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Resistance of *Busseola fusca* to Cry1Ab Bt Maize Plants in South Africa and Challenges to Insect Resistance Management in Africa

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Summary

The evolution of resistance to *Bacillus thuringiensis* (Bt) maize by the African stem borer, *Busseola fusca*, in South Africa highlighted the importance of the development of appropriate integrated resistance management (IRM) strategies for stem borers in Africa. Landscape heterogeneity is characteristic of African agroecosystems. This heterogeneity, in addition to between-field and within-field spatial mosaics resulting from variable gene expression in Bt maize, will provide challenges to managing resistance evolution of the lepidopteran stem borers that attack maize. Adding to this landscape heterogeneity is the cultivation of open-pollinated maize varieties (OPVs) and bimodal rainfall patterns that allow two maize cropping seasons each year in many subtropical and tropical areas. The role that these factors, as well as aspects such as low-dose expression events, refuge compliance, the genetic bases of resistance, pest behaviour, host plant range and farming practices, may play in the evolution of stem borers to Bt maize in Africa are addressed in this chapter.

4.1 Importance of Maize in Africa: From Small- to Large-scale Farming Systems

Maize is the most important cereal crop in Africa and sustains more than 300 million of Africa's most vulnerable people (La Rovere *et al.*, 2010). Maize farming within the African context is mostly done on small plots of land with low productivity. Increasing the productivity of these small-scale farming systems would ensure both food security in various contexts and an increased contribution to the economies of African countries (Byerlee and Heisey, 1996).

Production systems for maize in Africa are highly diverse. The crop is grown in a wide range of agroecologies, which may vary between unimodal and bimodal rainfall regions, forest zones, lowland tropics and highland temperate regions, each with its unique challenges. The sizes of maize fields also vary greatly. Numerous small fields are characteristic of small-scale maize farming systems in Africa. For example, Aheto *et al.* (2013) reported 58 (average size = 0.81 ha) and 97 (average size = 0.41 ha) maize fields

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km^{-2} in Ghana and Zambia, respectively. The use of fertilizers, herbicides and insecticides in these fields is minimal and the planting of open pollinated varieties (OPVs) is common. In some areas of the continent, large-scale commercial agriculture is practised, both under rainfed and irrigated conditions. In these mechanized systems, the use of fertilizers, herbicides and insecticides is high, and the maize is produced as a cash crop rather than for local consumption, as in most of the small-scale farming systems. In South Africa, for example, most of the maize (2.5 million ha) is cultivated in large-scale high-input farming systems in which the size of the maize fields can be several hundreds of hectares. There are, however, also large numbers of small-scale farmers with field sizes similar to those reported in the rest of Africa (Van den Berg, 2013).

The average maize yields in several African countries, where maize is a highly important staple food crop, are below 1 t ha^{-1}, which compares with a world average of 4.7 t ha^{-1} in 2005 and an average of 9.4 t ha^{-1} in the USA (Worku *et al.*, 2012). These low yields are ascribed to poor soil fertility, droughts, a high incidence of insect pests, diseases and weeds and limited access to fertilizer and improved maize seed.

4.2 Stem Borer Pests of Maize in Africa: Distribution and Pest Status

Stem borers seriously limit potentially attainable maize yields by infesting the crop from the seedling stage to maturity. Many species belonging to the families Pyralidae and Noctuidae have been found to attack maize in various parts of Africa. The most important of these are *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (Kfir *et al.*, 2002). Of these species, *B. fusca* and *S. calamistis* occur virtually throughout sub-Saharan Africa. *C. partellus* occurs mostly in the eastern and southern parts of the continent, while another species of *Chilo*, *C. orichalociliellus* (Strand), seem to be confined to coastal low altitude areas of East and southern Africa. In addition to the above-mentioned stem borer pest species, several noctuids, *B. segeta* (Bowden), *B. phaia* (Bowden) and *Pirateolea piscator* (Fletcher) have also recently been reported from maize in East Africa (Le Ru *et al.*, 2006a; Ong'amo *et al.*, 2013).

Estimates of crop losses vary greatly in different regions and agroecological zones. In Kenya alone, losses due to *B. fusca* damage fluctuate around 14% on average (De Groote, 2002), but can be as high as 73% (Mailafiya *et al.*, 2009). Kfir (1998) reported maize yield losses due to *B. fusca* to be between 10 and 60%. A review of *C. partellus* yield loss studies indicated losses between 4 and 73% depending on cultivar, planting date, pest population density and phenological stage of the crop at the time of infestation (Seshu-Reddy and Walker, 1990). In West Africa, stem borers are responsible for losses ranging between 25 and 55% in maize (Ndemah *et al.*, 2007).

While *Bacillus thuringiensis* (Bt) maize targets all stem borer species that attack the crop, the main species targeted differs between regions. In East and southern Africa, *B. fusca* and *C. partellus* are the main target species, while in Egypt they are *Sesamia cretica* (Lederer) (Lepidoptera: Noctuidae) and *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) (El-Shazlya *et al.*, 2013). The major stem borers of maize in West Africa include *B. fusca*, *S. calamistis* and *E. saccharina* (Bosque-Pèrez and Schulthess, 1998).

4.3 Commercial Release of Bt Maize in Africa

South Africa and Egypt are the only African countries that currently cultivate Bt maize, with cultivation having commenced in 1997 (Gouse *et al.*, 2005) and 2008 (El-Shazlya *et al.*, 2013), respectively. While Bt maize was quickly adopted in South Africa, the area of its cultivation in Egypt reportedly grew from 700 ha in 2008 to only 2000 ha in 2010 (James, 2010). However, recent reports do not list Egypt as a genetically modified (GM) maize-producing country

(James, 2013). The adoption rates of GM maize among commercial farmers in South Africa were high and in certain areas the market penetration of the Bt trait is nearly 100%. Today, Bt maize is planted on approximately 1.8 million ha in South Africa, making it the eighth largest producer of GM crops in the world (James, 2013). This high adoption rate has been ascribed to significant benefits of GM maize, such as the convenience of target pest management (Kruger *et al.*, 2009) and economic benefits. Gouse *et al.* (2005) reported that despite paying more for seed, adopters of GM maize enjoyed increased income over non-GM maize adopters through savings on pesticides and protection from yield losses due to target pest species. In order for these benefits to be realized in the future, insect resistance management (IRM) strategies need to be effectively implemented.

4.4 Resistance Evolution to Bt Cry1Ab Maize

Until 2006, when event Bt11 was commercially released in South Africa, all Bt maize hybrids contained event MON810 (an event is a specific genetic modification in a specific species). From the 2012/13 growing season onwards, the pyramided event MON89034, which expresses two different Cry (crystal) proteins, Cry1A.105 and Cry2Ab2, was planted on a large scale in South Africa.

The first evaluation of the efficacy of several Bt maize events against *B. fusca* was conducted between 1994 and 1997 (van Rensburg, 1999). Under field conditions, the ranges of survival of *B. fusca* after 14 days of feeding on MON810 hybrids were 0.5–0.9% after early infestations and 1.3–2.4% after late infestations (van Rensburg, 2001). Although *B. fusca* survival was higher than the high-dose requirement (see Section 4.5.2), the efficacy of the event MON810 was considered sufficient to protect maize from stem borer damage. Bt maize hybrids have since been shown to provide effective control of *C. partellus* and to provide partial to very good control of *S. calamistis* and *B. fusca* (van Rensburg, 1999; Van den Berg and Van Wyk, 2007; Tende *et al.*, 2010).

Damage caused by *B. fusca* to Bt maize was observed at a number of localities during the first harvest season (1999) after the first commercial plantings of Bt maize in South Africa (van Rensburg, 2001). The first official report of field resistance to Bt maize in *B. fusca* was subsequently made in 2006, when van Rensburg (2007) showed that significant numbers of the F_1 generation of diapause larvae collected on Bt maize in the Christiana area (27° 57′ S, 25° 05′ E) in the Northern Cape Province survived on Bt maize. A year after this first official report of resistance, other cases of resistance were reported and confirmed in the Vaalharts area, approximately 50 km from the initial site (Kruger *et al.*, 2011, 2012, 2014). Results from an extensive farmer survey conducted during 2010 indicated the presence of resistant populations in the maize production region and also showed that borer damage to Bt maize had been observed over a number of cropping seasons between 2003 and 2008 (Kruger *et al.*, 2011). During these surveys, farmers indicated that stem borers were generally effectively controlled with Bt maize, but in several districts it was indicated that stem borer damage to Bt maize was already prevalent from the 2003/04 season onward (Kruger *et al.*, 2011). A conservative estimate is that approximately 250 cases of product failure have been reported annually for the 2010/11 and 2011/2012 growing seasons (Van den Berg *et al.*, 2013).

Farmer's observations on the levels of stem borer infestation in Bt maize fields over time (Kruger *et al.*, 2012), show that infestation levels decrease with distance away from the initial site where resistance was reported (Fig. 4.1). These infestation levels are also below 10% in most cases, which is also below the economic threshold value of 10% for the application of chemical control measures. Despite the low stem borer infestation levels observed further than 100 km away from the original site (up to 2011), the numbers of farmers reporting the presence of stem borer damage in Bt maize fields were high (Fig. 4.2). Today, *B. fusca* populations with resistance to

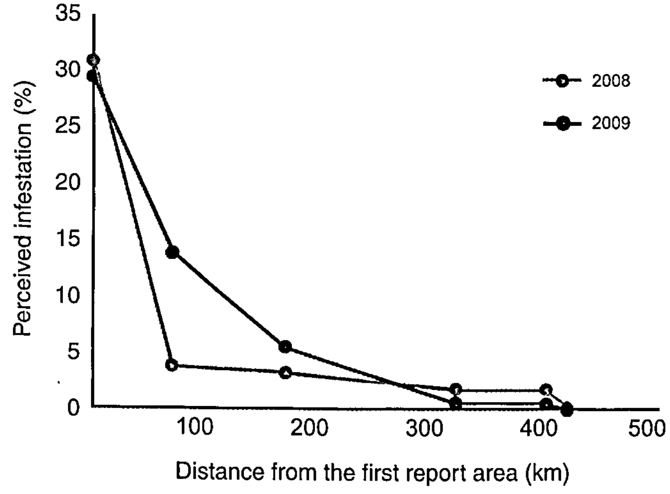

Fig. 4.1. Average infestation level of Bt maize perceived by farmers as a function of the distance from the site in South Africa where resistance of *Busseola fusca* was first reported. (Based on Kruger *et al.*, 2012.)

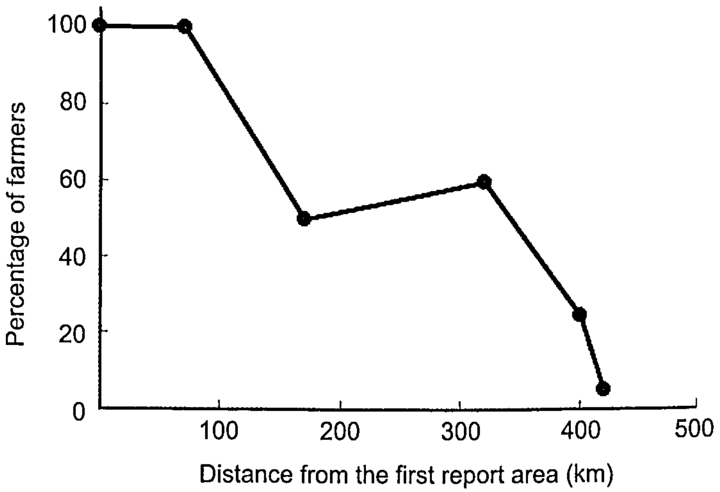

Fig. 4.2. Proportion of farmers who observed signs of *Busseola fusca* infestation on *Bt* maize in 2009, as a function of the distance from the site in South Africa where resistance of *Busseola fusca* was first reported. (Based on Kruger *et al.*, 2012.)

Cry1Ab-expressing maize occur throughout the maize production region. Although this is the case, the nature of the distribution is such that product failure is not reported on all farms, but rather on particular farms throughout certain geographical regions (Van den Berg *et al.*, 2013).

4.5 The High-dose Refuge Strategy

The current success of Bt maize in sustaining the susceptibility of some of the major pests is ascribed to the implementation of the 'high dose/refuge' IRM strategy (Tabashnik *et al.*, 2013). This is also the IRM strategy deployed in South Africa. The principle of this strategy is to promote survival of susceptible insects in refuges of non-Bt plants and random mating between the few resistant insects that survive on Bt plants with the many susceptible insects originating from the non-Bt refuge maintained nearby (Gould, 1998).

This strategy has certain requirements and is based on several assumptions and the interactions between these. These assumptions, as summarized by Roush (1994), Gould (1998), the US Environmental Protection Agency (US EPA, 1998, 2001), Ferré and Van Rie (2002) and Glaser and Matten (2003), are that:

- Non-transgenic refugia sustain a susceptible pest population.
- The crop expresses a high toxin concentration and resistance is recessive.
- Resistance genes are initially rare.
- There is random mating between resistant and susceptible adults.

Although the evolution of resistance by *B. fusca* has largely been ascribed to non-compliance with refuge requirements (Kruger *et al.*, 2011, 2012), other important factors have contributed. Indeed, IRM strategies cannot only be based on assumptions of single traits with simple effects (Gassmann *et al.*, 2009). The success of the high-dose refuge strategy in Africa may depend on how IRM strategies are adapted to a variety of farming contexts. The above-mentioned assumptions, as well as other possible contributing factors, such as pest biology and behaviour, are therefore revisited below.

4.5.1 Non-transgenic refugia

Refuges are habitats in which the target pest is not under selection pressure from the toxin, and which provide a sustainable habitat where pest development can take place and where a susceptible pest population is sustained. The planting of non-Bt maize is the most common form of providing a refuge. As noted above, it is widely accepted that non-compliance with refuge requirements contributed towards resistance evolution in *B. fusca*. Throughout the maize production region of South Africa, compliance with refuge requirements was low for the first 8 years after the release of Bt maize (Kruger *et al.*, 2009, 2011). Planting dedicated areas of non-Bt maize in close proximity to the crop is the prescribed strategy in South Africa and is generally considered the appropriate strategy to adopt in large-scale farming systems. However, unstructured refugia in the form of non-Bt maize fields of non-adopters of the technology, which will be a common occurrence in Africa, would also suffice as refugia, provided that certain measures are put in place so as not to allow a too high a proportion of Bt maize compared with non-Bt maize in a particular geographical area.

Wild host plants and alternative host plants have been suggested as a refuge strategy for stem borers in Africa (Mulaa *et al.*, 2011). Until recently, *B. fusca* was considered to be a species feeding not only on maize and cultivated and wild sorghums but also on many wild grasses. Wild host plants, mostly thick-stemmed grasses, were therefore considered to host sufficient numbers of *B. fusca* larvae to serve as a refuge. In contradiction of this, extensive field surveys in East and southern African over the past decade have shown that there is a low diversity of wild hosts for this stem borer (Le Ru *et al.*, 2006a,b; Ong'amo *et al.*, 2006; Moolman *et al.*, 2013), and that these wild hosts do not suffice as a refuge for stem borers in Africa.

4.5.2 High dose and functionally recessive resistance

The high-dose component of the high-dose/refuge strategy requires that the dose should theoretically be 25 times the dose needed to kill 99% of the susceptible individuals (Roush, 1994; Gould, 1998; US EPA, 1998, 2001; Glaser and Matten, 2003). This high-dose requirement is challenged by the deployment of Bt maize against a range of pests throughout the world, because a high dose for one pest may not be a high dose for another.

Although data have not been reported on the dominance of *B. fusca* resistance to Cry1Ab, pre-commercialization field data implied that the Cry1Ab maize did not kill 99% of larvae (van Rensburg, 1999). According to a review by Tabashnik *et al.* (2009), available pre-commercialization field data showed that the high-dose standard was not met by event MON810.

The diversity of the genetic bases of resistance and their inheritance is an important aspect in resistance management. A previous study (Campagne *et al.*, 2013) showed that resistance to Cry 1Ab in *B. fusca* was not recessively inherited, contrary to the important assumptions of the 'high dose/refuge' resistance management strategy. The study was carried out with maize stems under laboratory conditions, which did not directly reflect survival on plants in the field. Nevertheless, the results were consistent with those of a previous study showing the resistance of larvae originating from the Vaalharts area in whole plant bioassays conducted in greenhouses (van Rensburg, 1999) and are further supported by farmers' observations (Kruger *et al.*, 2011). Functionally non-recessive resistance results in a higher proportion of resistant phenotypes in a population in comparison with a recessively inherited resistance, all other things being equal. It is, therefore, expected to lead to rapid evolution of resistance in a pest population and to a drastic reduction in the efficiency of the refuge strategy.

When resistance is not recessive, refuges need to be much more abundant to delay resistance evolution effectively (Tabashnik and Gould, 2012; Brévault *et al.*, 2013). Because the high-dose standard was not met by event MON810, non-recessive inheritance of resistance appears to have hastened the evolution of *B. fusca* resistance to Cry 1Ab in Bt maize (Tabashnik *et al.*, 2009). Simple population genetics models (Wright, 1942) may be used to assess the effects of non-recessive inheritance of resistance traits on the rate at which resistance evolves.

4.5.3 Effects of selection and dominance on the evolution of resistance

Let us consider resistance due to a single locus, where R denotes the resistance allele and S is the susceptible allele in a landscape where *Bt* plants and non-*Bt* plants would be randomly distributed. Considering a monogenic resistance, the relative fitness of different genotypes under the selection operated by Bt toxins may be described by the following equations:

$$RR, s_1 = 1 + s \tag{1}$$

$$RS, s_2 = 1 + hs \tag{2}$$

$$SS, 1 \tag{3}$$

where s is the selection coefficient ($s > 0$, in this case) and h is the dominance level ($0 < h < 1$) of resistance. Assuming no effect of spatial structure, the selection coefficient s may be modelled as a function of the proportion of the Bt crop (β) in the system.

$$s = \frac{1}{1-\beta} - 1 \tag{4}$$

The change in the frequency of the resistance allele (p) from one to the next generation (i.e. t to $t + 1$) is described by the equation of Wright (1942), as a function of dominance and selection:

$$p_{t+1} = p_t \frac{1 + s_1 p_t + s_2 (1 - p_t)}{1 + s_1 p_t^2 + 2 s_2 p_t (1 - p_t)} \tag{5}$$

Dominance of a trait has a strong effect on the dynamics of change of allele frequency across generations, as suggested by the latter model. Across generations, non-recessive resistance is characterized by a strong increase in allele frequency, especially when the frequency of the resistance allele is low in comparison with a strictly recessive case. In contrast, the frequency increase of a recessive resistance allele will result in a fast elimination of the susceptible allele in comparison with non-recessive cases ($h > 0$) (Fig. 4.3) (Felsenstein, 2007).

As a consequence, non-recessive resistance alleles are expected to invade the

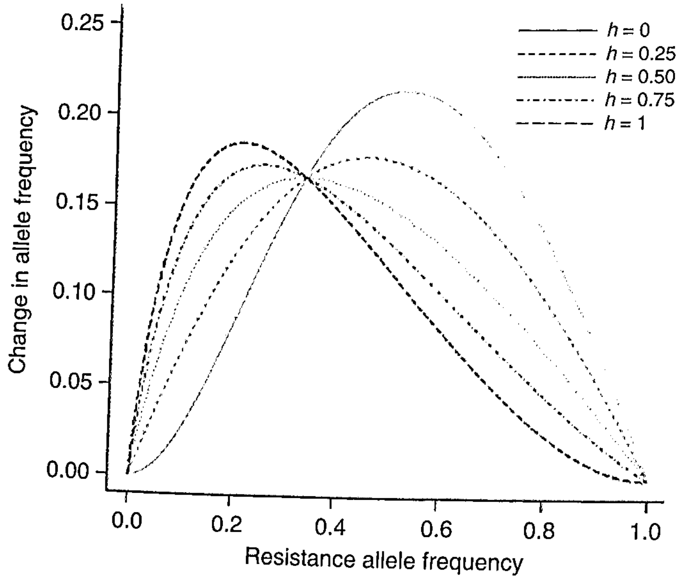

Fig. 4.3. Change in allele frequency as a function of frequency the resistance allele, in various cases: resistance allele is fully recessive ($h = 0$) to fully dominant ($h = 1$). The proportion of Bt crop in the model is $\beta = 0.8$.

pest population much more quickly than recessive resistance alleles (Tabashnik *et al.*, 2008). The time required for a change of gene frequency (from an initial frequency of p_0 to a much higher frequency p_T) cannot be analytically derived from the Wright model. However, approximations using a time continuous model may be obtained for the strictly recessive and dominant cases, T_{res} and T_{dom}, respectively (see Felsenstein, 2007):

$$T_{res} = \frac{1}{s}\left[-\frac{1}{p_T} + ln\left(\frac{p_T}{1-p_T}\right) + \frac{1}{p_0} - \ln\left(\frac{p_0}{1-p_0}\right) + 2s\,ln\left(\frac{1-p_T}{1-p_0}\right)\right] \quad (6)$$

$$T_{dom} = \frac{1}{s}\left[-\frac{1}{1-p_T} + ln\left(\frac{p_T}{1-p_T}\right) + \frac{1}{1-p_0} - ln\left(\frac{p_0}{1-p_0}\right)\right] \quad (7)$$

Setting p_T at 0.25, for the sake of simplicity, and p_0 at 10^{-x} (with $x > 3$), the previous equations (6) and (7) may numerically be reduced to:

$$T_{res} \approx 10^{x}.\left(\frac{1}{\beta} - 1\right) \quad (8)$$

and

$$T_{dom} \approx \left[x \ln(10) - 0.765\right]\left(\frac{1}{\beta} - 1\right) \quad (9)$$

Although both of these equations depend on the initial frequency of the resistance allele, they exhibit strong contrasts. Dominant resistance is expected to evolve with a time lapse that is proportional to the logarithm of the initial frequency of the resistance allele, while in the recessive case, the corresponding time would be inversely proportional to the initial resistance allele frequency. For example, the time required for a change in resistance allele frequency from $p_0 = 10^{-4}$ to $p_T = 0.25$ would be:

$$T_{res} \approx 10000\left(\frac{1}{\beta} - 1\right) \quad (10)$$

and

$$T_{dom} \approx 8.45\left(\frac{1}{\beta}-1\right) \qquad (11)$$

in the recessive and dominant cases, respectively. In other words, irrespective of the proportion of Bt crop in the system (which determines the selection coefficient), the model predicts that a fully dominant resistance would be acquired 1000 times faster than a recessive resistance.

Intermediate cases of dominance may be numerically explored using Eqn (6) (see Table 4.1). In line with previous results (e.g. Tabashnik *et al.*, 2008) the Wright (1942) model predicts that non-recessive resistance will drastically increase the rate of resistance evolution compared with the recessive case.

In a context where the genetic bases of resistance to Bt crops are not necessarily uniform (Campagne *et al.*, 2013; Jin *et al.*, 2013), possible high-dose failures in maize cultivated in small-scale African farming systems should be anticipated. The use of the technology by farmers in various agroecosystems and intrinsically non-recessive resistance are indeed crucial components in the evolution of resistance.

4.5.4 Pest biology and larval migration

Because pest biology, feeding behaviour and movement between plants determines the level of exposure to Bt plants, these aspects should be considered in resistance management strategies. They are discussed below for *B. fusca*.

Larval behaviour of Busseola fusca

The high level of between-plant larval migration by *B. fusca* (Calatayud *et al.*, 2014) will provide challenges regarding resistance management. The movement of larvae between plants in the refuge area and in adjacent Bt maize blocks (or between plants in a seed mixture planting) pose a potential concern for resistance evolution.

B. fusca larvae migrate throughout all their larval stages. This migration commences immediately after egg hatch and ceases during the last instar, 4 to 5 weeks post hatching, when the larvae prepare pupal cells in which they become pupae, or go into diapause (van Rensburg *et al.*, 1987a). Migration does not cease after the larvae leave plant whorls to feed inside maize stems. The larvae migrate until the

Table 4.1. Time required for a change in resistance allele frequency, from p_0 to $p_T = 0.25$ as a function of different levels of allele dominance (h) and the proportion of Bt crop in the system (β). T_{res} and T_{dom} are the strictly recessive and dominant cases.

Proportion of Bt crop	Initial frequency	No. generations to $p_T = 0.25$						
β	p_0	$h = 0$	$h = 0.25$	$h = 0.5$	$h = 0.75$	$h = 1$	T_{res}	T_{dom}
0.50	1×10^{-6}	$>1 \times 10^{6}$	56	32	24	19	$>1 \times 10^{6}$	13
0.50	1×10^{-5}	100,017	46	26	20	16	100,006	11
0.50	1×10^{-4}	10,012	35	21	15	13	10,003	8
0.50	1×10^{-3}	1008	25	15	11	9	1001	6
0.75	1×10^{-6}	333,349	23	15	12	10	333,335.7	4
0.75	1×10^{-5}	33,346	19	12	10	8	33,335	4
0.75	1×10^{-4}	3343	15	10	8	7	3334	3
0.75	1×10^{-3}	340	11	7	6	5	333	2
0.90	1×10^{-6}	111,125	11	8	7	6	111,112	2
0.90	1×10^{-5}	11,122	9	7	6	5	11,111	1
0.90	1×10^{-4}	1119	7	5	5	4	1111.0	1
0.90	1×10^{-3}	117	5	4	4	3	111	1

6th instar, a behaviour which is density dependent (van Rensburg *et al.*, 1987a). Furthermore, a significant positive relationship has been observed between the plant stand (which is very high, especially in irrigated systems where *B. fusca* resistance in South Africa is problematic) and the number of larvae that migrate successfully and survive on maize plants (van Rensburg *et al.*, 1988).

A reduction in the numbers of susceptible individuals due to pre-feeding movement from non-Bt to Bt plants could increase the potential for heterozygous (*RS*) larvae to survive and adults to mate, which could, in turn, lead to an increase in the incidence of homozygous (*RR*) resistant individuals in the offspring (Murphy *et al.*, 2010). The development of resistance may also occur when more mature larger larvae move from non-Bt to Bt plants, and in consequence are exposed to sublethal dosages of Bt proteins (Murphy *et al.*, 2010). This may lead to an increased risk of resistance development over time. A target pest exhibiting a more sedentary behaviour might, in contrast, not be affected in this way. Such insects (for example aphids) would endure longer exposure to Bt proteins throughout their life cycle (Gould, 2000).

Accordingly, the larval behaviour of all stem borer species that will be exposed to Bt maize in Africa will have to be considered in the development of IRM strategies.

Adult behaviour of Busseola fusca

Variability in pest biology and behaviour between different geographical regions may affect the exposure of pests to Bt maize, thereby influencing the rate of resistance evolution. Guse *et al.* (2002) and Onstad *et al.* (2002) showed that different stem borer species exhibit different adult behaviour patterns, particularly in mating, oviposition and male moth dispersal, and indicated that these interactions of landscape and insect behaviour must be understood in order to develop suitable resistance management strategies.

Agronomic practices such as fertilizer use, cultivation and irrigation practices all influence the landscape. The behaviours of *Diatraea grandiosella* (Lepidoptera: Crambidae) and *O. nubilalis*, for example, differ in irrigated and non-irrigated maize fields (Guse *et al.*, 2002; Onstad *et al.*, 2002). *B. fusca* moths also prefer to mate and move within moister vegetation than drier vegetation. This behaviour was indicated by van Rensburg (2007) as a possible factor that could have resulted in higher pest numbers in Bt maize than in non-Bt maize in South Africa, and thus contributed to resistance evolution. Guse *et al.* (2002) reported that this type of adult behaviour would strongly influence the evolution of resistance to Bt maize and that practices that would increase oviposition in natural refuges would delay resistance evolution because they would increase the source potential of refuges and reduce the intensity of selection.

Whereas *B. fusca* has only three generations per season in temperate South Africa, this situation is different in subtropical and tropical regions. Flight patterns of *B. fusca* moths in areas where only one rainy season occurs show two to three distinct generations with zero moth activity during the winter period (van Rensburg *et al.*, 1987b). However, less discernible patterns are observed in areas where maize is cultivated throughout the year (van Rensburg, 1997). Bimodal rainfall patterns, in effect, result in two cropping seasons a year, with large-scale staggering of planting dates. This phenomenon adds to landscape heterogeneity and consequently provides longer periods of availability of maize suitable for borer infestation. The possible effects of landscape heterogeneity on *B. fusca* exposure to Bt maize have not been considered before.

Other factors that can contribute to resistance evolution in *B. fusca* are that moths need no sexual maturation time and mating may start a few hours after moth emergence (Calatayud *et al.*, 2014), hence increasing the likelihood of non-random mating. Males can also mate several times, although only once a night, and polyandry is not obligatory and not necessary (Unnithan and Paye, 1990).

4.6 Insect Resistance Management in the African Context

Landscape heterogeneity is characteristic of African agroecosystems. This heterogeneity, in addition to between-field and within-field spatial mosaics resulting from variable gene expression in Bt maize, will be important in IRM. Adding to this landscape heterogeneity is the cultivation of OPV maize varieties and bimodal rainfall patterns that allow two maize cropping seasons a year in many subtropical and tropical areas. The role that these factors may play in resistance evolution to Bt maize in Africa will not have previously been considered.

Spatial mosaics resulting from variable gene expression within a field could significantly affect resistance evolution (Onstad and Carrière, 2013). For example, plant-gene expression and pollen dispersal, as well as typical African agricultural practices such as planting OPVs and seed saving, can significantly contribute to resistance evolution. Onstad and Carrière (2013) indicated that soil moisture, soil nutrients, herbivory and topography vary over space and influence the growth of plants and the production of toxin in these plants. Hence, expression of a gene for Bt production may vary among plants from a single cultivar over a crop field, creating a spatial mosaic of toxin doses. This type of spatial mosaic would be especially important in cases where neighbouring farmers plant multiple cultivars and where individual farmers plant both hybrid maize and OPVs on small plots of land.

One of the biggest potential contributors to both in-field and landscape-level spatial mosaics in terms of Bt protein expression levels is the cultivation of OPVs. Maize has a high risk of gene flow through cross-pollination, particularly when landholdings are fragmented, varieties are planted continuously and farmers recycle, exchange or mix maize seeds (Smale and De Groote, 2003).

Pollen dispersal between Bt and non-Bt plants (OPVs) and subsequent cultivation of F_1 and F_2 OPV seed by small farmers is a serious threat to the sustainable use of Bt maize. Cross-pollination could transform external refuges, in this case neighbouring farms on which OPVs are planted, into seed mixtures in subsequent cropping seasons. When larvae move between plants in such fields, the adventitious presence of Bt plants in OPV fields can be expected to accelerate resistance evolution. Aheto *et al.* (2013) showed that the potential for gene flow, be it in the form of pollen flow between fields or seed exchange between farmers, has significant potential to spread transgenes across landscapes. The combination of low-dose expression resulting from the cross-pollination of OPVs with Bt hybrids provides unique challenges to IRM in African farming systems.

Africa at large is particularly vulnerable to potential unintended and undesirable spread of genetically modified organisms (GMOs), with a consecutive mixing with non-GM material. Maize seed is easy to store and transport, and through pollen flow, traits can easily be transferred between varieties (Smale and De Groote, 2003). Throughout Africa, the cultivation of OPVs, seed saving and seed exchange are common (Smale and Phiri, 1998; Smale and DeGroote, 2003; Aheto *et al.*, 2013). This is also the case in South Africa, despite a strong and regulated private seed sector and seed production and marketing system (Mphinyane and Terblanché, 2005; Van den Berg, 2013).

4.7 Conclusion

Small-scale farming systems in Africa provide unique challenges to IRM strategies. Lessons learned from the case of *B. fusca* have shown that its biology is poorly understood and that the assumptions on which the high-dose refuge strategy is based are not warranted for this pest. Other challenges are in the form of large variability in stem borer ecology between regions, farming practices and the cultivation of OPVs.

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