-cropping season and they could play an important role in pest ecology in maize agro-ecosystems. In the past, because of the general perception that stem borer pest species such as *B. fusca* and *Chilo partellus* (Lepidoptera: Crambidae) were common in wild host plants, removal of these plants adjacent to cereal fields was often recommended as part of IPM strategies (Seshu Reddy, 1983; 1990). Removal of wild hosts would therefore prevent early infestation of cultivated crops in the following season. However, recent surveys indicated that wild host plants play an insignificant role as a carry-over reservoir for economically important species (Ong'amo *et al*., 2006a; Oteino *et al*., 2008). Fewer than 10 % of larvae survive on wild host plants as opposed to 20-30 % on cultivated crops (Ong'amo *et al*., 2006a). Only 0.06% of the total number of stem borers collected in wild host plants during country-wide surveys in South Africa between 2005 – 2010, was found to be economically important species (*B. fusca*, *C. partellus* and *S. calamistis*) (Moolman, Van den Berg and Le Rü, unpublished data). The above mentioned reports indicate that large gaps exist in information 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).

The extensive survey of the diversity of lepidopteran stem borers on monocotyledonous plants in eastern Africa, conducted by Le Rü *et al*. (2006) reported 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. Careful consideration of the host exploitation strategy (monophagous vs. polyphagous) as well as the actual importance and presence of wild hosts at a regional scale should be done during development of IRM strategies. This is especially important in areas such as Africa where the “presence of wild host plants” seem to be a serious consideration as part of the IRM strategy, in spite of the fact that no basic information is available on these topics for most of the continent.

The misconception that wild host plants will be solution to IRM problems does not seem to get through to even leading scientists which are active in the field or GM crops and IRM in Africa. For example, a recent study of IRM possibilities in Kenya before the introduction of Bt crops in that country still described the value of wild hosts in stem borer IRM (Mulaa *et al.*, 2011). These authors reported that wild sorghum species (*Sorghum versicolor* Anderson and *Sorghum arundinaceum* Stapf) as well as Napier grass (*Pinnesetum purpureum* Schumach) were suitable refuge options and that these wild hosts could easily be incorporated into an IPM program. These authors went as far as to indicate that the presence of natural refugia in some areas was sufficient enough not to require additional planting of refugia (Mulaa *et al.*, 2011).

For polyphagous pests such as *H. armigera*, the possibility of using other crops as refuges for Bt cotton seems viable. A study of the population dynamics of *H. armigera* in China showed that other crops can be used as refuge for *H. armigera* in Bt cotton growing areas (Shengjiang *et al.*, 2001). However, the inter-planting of maize and Bt cotton may impact negatively on the release of Bt maize in the future. This is because Bt cotton and Bt maize expresses very similar toxins, which will result in exposure of the majority of the pest population in an area to similar *cry* toxins, even if different generations of the pest alternate between crops. This continued exposure may accelerate the development of resistance in *H. armigera* populations (shengjiang *et al.*, 2001). The use of other plants as refuge was approved by the USEPA in areas where Bollgard® II cotton, producing the Cry1Ac and Cry2Ab toxins, is planted. In certain eligible regions of the USA, cotton producers can now take advantage of non-cotton crops and other plants as refugia for certain pests and are not required to plant a non-Bt cotton refuge for Bollgard® II cotton (USEPA, 2007). Refuge requirements for Bt maize grown in the ‘corn-belt’ in the USA are a 20% non-Bt field maize refuge, and 50% non-Bt maize refuge for Bt maize grown in cotton-growing areas. This approach was described as scientifically-sound, protective, feasible, sustainable, and practical to growers (USEPA, 2001).

Nibouche *et al.* (2007), through the use of models, illustrated that in the absence of a non-Bt cotton refuge and with Bt maize occupying 75% of the cropping area in West Africa, that the period to resistance development of *H. armigera* could be relatively short. This demonstrates that non-cotton host plants such as Bt maize cannot act as refugia and that non-Bt cotton refugia must be set up to ensure the sustainability of Bt cotton crops in this area. The contribution of wild host plants and weeds towards population numbers of polyphagous pests such as those in the bollworm complex of cotton was illustrated by Green *et al*. (2003). The latter authors indicated that several species of indigenous plants and weeds in the cotton producing area on the Makhathini Flats area of South Africa served as natural refugia for species of the bollworm complex. These species were *H. armigera* and *Diparopsis castanea* (Lepidoptera: Noctuidae). Green *et al*. (2003) further indicated that bollworm numbers on weeds were similar to those on non-transgenic cotton. However, the studies done by Nibouche *et al.* (2007) in west Africa showed that gene flow between *H. armigera* populations attacking cotton and vegetables only occurs during long range migrations at the end of the rainy season and that it was not sufficient to allow vegetables to play the role of refuges required by the high-dose refuge strategy (Nibouche *et al.*, 2007). Whether mating between bollworm moths originating from wild host plants and those that originate from crops is random, which is a requirement for effective refuge strategies, is not known.

The quick development and spread of resistant pest populations observed in the case of in South Africa (Kruger *et al*., 2011a) is difficult to explain and highlights the knowledge gaps in the biology of even the most important insect pests. The distance that moths move prior to and after mating as well as whether they mate before or after a significant first flight away from the eclosion site, is also unknown. This data on target pest species could provide valuable information that could assist in improved IRM strategies. These aspects and knowledge gaps were also highlighted in the ecological risk assessment done 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. However, 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 this 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).

4.2.3. Grower behaviour and attitudes

There are a few questions that need to be answered about the behaviour and attitudes of growers: 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?

The act of pest management drives the evolution of pest resistance, because pest management is fundamentally a human activity in agricultural production. Human behaviour is just as important to consider as pest biology and behaviour when investigating resistance development (Hurley & Mitchell, 2008). For individual farmers IRM is more of a public concern and no real incentive exists to manage it properly on their own (Clark & Carlson, 1990). Therefore institutions, like the United States Environmental Protection Agency (USEPA), have formulated and are implementing IRM policies in order to promote proper pest and resistance management (Hurley & Mitchell, 2008).

One of these strategies is the high-dose/refuge strategy (Chapter 2) to manage resistance developing in genetically modified crops. The USEPA oblige farmers to plant conventional crop varieties (refugia) along with the transgenic varieties and also prescribes spatial arrangement of refugia in relation to transgenic varieties. This is implemented by contractual agreements between the seed companies and farmers (Hurley & Mitchell, 2008). The additional risk of pest damage and added management responsibilities with planting of refugia, however, provides an incentive to ignore these requirements. Farmers are generally reluctant to employ the prescribed IRM strategies and in the case of South Africa companies, did not emphasize and enforce compliance to refuge strategies during the seven year period after release of Bt maize (Kruger *et al.*, 2009). Since compliance to refuge requirements in South Africa 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 (Kruger *et al.*, 2009, Kruger *et al.*, 2011b).

To be accepted by farmers, the IRM strategies must be compatible with existing cropping systems and, where relevant, the alternative crops used as refuge must be economically viable and socially acceptable. It should also be easy to implement, otherwise farmers may ignore or modify it, thereby reducing its effectiveness (Mulaa *et al.*, 2007; 2011). Continuous surveys will give reasons for non-compliance so that modifications can be made to improve IRM plans and the information can also be used to design further education programs (Mulaa *et al.*, 2011).

Most of African agriculture remains traditional, land holdings are small and crop production is labour intensive. Arthropod pests are also one of the major constraints to African farming systems (Abate *et al.*, 2000). The effective management of these pests and also delaying the development of resistance of these pests to control methods is of utmost importance. Small scale farmers in Africa generally still use traditional control methods such as crop rotation, intercropping or specific responsive actions to reduce pest attacks (Abate *et al.*, 2000). The use of Bt technology will obviously reduce these control methods significantly. For an IRM strategy to be effective in these systems it should fit into this traditional setup. Since these farmers effectively manage a mixed cropping system, the planting of refuge crops should fit into their practices reasonably well.

Farmers therefore need to be educated with regard to management of transgenic maize and regulatory requirements in order to delay the evolution of resistance. In countries where GM crops are not yet cultivated, sensitisation with regard to IRM should be done and systems developed to ensure good stewardship. If farmers are not educated in this regard, it may compromise other GM insecticidal crop events that may be introduced into the country in future. Farmers should also 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.

4.2.4. Product performance

Questions that need to be answered concerning the performance of the product are: 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 insecticide applications still be needed on the Bt crop for the target pest?

During the past decade Bt maize was perceived to provide effective control of stem borers in the Vaalharts area and in the Highveld region in South Africa before resistance became a problem in several areas (Kruger *et al.*, 2011a). Since the level of resistance to Cry1Ab (MON810) 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 (pyramid genes) is currently the only viable alternative option.

It is predicted that stacked genes will result in a significant delay in development of resistance in insecticidal GM crops. Theoretically, if resistant individuals are rare where one toxin is involved, then resistant individuals where two toxins are involved would be extremely rare (Bates *et al.*, 2005). This strategy requires that the initial frequency of resistance genes is low and that cross resistance does not occur (Ferré *et al.*, 2008).

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 ensure the effective management of IRM strategies so that farmers can continue to benefit from the advantages of Bt crops.

4.3. Conclusion

The information available with regard to resistance management in Bt crops mostly originates from laboratory and small-plot research (McGaughey *et al.*, 1998). Large scale experimentation is needed to attempt implementation of resistance management plans over large areas and in different farming systems. More information about the following topics can aid in the development of area-specific IRM strategies:

- Pest genetics and resistance mechanisms.
- Cross-resistance among Bt toxins and cross-resistance to unrelated toxins.
- Limited understanding of the behaviour and biology of pests.
- Resistance risk assessment in specific crops and specific geographical areas.
- The practical aspects of implementing and managing refuges.
- Effects of transgenic crop varieties on pest population levels.
- Development of methods for early detection of resistance.

Chapter 5

Recommendations and conclusions

The development of field resistance of target pests to GM crops has highlighted the necessity for effective IRM strategies. It was therefore necessary to investigate the development of the refuge concept and its application in IRM, to document new developments in this field and to record current insect resistance management strategies suggested for crops/pests relevant to Africa. Not only will this information facilitate appropriate risk assessment for GM crops but it will also be assessed for its relevance and applicability to both commercial and small-scale farming systems in Africa.

As with conventional insecticides the best way to prolong the efficacy of insecticidal proteins produced by transgenic crops is to use them in conjunction with alternative control methods and thereby minimize the exposure of pests to toxins. Insect resistance management would be most effective and sustainable when it is regarded as part of an integrated pest management program (Onstad, 2008; McGaughey & Whalon, 1992; Glaser & Matten, 2003). Since small-holder African farmers rely hugely on pest control methods other than insecticides, the implementation of such an IRM strategy should be feasible. Onstad (2008) indicated four factors on which the above mentioned statement is based:

- a diversification of causes of mortality so that a pest is not selected by a single mechanism;
- a reduction of selection pressure for each mortality mechanism;
- maintenance of a refuge or promoting immigration to promote mixing of susceptible and resistant individuals and
- prediction using monitoring and models.

The high-dose/refuge strategy is currently the IRM strategy used in most of the world, including South Africa. Most of the refuge requirements are the same for different crops and do not take into account the wide range of pest species and large differences in biology. The target pests of specific Bt crops also differ between countries. Although the development of Bt crop cultivars that produce specific Cry proteins is largely done with North American pest species in mind, these cultivars are also deployed to other regions of the world if found effective against similar pests of these crops.

The approach to have a similar management strategy for different pest species on one crop is illustrated through the examples referred to below. For example, the target pests for Bt maize containing the *cry1F, cry34Ab1* and *cry35Ab1* genes (Herculex® XTRA), are *D. v. virgifera*, *Diabrotica barberi* (Coleoptera: Chrysomelidae) and *Diabrotica virgifera zea* (Coleoptera: Chrysomelidae) (USEPA, 2010). Cullen (2010) on the other hand listed *O. nubilalis*, *D. v. virgifera*, *S. frugiperda*, *Agrotis ipsilon* (Lepidoptera: Noctuidae) and *Richia albicosta* (Lepidoptera: Noctuidae) as target pests. Other pests listed, in addition to those indicated above, are *Diatraea grandiosella* (Lepidoptera: Crambidae), *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae), *Diatraea crambidoides* (Lepidoptera: Crambidae) and *Diatraea saccharalis* (Lepidoptera: Crambidae) (Pioneer, 2011). For all of these pests and in some cases crops also mentioned, the refuge requirements are mostly uniform. The large differences in life history strategies of different pest species as well as the fact that even different life stages of pests are subject to the identical IRM strategies, leads to the realization that one strategy cannot serve all and that more pest-specific management strategies should be developed.

The spatial arrangement of the refuge area should take into account the biology of the target pest. Within-field mixtures of Bt and non-Bt plants are not the optimum refuge design for insects that move between plants during development, such as *Busseola fusca* (Lepidoptera: Noctuidae) and *Helicoverpa armigera* (Lepidoptera: Noctuidae). Separate refuge fields would thus better suit the biology of these target pests. These separate refuge fields may also not be too far from the Bt field, or else there might not be random mating between resistant and susceptible insects. In this case it is also important to know how far the adult insects will move before mating. Behavioural differences between species, especially during the damaging stages (larvae or adult) make uniform refuge requirements very unpractical and could even increase the rate of resistance development in some species. Current IRM strategies have largely been developed to suit commercial farming systems in developed countries.

GM crops will be introduced into developing countries on the African continent on a large scale during the following five to ten years. IRM faces particular challenges in these countries, due to unique cropping systems cultural practices as well as pest complexes against which the current Bt cry proteins are not very effective. Valuable lessons were learned in South Africa with regards to IRM of Bt cotton and maize over the past thirteen years. These experiences highlighted the importance of the following aspects in IRM:

- monitoring of resistance development
- monitoring of adoption rates
- stewardship responsibilities
- refugia compliance
- understanding of farmer behaviour
- understanding the farming system.

IRM in Africa is currently not addressed in a holistic manner and it is not foreseen that this will change in the near future. It is recommended that crop ecologists and social scientists also become involved in these issues. Only if GM crop stewardship in general and IRM in particular is addressed in an honest way will the benefits provided by this technology remain available to those who need it for a long time. Capacity development in research and training as well as farmer training is essential to ensure proper use of GM technology. Without an understanding of African farming systems or at least an attempt to do so, improved technologies such as GM crops could fail in Africa (Van den Berg, accepted), similar to the experiences with the failure of the Green revolution in Africa (Eicher, 1984).

References

Abate, T., Van Huis, A. and Ampofo, J.K.O. 2000. Pest management strategies in traditional agriculture: an African perspective. *Annual Review of Entomology* 45: 631-659.

Alyokhin, A.V. 2008. Colorado potato beetle biology and management. http://www.potatobeetle.org/overview.html. Date of access: 1 June 2011.

Allyokhin, A.V. 2009. Colorado potato beetle management on potatoes: current challenges and future prospects. *Fruit, Vegetable and Cereal Science and Biotechnology* 3:10-19.

Alyokhin, A.V. and Ferro, D.N. 1999. Reproduction and dispersal of summer-generation Colorado potato beetle (Coleoptera: Chrysomelidae). *Population Ecology* 28: 425-430.

Andow, D.A. 1991. Vegetational diversity and arthropod population response. *Annual Review of Entomology* 36: 561-586.

Andow, D.A. and Hilbeck, A. 2004. Environmental risk assessment of genetically modified organisms: Vol. 1. A case study of Bt maize in Kenya. CAB International. Wallingford. UK.

Andow, D.A. and Ives, A.R. 2002. Monitoring and adaptive resistance management. *Ecological Applications* 12: 1378-1390.

Anon. 2006. IPM - Understanding *Helicoverpa* ecology and biology in southern Queensland: know the enemy to manage it better. http://www2.dpi.qld.gov.au/fieldcrops/17696.html. Date of access: 31 May 2011.

Anon. 2007. *Helicoverpa*: *Helicoverpa armigera* and *Helicoverpa punctigera.* http://www.cottoncrc.org.au/content/Industry/Publications/Pests_and_Beneficials/Cotton_Insect_Pest_and_Beneficial_Guide/Pests_by_common_name/Helicoverpa.aspx. Date of access: 31 May 2011

Ball, H.J. 1957. On the biology and egg-laying habits of the western corn rootworm. *Journal of Economic Entomology* 50: 126-128.

Bates, S.L., Zhao, J-Z., Roush, R.T. and Shelton, A.M. 2005. Insect resistance management in GM crops: past, present and future. *Nature Biotechnology* 23: 57-62.

Begon, M., Townsend, C.R. and Harper, J.L. 2006. Ecology: from individuals to ecosystems. 4th ed. Blackwell Publishing. UK. 738 pp.

Blanco, C.A., Andow, D.A., Abel, C.A., Sumerford, D.V., Hernandez, G., López, J.D., Adams, L., Groot, A., Leonard, R., Parker, R., Payne, G., Perera, O.P., Terán-Vargas, A.P. and Azuara-Domígues, A. 2009. *Bacillus thuringiensis* Cry1Ac resistance frequency in tobacco budworm (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 102: 381-387.

Bourguet, D., Chaufux, J., Séguin, M., Buisson, C., Hinton, J.L., Sodola, T.J., Porter, P., Cronholm, G., Buschman, L.L. and Andow, D.A. 2003. Frequency of alleles conferring resistance to Bt maize in French and US corn belt populations of the European corn borer, *Ostrinia nubilalis*. *Theoretical and Applied Genetics* 106: 1225-1233.

Bourguet, D., Genissel, A. and Raymond, M. 2000. Insecticide resistance and dominance levels. *Journal of Economic Entomology* 93: 1588-1595.

Casida, J.E. and Quistad, G. B. 1998. Golden age of insecticide research: Past, present, or future? *Annual Review of Entomology* 43: 1-16.

Chege, P.G., Clark, T.L. and Hibbard, B.E. 2009. Initial larval feeding on an alternate host enhances eastern corn rootworm (Coleoptera: Chrysomelidae) beetle emergence on *Cry3Bb1*-expressing maize. *Journal of the Kansas Entomological Society* 82: 63-75.

Chen, J., Hua, G., Jurat-Fuentes, J.L., Abdullah, M.A. and Adang, M.J. 2007. Synergism of *Bacillus thuringiensis* toxins by a fragment of a toxin-binding cadherin. *Proceedings of the National Academy of Sciences* 104: 13901-13906.

Clark, J.S. and Carlson, G.A. 1990. Testing for common versus private property: The case of pesticide resistance. *Journal of Environmental Economics and Management* 19: 45-60.

Cohen, M.B. 2000. Bt rice: practical steps to sustainable use. *International Rice Research Notes* 25: 4-10.

Comins, H.N. 1977. The management of pesticide resistance. *Journal of Theoretical Biology* 65: 399-420.

Cullen, E. 2010. Keeping up with Bt corn insect traits and refuge requirements. University of Wisconsin Cooperative Extension.

http://fyi.uwex.edu/grain/files/2010/05/Keeping-up-with-Bt-corn-traits1.pdf. Date of access: 23 November 2010.

Denholm, I. and Rowland, M.W. 1992. Tactics for managing pesticide resistance in arthropods: Theory and practice. *Annual Review of Entomology* 37: 91-112.

Erasmus, A., Van Rensburg, J.B.J. and Van den Berg, J. In review. Response of the African bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) to Bt maize in South Africa and its implications for resistance development.

Farrow, R.A. and Daly, J.C. 1987. Long-range movements as an adaptive strategy in the genus *Heliothis* (Lepidoptera: Noctuidae): a review of its occurrence and detection in four pest species. *Australian Journal of Zoology* 35: 1-24.

Ferré, J. and Van Rie, J. 2002. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* 47: 501-533.

Ferré, J., Van Rie, J. and MacIntosh, S.C. 2008. Insecticidal genetically modified crops and insect resistance management (IRM). In: Integration of insect resistant genetically modified crops (Eds. J. Romeis, A.M. Shelton and G.G. Kennedy). Springer Science and Business Media B.V. 41-85pp.

Firempong, S. and Zalucki, M.P. 1990. Host plant preferences of populations of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) from different geographic locations. *Australian Journal of Zoology* 37: 665-673.

Fitt.G.P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. *Annual Review of Entomology* 34: 17-52.

Fitt, G.P. 2004. Implementation and impact of transgenic Bt cottons in Australia. *In* proceedings, world cotton research conference-3, Cape Town 2003. Agricultural Research Council, Institute for Industrial Crops, Pretoria, Republic of South Africa.

Fitt, G.P. 2008. Resistance risk assessment and management of Bt cotton in Vietnam. In: *Environmental Risk Assessment of Genetically Modified Organisms, Volume 4: Challenges and Opportunities with Bt cotton in Vietnam* (Eds. D.A. Andow, A. Hilbeck and N. Văn Tuất). CAB International. Wallingford. UK. 296-329 pp.

Forrester, N.W. 1990. Designing, implementing and servicing an insecticide resistance management strategy. *Pesticide Science* 28: 167-179.

Gassmann, A.J., Carrière, Y. and Tabashnik, B. E. 2009. Fitness costs of insect resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* 54: 147-163.

Gassmann, A.J., Petzold-Maxwell, J.L., Keweshan, R.S. and Dunbar, M.W. 2011. Field-evolved resistance to Bt maize by western corn rootworm. *PLoS ONE* 6: e22629. doi:10.1371/journal.pone.0022629.

Georghiou, G.P. and Taylor, C.E. 1977a. Genetic and biological influences in the evolution of insecticide resistance. *Journal of Economic Entomology* 70: 319-323.

Georghiou, G.P. and Taylor, C.E. 1977b. Operational influences in the evolution of insecticide resistance. *Journal of Economic Entomology* 70: 653-658.

Glaser, J.A. and Matten, S.R. 2003. Sustainability of insect resistance management strategies for transgenic Bt corn. *Biotechnology Advances* 22: 45-69.

Gould, F. 1988. Evolutionary biology and genetically engineered crops: Consideration of evolutionary theory can aid in crop design. *BioScience* 38: 26-33.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annual Review of Entomology* 43: 701-726.

Gould, F. 2000. Testing Bt refuge strategies in the field. *Nature Biotechnology* 18: 266-267.

Green, W.M., De Billot, M.C., Joffe, T., Van Staden, L., Bennet-Nel, A., Du Toit, C.L.N. and Van Der Westhuizen, L. 2003. Indigenous plants and weeds on the Makhathini Flats as refuge hosts to maintain bollworm population susceptibility to transgenic cotton (Bollgard^{TM}). *African Entomology* 11: 21-29.

Haile, A. and Hofsvang, T. 2002. Host plant preference of the stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Crop Protection* 21: 227-233.

Hardwick, D.F. 1965. The corn earworm complex. *Memoirs of the Entomological Society of Canada* 40.247 pp.

Hare, J.D. 1990. Ecology and management of the Colorado potato beetle. *Annual Review of Entomology* 35: 81-100.

Harris, K.M. and Nwanze, K.F. 1992. *Busseola fusca* (Fuller), the African maze stem borer: a handbook of information. International Crops Research Institute for the Semi-Arid Tropics. Patancheru. India. 84 pp.

Head, G. 2010. Adapting insect resistance management strategies for transgenic Bt crops to developing world needs. www.gmo-safety.eu/pdf/biosafenet/Head.pdf. Date of access 5 May 2011.

Head, G. and Savinelli, C. 2008. Adapting insect resistance management programs to local needs. In: Insect resistance management: biology, economics and predictions (Ed. D.W. Onstad). Elsevier, USA. 89-106 pp.

Hibbard, B.E., Duran, D.P., Ellersieck, M.R. and Ellsbury, M.M. 2003. Post-establishment movement of Western corn rootworm larvae (Coleoptera: Chrysomelidae) in central Missouri corn. *Journal of Economic Entomology* 96: 599-608.

Hodgson, E.W. 2008. Western corn rootworm. *Utah Pests Fact Sheet.* Utah State University Extension and Utah Plant Pest Diagnostic Laboratory. http://extension.usu.edu/files/publications/factsheet/western-corn-rootworm.pdf. Date of access: 31 May 2011.

Hoy, C.W. 1999. Colorado potato beetle resistance management strategies for transgenic potatoes. *American Journal of Potato Research* 76: 215-219.

Huang, F., Andow, D.A. and Buschman, L.L. 2011. Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America. *Entomologia Experimentalis et Applicata* 140: 1-16.

Huang, F., Leonard, B.R., Cook, D.R., Lee, D.R., Andow, D.A., Baldwin, J.L., Tindall, K.V. and Wu, X. 2007. Frequency of alleles conferring resistance to *Bacillus thuringiensis* maize in Louisiana populations of south-western corn borer (Lepidoptera: Crambidae). *Entomologia Experimentalis et Applicata* 122: 53-58.

Hurley, T.M. and Mitchell, P.D. 2008. Insect resistance management: adoption and compliance. In: Insect resistance management; biology, economics and predictions (Ed. D.W. Onstad). Elsevier, USA. 227-253 pp.

Insecticide Resistance Action Committee. 2010. Resistance management for sustainable agriculture and improved public health, 2nd Ed. http://www.irac-online.org/wp-content/uploads/2009/09/VM-Layout-v2.6_LR.pdf.

Jacques Jr., R.L. and Fasulo, T.R. 2000. Colorado potato beetle: *Leptinotarsa decemlineata* (Say) (Insecta: Coleoptera: Chrysomelidae). *DPI Entomology Circular 271*. University of Florida Insitute of Food and Agricultural Sciences:

Department of Entomology and Nematology and Florida Department of Agriculture and Consumer Services: Division of Plant Industry. http://entnemdept.ufl.edu/creatures/veg/leaf/potato_beetles.htm. Date of access: 31 May 2011.

James, C. 2010. Global status of commercialized/GM crops. ISAAA Briefs No 41. ISAAA: Ithaca, NY. www.isaaa.org.

Kfir, R. 1998. Maize and grain sorghum: southern Africa. In: *African Cereal Stem Borers: Economic Importance, Taxonomy, Natural Enemies and Control* (Ed. A. Polaszek). Cambridge. UK. 29-37 pp.

Kruger, M., J.B.J. Van Rensburg, and J. Van den Berg. (In review). Reproductive biology of Bt-resistant and susceptible field-collected larvae of the maize stem borer, *Busseola fusca* (Lepidoptera: Noctuidae). *African Entomololgy.*

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.

Kruger, M., Van Rensburg, J.B.J. and Van den Berg, J. 2011a. Resistance to Bt maize in *Busseola fusca* (Lepidoptera: Noctuidae) from Vaalharts, South Africa. *Environmental Entomology* 40: 477-483.

Kruger, M., Van Rensburg, J.B.J. and Van den Berg, J. 2011b. Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa. *Journal of Applied Entomology* doi: 10.1111/j.1439-0418.2011.01614.x

Lauer, J. 2003. Refuge requirements for Bt corn. Wisconsin Crop Manager 10: 15-16. http://corn.agronomy.wisc.edu/WCM/W114.aspx. Date of access: 4 August 2010.

Le Rü, B.P., Ong'amo, G.O., Moyal, P., Ngala, L., Musyoka, B., Abdullah, Z., Cugala, D., Defabachew, B., Haile, T.A., Matama, T.K., Lada, V.Y., Negassi, B., Pallangyo, K., Ravolonandrianina, J., Sidumo, A., Omwega, C.O., Schulthess, F., Calatayud, P.A. and Silvain, J.F. 2006. Diversity of lepidopteran stem borers on monocotyledonous plants in eastern Africa and the islands of Madagascar and Zanzibar revisited. *Bulletin of Entomological Research* 96: 555-563.

Liu, Y-B., Tabashnik, B.E., Dennehy, T.J., Patin, A.M. and Bartlett, A.C. 1999. Development time and resistance to Bt crops. *Nature* 400: 519.

Liu, F., Xu, Z., Zhu, Y.C., Huang, F., Wang, Y., Li, H., Gao, C., Zhou, W. and Shen, J. 2008. Evidence of field-evolved resistance to Cry1Ac-expressing Bt cotton in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in northern China. *Pest Management Science* 66: 155-161.

Luttrell, R.G., Ali, I., Allen, K.C., Young III, S.Y., Szalanski, A., Williams, K., Lorenz, G., Parker, C.D. Jr. and Blanco, C. 2004. Resistance to Bt in Arkansas populations of cotton bollworm. *Proceedings of the 2004 Beltwide Cotton Conferences*, San Antonio, TX, January 5-9, 2004, ed D.A. Richter (National Cotton Council of America, Memphis, TN). 1373-1383pp.

Mallet, J. and Porter, P. 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? *Proceedings of the Royal Society of London* B250: 165-169.

Matten, S.R., Head, G.P. and Quemada, H.D. 2008. How governmental regulation can help or hinder the integration of Bt crops into IPM programs. *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs* (Eds. J. Romeis, A.M. Shelton, G.G. Kennedy). Springer, New York. 27-39pp.

Mbugi, J.P. 1999. Comparative studies on the adult dispersal behavior of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). Kenya Information Preservation Society. www.researchkenya.org/?ID=1602&search=Busseola+fusca. Date of access: 2 June 2011.

McGaughey, W.H. and Whalon, M.E. 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. *Science* 258: 1451-1455.

McGaughey, W.H., Gould, F. and Gelernter, W. 1998. Bt resistance management, a plan for reconciling the needs of the many stakeholders in Bt-based products. *Nature Biotechnology* 16: 144-146.

Meihls, L.N., Higdon, M.L., Siegfried, B.D., Miller, N.J., Sappington, T.W., Ellersieck, M.R., Spencer, T.A. and Hibbard, B.E. 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. *Proceedings of the National Academy of Sciences* 105: 19177-19182.

Monsanto. 2010. Monsanto – Cry1Ac resistance in Indian pink bollworms. http://www.monsanto.com/newsviews/Pages/india-pink-bollworm.aspx. Date of access: 5 May 2011.

Monsanto.2011a. User guideline for the production of genetically modified maize. http://www.monsanto.co.za/en/content/biotech/prod_guides/yieldgard_eng.pdf Date of access: 22 February 2011.

Monsanto. 2011b. Your pocket sized users guide for Bollgard with Roundup Ready® cotton (stacked gene cotton). http://www.monsanto.co.za/en/content/biotech/prod_guides/dubbelgeen_eng.pdf. Date of access: 22 February 2011.

Mulaa, M.A., Bergvison, D.J., Mugo, S.N. and Ngeny, J. 2007. Developing insect resistance management strategies for Bt maize in Kenya. *African Crop Science Conference Proceedings* 8: 1067-1070.

Mulaa, M.A., Bergvison, D.J., Mugo, S.N., Wanyama, J.M., Tende, R.M., De Groote, H. and Tefera, T.M. 2011. Evaluation of stem borer resistance management strategies for Bt maize in Kenya based on alternative host refugia. *African Journal of Biotechnology* 10: 4732-4740.

Nault, B.A. 2001. Survival and fecundity of Bt-susceptible Colorado potato beetle adults after consumption of transgenic potato containing *Bacillus thuringiensis* subsp. *tenebrionis* Cry3A toxin. *Entomologia Experimentalis et Applicata* 101: 265-272.

Nibouche, S., Guérard, N., Martin, P. and Vaissayre, M. 2007. Modelling the role of refuges for sustainable management of dual-gene Bt cotton in West African smallholder farming systems. *Crop Protection* 26: 828-836.

Olofsson, H., Ripa, J. and Jonzén, J. 2009. Bet-hedging as an evolutionary game: the trade-off between egg size and number. *Proceedings of the Royal Society Biological Sciences* doi: 10.1098/rspb.2009.0500.

Ong'amo, G. O., Le Ru, B., Dupas, S., Moyal, P., Muchugu, E., Calatayud, P. A. and Silvian, J. F. 2006. The role of wild host plants in the abundance of lepidopteran stem borers along altitude gradient in Kenya. *Annales de la Société Entomologique de France* 42: 363-370.

Onstad, D.W. 2008. Major issues in insect resistance management. In: Insect resistance management: biology, economics and prediction (Ed. D.W. Onstad). Elsevier, USA. 1-16 pp.

Onstad, D.W. and Gould, F. 1998. Modelling the dynamics of adaptation to transgenic maize by European corn borer (Lepidoptera: Pyralidae). *Journal of Economic Entomology* 91: 585-593.

Onstad, D.W. and Guse, C.A. 2008. Concepts and complexities of population genetics. In: Insect resistance management: biology, economics and predictions (Ed. D.W. Onstad). Elsevier, USA. 69-88 pp.

Onstad, D.W., Mitchell, P.D., Hurley, T.M., Lundgren, J.G., Porter, R.P., Krupke, C.H., Spencer, J.L., Difonzo, C.D., Baute, T.S., Hellmich, R.L., Buschman, L.L., Hutchison, W.D. and Tooker, J.F. 2011. Seeds of change: corn seed mixtures for resistance management and integrated pest management. *Journal of Economic Entomology* 104: 343-352.

Otieno, N.A., Le Ru, B.P., Ong'amo, G.O., Dupas, S., Calatayud, P.A., Makobe, M., Ochora, J. and Silvian, J.F. 2008. Diversity and abundance of wild host plants of lepidopteran stem borers in two different agro-ecological zones of Kenya. *International Journal of Biodiversity Science, Ecosystems Services and Management* 4: 92-103.

Pioneer. 2011. Herculex® XTRA (HXX) product use guide. http://www.pioneer.com/CMRoot/pioneer/us/products/stewardship/pugs/hxx.pdf. Date of access: 8 April 2011.

Raffa, K.F. and Priester, T.M. 1985. Synergists as research tools and control agents in agriculture. *Journal of Agricultural Entomology* 2: 27-45.

Ragsdale, D and Radcliff, E. 2011. Colorado potato beetle. The University of Minnesota. http://www.vegedge.umn.edu/vegpest/cpb.htm. Date of access: 2 June 2011.

Ratnadass, A., Fernandes, P., Avelino, J. and Habib, R. 2010. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems. *Agronomy for Sustainable Development* doi: 1007/s13593-011-0022-4

Renner, R. 1999. Will Bt-based pest resistance management plans work? *Environmental Science and Technology* 33: 410-415.

Roush, R.T. 1994. Managing pests and their resistance to *Bacillus thuringiensis*: can transgenic crops be better than sprays? *Biocontrol Science and Technology* 4: 501-516.

Roush, R.T. 1997. Bt-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? *Pesticide Science* 51: 328-334.

Roush, R.T. and Daly, J.C. 1990. The role of population genetics in resistance research and management. In: Pesticide resistance in arthropods (Eds. R.T. Roush and B.E. Tabashnik). Routledge, Chapman and Hall, New York. 97-152pp.

Seshu Reddy, K. V. 1983. Studies on the stem borer complex of sorghum in Kenya. *Insect Science and its Application* 4: 3-10.

Seshu Reddy, K. V. 1990. Cultural control of *Chilo* spp. in graminaceous crops. *Insect Science and its Application* 11: 703-712.

Shengjiang, T., Xiaofeng, C., Dianmo, L. and Huizhen, Z. 2001. Can other host species of cotton bollworm be non-Bt refuges to prolong the effectiveness of Bt-cotton. *Chinese Science Bulletin* 46: 1804-1807.

Spencer, J.L., Hibbard, B.E., Moeser, J. and Onstad, D.W. 2009. Behaviour and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *Agricultural and Forest Entomology* 11: 9-27.

Spencer, J.L., Levine, E., Isard, S.A. and Mabry, T.R. 2005. Movement, dispersal and behaviour of Western corn rootworm adults in rotated maize and soybean fields. In: *Western corn rootworm: ecology and management* (Eds. S. Vidal, U. Kuhlmann and C.R. Edwards). CAB International. UK. 121-144 pp.

Tabashnik, B.E. 1989. Managing resistance with multiple pesticide tactics: Theory, evidence, and recommendations. *Journal of Economic Entomology* 82: 1263-1269.

Tabashnik, B.E. 1994a. Delaying insect adaptation to transgenic plants: Seed mixtures and refugia reconsidered. *Proceedings of the Royal Society of London* 255: 7-12.

Tabashnik, B.E. 1994b. Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* 39: 47-79.

Tabashnik, B.E. 1994c. Delaying insect adaptation to transgenic plants: seed mixtures and refugia reconsidered. *Proceedings of the Royal Society of London* B 255: 7-12.

Tabashnik, B.E. 2008. Delaying insect resistance to transgenic crops. *Proceedings of the National Academy of Science USA* 105: 19029-19030.

Tabashnik, B.E., Carriére, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M. and Zhao, J-Z. 2003. Insect resistance to transgenic Bt crops: Lessons from the laboratory and field. *Journal of Economic Entomology* 96: 1031-1038.

Tabashnik, B.E. and Croft, B.A. 1982. Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. *Environmental Entomology* 11: 1137-1144.

Tabashnik, B.E., Cushing, N.L., Finson, N. and Johnson, M.W. 1990. Field development of resistance to *Bacillus thuringiensis* in Diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology* 83: 1671-1676.

Tabashnik, B.E., Fabrick, J.A., Henderson, S., Biggs, R.W., Yafuso, C.M., Nyboer, M.E., Manhardt, N.M., Coughlin, L.A., Sollome, J., Carrière, Y., Dennehy, T.J. and Morin, S. 2006. DNA screening reveals pink bollworm resistance to Bt cotton remains rare after a decade of exposure. *Journal of Economic Entomology* 99: 1525-1530.

Tabashnik, B.E., Finson, N., Groeters, F.R., Moar, W.J., Johnson, M.W., Luo, K. and Adang, M.J. 1994. Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella*. *Proceedings of the National Academy of Sciences USA* 91: 4120-4124.

Tabashnik, B.E., Gassmann, A.J., Crowder, D.W. and Carrière, Y. 2008. Insect resistance to Bt crops: evidence versus theory. *Nature Biotechnology* 26: 199-202.

Tabashnik, B.E., Gould, F. and Carriére, Y. 2004. Delaying evolution of insect resistance to transgenic crops by decreasing dominance and heritability. *Journal of Evolutionary Biology* 17: 904-912.

Tabashnik, B.E., Liu, Y-B., Finson, N., Mason, L. and Heckel, D.G. 1997. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. *Proceedings of the National Academy of Sciences USA* 94: 1640-1644.

Tabashnik, B.E., Van Rensburg, J.B.J., Carriére, Y. 2009. Field-evolved insect resistance to Bt crops: definition, theory, and data. *Journal of Economic Entomology* 102: 2011-2025.

Taylor, C.E. and Georghiou, G.P. 1979. Suppression of insecticide resistance by alteration of gene dominance and migration. *Journal of Economic Entomology* 72: 105-109.

Tende, R.M., Mugo, S.N., Nderitu, J.H., Olubayo, F.M., Songa, J.M., Bergvinson, D.J. 2010. Evaluation of *Chilo partellus* and *Busseola fusca* susceptibility to δ-endotoxins in Bt-maize. *Crop Protection* 29: 115-120.

Turner, G., Bergvison, D. and Das, B. 2011. African maize stem borer (extended information: life cycle and description. International Maize and Wheat Improvement Centre, CIMMYT, Mexico. http://maizedoctor.cimmyt.org/index.php?id=310&option=com_content&task=view. Date of access: 2 June 2011.

United States Environmental Protection Agency. 1998. The white paper on Bt plant pesticide resistance management. Washington. USA. http://www.epa.gov/biopesticides. Date of access: 22 November 2010.

United States Environmental Protection Agency. 2001. Biopesticides registration action document: *Bacillus thuringiensis* plant-incorporated protectants. Washington. USA. http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm. Date of access: 22 November 2010.

United States Environmental Protection Agency. 2007. Pesticide News Story: EPA Approves Natural Refuge for Insect Resistance Management in Bollgard II Cotton. http://www.epa.gov/oppfead1/cb/csb_page/updates/2007/bollgard-cotton.htm. Date of access: 12 August 2011.

United States Environmental Protection Agency – Scientific Advisory Panel. 1998. Final report of the FIFRA scientific advisory panel subpanel on *Bacillus thuringiensis* (Bt) plant-pesticides and resistance management. Washington. USA. http://www.epa.gov/scipoly/sap/meetings/1998/february/finalfeb.pdf. Date of access: 9 May 2011.

Vacher, C., Bourguet, D., Rousset, F., Chevillon, C. and Hochberg, M.E. 2003. Modelling the spatial configuration of refuges for a sustainable control of pests: a case study of *Bt* cotton. *Journal of Evolutionary Biology* 16: 378-387.

Van den Berg, J. and Rebe, M. 2001. Infestation patterns of stem borers in maize surrounded by graminaceous trap crops. (*In* Van den Berg, J. and Uys, V.M. eds. Proceedings of the Final Workshop of the Southern African Stem Borer Management). Programme: 2001, 13th Congress of the Entomological Society of Southern Africa, Pietermaritzburg. p. 41-50.

Van Rensburg, J.B.J. 2001. Larval mortality and injury patterns of the African stalk borer, *Busseola fusca* (Fuller) on various plant parts of Bt-transgenic maize. *South African Journal of Plant and Soil* 18: 62-68.

Van Rensburg, J.B.J. 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt transgenic maize. *South African Journal of Plant and Soil* 24: 147-151.

Van Wyk, A., Van den Berg, J. and Van Hamburg, H. 2007. Selection of non-target Lepidoptera species for ecological risk assessment of Bt maize in South Africa. *African Entomology* 15: 356-366.

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

Whalon, M.E., Mota-Sanchez, D. and Hollingworth, R.M. 2008. Analysis of global pesticide resistance in arthropods. In: Global pesticide resistance in arthropods (Eds. M.E. Whalon, D. Mota-Sanchez and R.M. Hollingworth). CAB International, Cromwell Press, Trowbridge, UK. 5-31pp.

Whalon, M.E., Mota-Sanchez, D., Hollingworth, R.M. and Duynslager, L. 2011. Arthropod resistance database. www.pesticideresistance.com. Date of access: 3 March 2011.

Wu, K.M. and Guo, Y.Y. 2005. The evolution of cotton pest management practices in China. *Annual Review of Entomology* 50: 31 – 52.

Zhang, J-H., Wang, C-Z., Qin, J-D. and Guo, S-D. 2004. Feeding behaviour of *Helicoverpa armigera* larvae on insect-resistant transgenic cotton and non-transgenic cotton. *Journal of Applied Entomology* 128: 218-225.