Toxicity of four insecticides to *Spodoptera frugiperda* in South Africa

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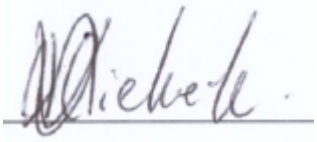
DECLARATION BY THE CANDIDATE

I, D.C. van Niekerk, declare that the work presented in this MSc thesis is my own work, that it is not been submitted for any degree or examination at any other University and that all the sources I have used or cited have been acknowledged by the complete reference.

Signature  Date: 03/12/2020

DECLARATION AND APPROVAL BY SUPERVISORS

We declare that the work presented in this thesis was carried out by the candidate under our supervision and we approve this submission.

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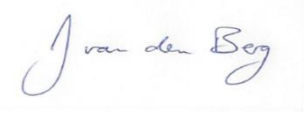
Signature:  Date: 03/12/2020

Abstract

The Fall Armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is described as one of the most important noctuid pests in the Americas. The invasion of the FAW in South Africa during 2017 poses a serious risk, especially for maize production. With 350 larval host plants reported, it is also described as one of the most destructive pests in agriculture. The high dependency on chemical control to effectively manage this pest has resulted in the development of resistance to numerous chemical classes. Since there is no baseline insecticide susceptibility data to the insecticides registered in South Africa, one of the objectives of this study was to estimate baseline susceptibility of three FAW populations to emamectin benzoate, spinetoram/methoxyfenozide and chlorpyrifos, using a leaf dip bioassay method as prescribed by the Insecticide Resistant Action Committee (IRAC). All three populations exhibited high susceptibility to emamectin benzoate and spinetoram/methoxyfenozide, while moderate to high levels of tolerance to chlorpyrifos were found. One of the many insecticides registered for FAW control in South Africa is pyridalyl, an insecticide which is considered to have a novel mode of action. Although pyridalyl has been reported to effectively control FAW in the Americas, no specific method is prescribed by which FAW susceptibility to pyridalyl can be evaluated in bioassays. In this study different bioassay methods, *viz*. leaf dipping, topical application, diet incorporation and diet overlay, were evaluated to find the most suitable method to assess the toxicity of pyridalyl to *S. frugiperda* larvae. The topical application bioassay proved to be the most suitable method for susceptibility evaluations of FAW. Fall armyworm was also shown to be highly susceptible to pyridalyl, and all these insecticides can therefore be included in an insect resistant management program for this pest in South Africa.

Key words: Fall armyworm, chemical control, baseline susceptibility, bioassay, IRAC

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Chapter 1: Introduction and Literature review

1.1 General introduction

With a global human population of approximately 7.7 billion and a growth rate of 1.2% per year (Roser *et al*., 2019), agriculture is under tremendous pressure to supply enough food and fiber (FAO, 2009; Kumar and Singh, 2014; Campos *et al.*, 2019). The potential that agriculture can offer must therefore be utilized to the maximum in a sustainable manner (Oerke, 2006; Kumar and Singh, 2014; Campos *et al.*, 2019). With most of the lands already cultivated, and many crop varieties already at their yield plateau, other measures need to be taken to ascertain that the maximum yield is harvested (Kumar and Singh, 2014; Campos *et al.*, 2019).

One of the aspects preventing maximum crop harvest, is damage by agricultural pests (Donatelli *et al.*, 2017; Campos *et al.*, 2019). Approximately 67 000 agricultural pests are responsible for a global reduction of 10-16% in agricultural yields before harvest (Kumar and Singh, 2014; Bradshaw *et al*., 2016). Without any prevention methods, these pests can cause a 70% reduction in production (Campos *et al.*, 2019).

Much of the damage done to crops globally can be attributed to invasive species (Cook *et al*., 2011; Paini *et al.*, 2016). The increase in international trade in the era of globalization, as well as global climate change, has increased the potential of organisms to invade and establish in new regions (Richardson *et al*., 2003; Hulme, 2009; Bradshaw *et al*., 2016). Biological invasions threaten biodiversity globally (Richardson *et al*., 2003), and is responsible for a minimum loss of US$70.0 billion per year (Bradshaw *et al*., 2016). In an estimation of the threat posed by invasive species to agriculture globally by Paini *et al*. (2016), South Africa is ranked as the 26th most threatened country to be invaded by alien species, which could have a potential economic impact of US$3.922.

The increase in infrastructure, rich biodiversity and well developed agricultural and forestry sectors in South Africa are all factors increasing the potential for the

introduction, establishment, and distribution of alien species in South Africa (Van Wilgen *et al*., 2001). The Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is one of these recent exotic species that invaded Africa (Goergen *et al*., 2016), including South Africa (IPPC, 2017).

1.2 Fall Armyworm, *Spodoptera frugiperda*

The Fall Armyworm (FAW) is a devastating polyphagous pest with more than 350 larval host plants reported (Montezano *et al.,* 2018). Fall Armyworm consists of two strains that differ from each other based on their host plant preferences, pheromone blend compositions, mating behaviour, and mating compatibility (Pashley, 1986; Schofl *et al.*, 2009; Schofl *et al.,* 2011; Nagoshi and Meagher, 2004; Unbehend *et al.,* 2013; Kost *et al.,* 2016). Although these strains are morphologically identical, they are genetically different due to different molecular markers at the nucleus and mitochondrial DNA (Pashley *et al.,* 1985; Lu and Adang, 1996; McMichael and Prowell, 1999; Nagoshi and Meagher, 2004; Prowell *et al.,* 2004; Nagoshi, 2010). Although both strains feed on maize (*Zea mays* (L.)), the rice strain (R strain) primarily feeds on rice (*Oryza sativa* (L.)) and various pasture grasses, while the maize strain (C strain) predominantly feeds on maize, cotton (*Gossypium hirsutum* (L.)), and sorghum (*Sorghum bicolor* (L.) Moench) (Pashley *et al.,* 1987; Veenstra *et al.,* 1995; Juarez *et al.,* 2014; Murúa *et al.,* 2015). Nagoshi *et al*. (2019) reported that FAW in Africa may be a novel interstrain hybrid population, with possible novel behavioral characteristics. As a result, data on the host range, long-distance migratory behaviour, and potential resistance to multiple pesticides depending on the origin of the initial population, are lacking, which could complicate its control in Africa (Nagoshi *et al*., 2019).

1.2.1 Biology and life cycle

Fall Armyworm is a multivoltine insect, (more than one generation per year) and a holometabolic life cycle (Figure 1.1) (Walton and Luginbill, 1916). Depending on environmental conditions, the life cycle can last from 30 to 90 days (Vickery, 1929). Generations of FAW can overlap and all life stages can therefore be found simultaneously in a single field (Harrison *et al.*, 2019). The pest does not enter into

diapause and can therefore infest cropping systems throughout the year (Johnson, 1987).

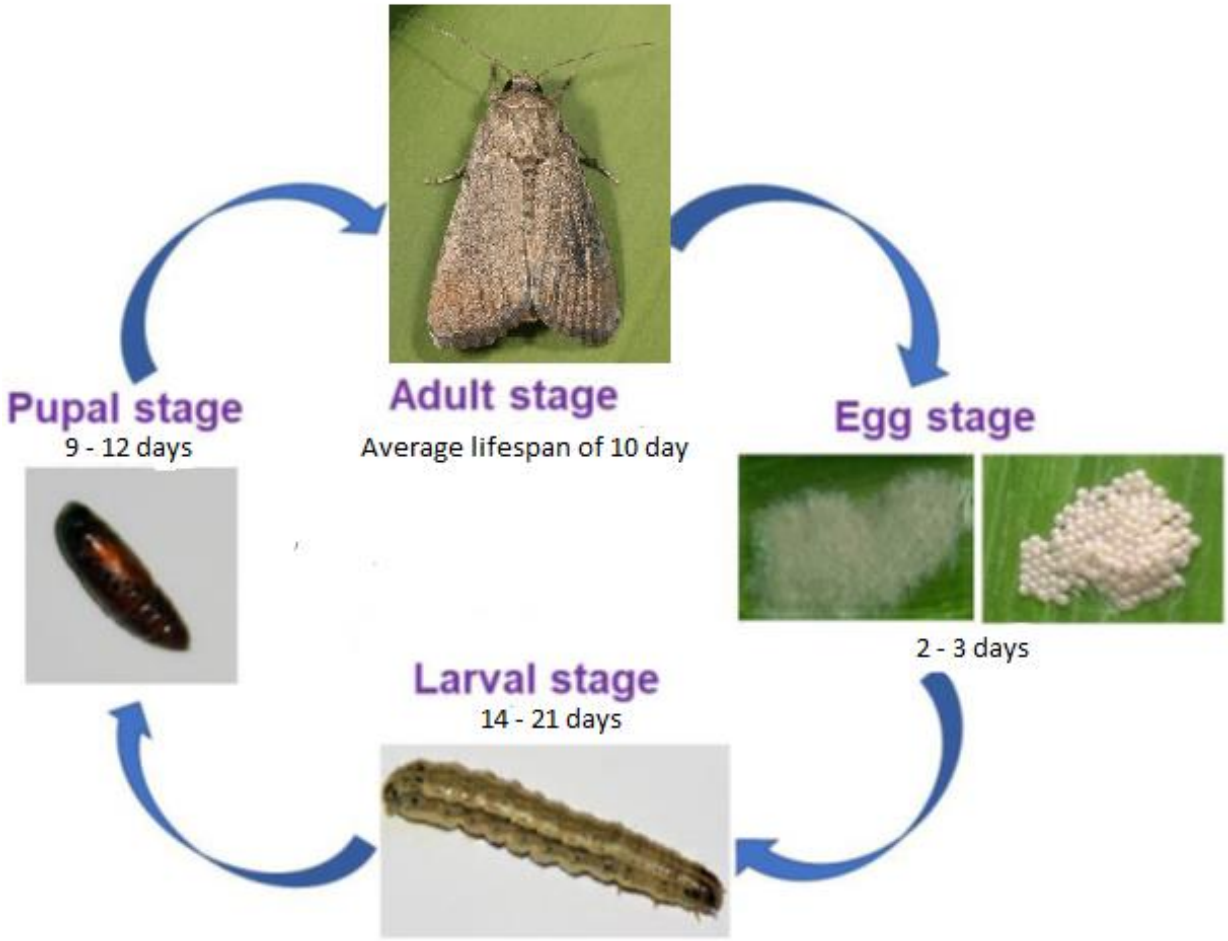

Figure 1.1: Life cycle of the Fall Armyworm (Tefera *et al*., 2019).

Female moths lay eggs in clusters of between 50 to several hundred at night and can produce approximately 1500 eggs over her lifespan, but can exceed 2000 (Walton and Luginbill, 1916; Johnson, 1987; Sharanabasappa *et al*., 2018b). Eggs are laid in a single layer or in several layers (Capinera, 2017). Eggs have a dome shape (oblate-spheroidal) with a height of approximately 0.3 mm and a diameter of 0.4 mm, and is sometimes covered with white scales from the moth (Figure 1.2) (Luginbill 1928; Deole and Paul, 2018). Newly laid eggs are white to green in colour and darken over time until brown and eventually black (Luginbill 1928; Sharanabasappa *et al*., 2018b). These eggs usually emerge after two to three days under optimal conditions (Capinera, 2017; Sharanabasappa *et al*., 2018b). As neonates eclose, they feed on their own egg mass and the surrounding plant material (Walton and Luginbill, 1916;

Hardke *et al*., 2015). To avoid interspecific competition and cannibalism, young larvae quickly disperse to surrounding plants by either crawling or ballooning (Pannuti *et al.,* 2016; Bentivenha *et al.*, 2017). By means of the wind, first and second instar larvae can distribute to neighbouring plants by means of ballooning on silk threads (Pannuti *et al.,* 2016).

Figure 1.2: (a) Eggs with scales, and (b) newly hatched larvae (Huesing *et al*., 2018).

Fall Armyworm has six to seven larval instars. The development time for larvae (from first instar to prepupa) can range from 14 to 21 days during warmer temperatures (Sharanabasappa *et al*., 2018b), and up to 50 days in colder temperatures, but it also depends on food availability (Luginbill, 1928; Pitre and Hogg, 1983; Hardke *et al*., 2015). First instar larvae are light green in colour with a black head capsule (Hardke *et al*., 2015; Deole and Paul, 2018; Sharanabasappa *et al*., 2018b). As larvae grow, colours vary from light green to brown with final instars dark in colour (Hardke *et al*., 2015). Average lengths of larvae can range from 1.7 mm, for first instars, to 34 mm for final instars (Hardke *et al*., 2015; Capinera, 2017). Fall Armyworm larvae are typically characterised by a prominent inverted “Y” on their head capsules, as well as four prominent spots on the 8th abdominal segment (Figure 1.3) (Hardke *et al*., 2015; Sharanabasappa *et al*., 2018b).

Figure 1.3: Characteristic marks on *S. frugiperda* (Huesing *et al*., 2018).

Final instar larvae leave the plant and burrow into the soil to pupate (Luginbill, 1928; Pitre and Hogg, 1983), usually at a depth of 2 – 8 cm, depending on the soil texture, moisture and temperature (Sparks, 1979; Pitre and Hogg, 1983; Hardke *et al*., 2015). Pupation can also occur on the host plant itself or on hard soil where loose debris is used to form a cocoon (Capinera, 2017). Pupae are initially light green in colour and darken to red-brownish (Hardke *et al*., 2015; Capinera, 2017; Deole and Paul, 2018). The period from the formation of the pupa until moth emerge can range from 9 – 12 days (Da Silva *et al.,* 2017; Sharanabasappa *et al*., 2018b).

Clear distinction between sexes can be seen in the adult phase. The forewing of male moths has a blotchy grey and brown appearance with a triangular white spot on the tip of the wing (apical region), as well as a circular white spot at the centre (Figure 1.4). The forewing of females is less prominently marked, with a more uniform greyish to brown colour (Figure 1.5). The hind wing from both sexes are silver to white in colour with a narrow brown border (Luginbill, 1928; Oliver and Chapin, 1981; Sharanabasappa *et al*., 2018b). The wingspan of adults can range from 25 to 35 mm (Oliver and Chapin, 1981; Sharanabasappa *et al*., 2018b). The average lifespan of a FAW adult is 10 days (Capinera, 2017). These moths are nocturnal, and most active at night (Johnson, 1987). Females can mate multiple times with different males. Oviposition usually occurs during the first four to five days of the female moth's life

(Sharanabasappa *et al*., 2018b), however oviposition after 17 days has been reported (Johnson, 1987).

Figure 1.4: Adult male *S. frugiperda*. Photograph by Lyle J. Buss, University of Florida.

Figure 1.5: Adult female *S. frugiperda*. Photograph by Lyle J. Buss, University of Florida.

1.2.2 Host plants and damage

Fall Armyworm larvae are classified as polyphagous at individual level, since a larva can feed on more than one host plant during its development (Montezano *et al.,* 2018). A recent literature review reported 353 *S. frugiperda* larval host plants in 76 plant families (Montezano *et al.,* 2018). Fall Armyworm larvae are known to attack grain

crops such as maize and sorghum (Huesing *et al*., 2018; Montezano *et al.,* 2018), but also other crops such as cotton (*Gossypium hirsutum* (L.)), soybeans (*Glycine max* (L.) Merr.), rice (*Oryza sativa* (L.)), tobacco (*Nicotiana tabacum* (L.)) and wheat (*Triticum aestivum* (L.)) (sorghum; Pashley, 1988; Hardke *et al*., 2015).

Larvae hide during the day and therefore, most of the damage is done in the evening, early in the morning and late afternoon (Hardke *et al*., 2015; Abrahams *et al.*, 2017). Host plant parts consumed by *S. frugiperda* larvae depend on the crop and its development phase, as well as the larval stage (Goergen *et al.,* 2016; Deole and Paul, 2018). On maize, first-instar larvae cause a characteristic window effect when feeding from one side of the leaf whilst the opposite epidermal layer remains intact (Figure 1.6) (Barlow and Kuhar, 2009; Abrahams *et al.*, 2017; Deole and Paul, 2018; Sisay *et al.,* 2019). Second and third-instar larvae are responsible for creating holes in the leaves and eat from the edges of the leaf inwards (Figure 1.6) (Barlow and Kuhar, 2009). The final instar can cause substantial defoliation, being able to consume up to 77% of the plant material, leaving it with a tattered and torn appearance (Abrahams *et al.,* 2017). Later instar larvae feed inside the whorl of maize plants where they are protected from insecticide applications (Abrahams *et al.*, 2017) and creating a frass plug, making control measures difficult (Deole and Paul, 2018). In young plants, larvae are able to cut stems, and also tunnel into developing reproductive structures, such as the growth point which can cause ‘dead heart’, preventing any ears from forming (Johnson, 1987; Goergen *et al.,* 2016; Abrahams *et al.*, 2017; Deole and Paul, 2018).

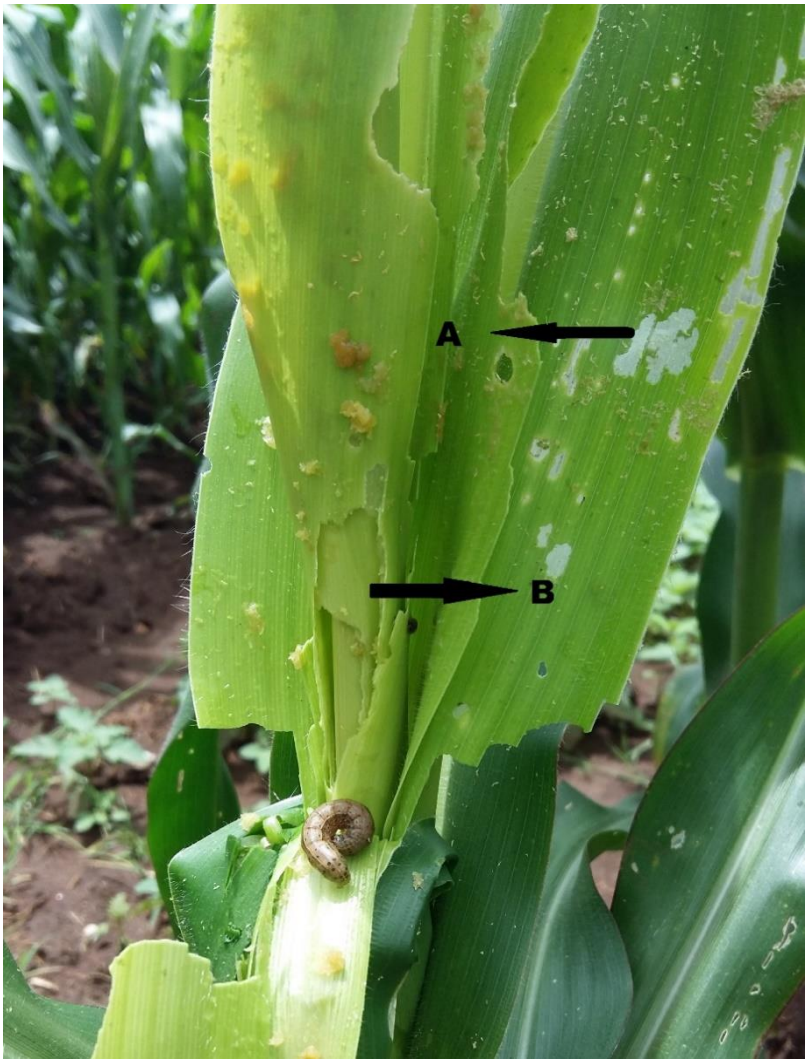

Figure 1.6: Damage caused by *S. frugiperda* larvae on maize: (A) windowing damage from first instars larvae; (B) holes caused by older larvae.

1.2.3 Distribution

Spodoptera frugiperda is considered to be one of the most important noctuid pests in the Americas, and occurs widespread across the Eastern and Central parts of North America as well as in South America (Luginbill, 1928; Vickery, 1929; Johnson, 1987; Nagoshi *et al.,* 2012; Murúa *et al.*, 2015). It is indigenous to the tropical and sub-tropical regions of the Americas (Sparks, 1986) and has recently invaded Africa, Asia, and Australia (Goergen *et al*., 2016; Sharanabasappa *et al.,* 2018a; Xiao-xu *et al*., 2019; IPPC, 2020).

The presence of FAW in Africa was first reported in early 2016 from several Western and Central African countries (Benin, Nigeria, Sao Tome and Principe, and Togo) (Goergen *et al*., 2016). From there it spread rapidly, invading a total of 44 countries in Africa in only two years (Feldmann *et al*., 2019). The first positive identification of the invasive FAW outside Africa was made in India in July 2018 (Ganiger *et al.,* 2018; Sharanabasappa *et al.,* 2018a) and the pest has since been present in many other countries in Asia, such as China (Jing *et al.,* 2019), Sri Lanka (Perera *et al.,* 2019) and Korea (Lee *et al.,* 2020), and Australia (IPPC, 2020) (Figure 1.7).

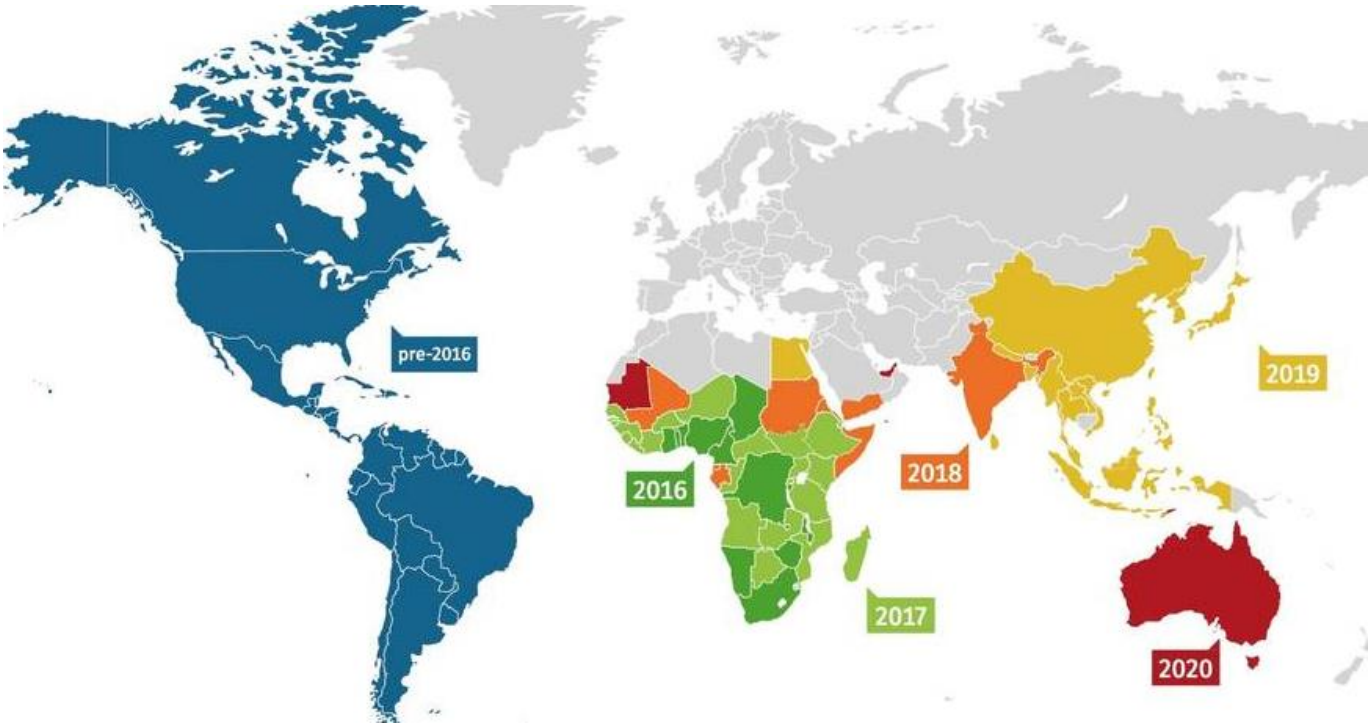

Figure 1.7: Worldwide distribution of *S. frugiperda* since 2016 (FAO, 2020).

Molecular data suggested that FAW introduced into Africa originates from the Caribbean region and Eastern parts of United States (Nagoshi *et al.,* 2017). Introduction into Africa could have been as eggs, larvae, pupae or moths, or a combination of these (Cock *et al*., 2017). A framework on types of pathways from Hulme *et al*. (2008) was used by Cock *et al*. (2017) to speculate on the possible pathways in which FAW entered Africa. Of the six possible pathways suggested, only three is applicable in this case: unaided dispersal, contaminant of a commodity, and stowaway on a vector (Cock *et al*., 2017). However, Early *et al.* (2018) stated that unaided dispersal is highly unlikely due to prevailing winds being from East to West. It is also highly unlikely that FAW entered as a contaminant of a commodity, as there is

minimal trade in fresh produce between America and Africa (Early *et al*., 2018). The more likely way of introduction into Africa was through transfer as a stowaway on a flight. The countries in West Africa which were initially invaded by FAW, is those which also have the major air transportation hubs and their warm, moist climates matchup with the climates of the regions many flights arriving from (Early *et al.,* 2018).

The rapid spread within Africa, can be attributed to unaided dispersal by flight, as well as national and international transportation (Faulkner *et al*., 2017; Early *et al.,* 2018). Lepidopteran species are known for their long dispersal distances (Johnson, 1969; Rose *et al.*, 1975; Westbrook *et al.,* 2016). Fall Armyworm is described as one of the most mobile noctuid pests in the Western Hemisphere (Johnson, 1969), able to migrate over long distances. It is a very important component of its survival strategy (Johnson, 1969; Nagoshi and Meagher, 2008). Fall Armyworm moths can fly up to 500 km before oviposition occurs (Ashley *et al*., 1989). However, the direction and speed largely depend on prevailing winds (Johnson, 1987). Fall Armyworm moths fly just above the lowest part of the atmosphere and can therefore disperse over long distances when the wind pattern is favourable (Glick, 1939). One of these FAW moth migrations of 1600 km in 30 hours was reported by Rose *et al.* (1975).

In South Africa, the first positive identification was made in January 2017 from farms in the Limpopo and North West provinces (IPPC, 2017). Currently, this pest has been identified in all nine provinces of South Africa (CropLife, 2018), where it causes major damage to maize crops. Since maize is the most important grain crop in South Africa, the presence of FAW holds serious economic risks for farmers in South Africa (IPPC, 2017).

1.2.4 Economic importance

Spodoptera frugiperda has the largest number of host plants compared to its congeneric species, *viz*. *Spodoptera albula* (Walker), *Spodoptera cosmioides* (Stoll), *Spodoptera dolichos* (Fabricius) and *Spodoptera eridania* (Stoll) (Montezano *et al.,* 2018). With its establishment in Africa, major damage to economically important crops such as rice, sorghum, maize, and sugarcane is done, with maize being the most

affected crop in all invaded countries (Goergen *et al.,* 2016; Kumela *et al.,* 2018). The damage by FAW to the maize industry in Africa in one year, was estimated at US$3 billion by Stokstad (2017). Without any control measures applied, the threat posed by FAW to maize yields in only 12 of Africa's maize producing countries, was estimated at 8.3 to 20.6 M metric tons per year, with a value of between US$2.48 billion and US$6.19 billion (Day *et al*., 2017). CIMMYT (2018) also stated that FAW could cause US$3-6 billion in damage to Africa's food industry annually.

Traits that make this pest more devastating than other invasive pests, includes its polyphagous nature (Montezano *et al*., 2018), migratory capability (Rose *et al.*, 1975; Nagoshi and Meagher, 2008; Westbrook *et al.,* 2016), multi-voltinism (Vickery, 1929), and short lifecycle (Walton and Luginbill, 1916). The pest can persist throughout the year where it is able to build up and maintain populations outside the main cropping season or area, due to its polyphagous nature (Favetti *et al.,* 2017; Montezano *et al.,* 2018).

Other potential economic impacts include: reduction of regional and international trade, a decrease in availability of seed to farmers in future growing seasons; consequent food security impacts; effects on natural and financial capital; and an increase in pest migration risk to Europe, Asia, and Australia (Abrahams *et al.,* 2017; Day *et al.,* 2017). Since there is a high likelihood that FAW will remain a serious problem in Africa, it is necessary to apply the correct crop protection measures (Huesing *et al*., 2018).

1.2.5 Control

Crop protection is defined as the methods used to prevent and control crop losses by agricultural pests (Oerke, 2006). Integrated pest management (IPM) is built on ecological and economic principles and social considerations, where several strategies are used to protect crops by keeping the numbers of the insect populations in check and limiting damage (Kogan, 1998). It is a sustainable, science-based, decision-making process that combines biological, cultural, physical, and chemical tools to identify, manage, and reduce risk from pests and pest management tools and

strategies in a way that minimizes overall economic, health, and environmental risks (USDA-ARS, 2018). Integrated pest management strategies that combines the use of biological, cultural, and chemical control is foreseen as the only sustainable strategy to manage FAW (Sisay *et al.,* 2018).

1.2.5.1 Biological control

Biological control is defined by Eilenberg *et al.* (2001) as “the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be”. Natural enemies can include predators, parasitoids, nematodes, fungi, bacteria, protozoa, and viruses (Tefera *et al.,* 2019). Biological control is a promising management tool against FAW, as many successful parasitoids have been documented (Ashley, 1979; Pair *et al.,* 1986; Wheeler *et al*., 1989; Murúa *et al.,* 2009; Meagher *et al.,* 2016). Molina-Ochoa (2003) put together an inventory of parasitoids and parasites of FAW in the Americas and Caribbean basin and reported approximately 150 species. Four new hymenopteran and one dipteran parasitoid species were reported for FAW in Africa by Sisay *et al*. (2018), which have not previously been reported from Africa, North or South America. Being a safe and sustainable option, biological control is an important pillar in the IPM approach against FAW (Tefera *et al.,* 2019).

1.2.5.2 Genetically modified crops

Genetically modified crops are hybrid plants that express crystal proteins (Cry-proteins) from the bacterium *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae), which is lethal to certain lepidopteran insects when ingested (Stewart *et al*., 2001; Wu *et al*., 2003; Kumar and Kumar, 2004; Chen *et al.,* 2016). Bt maize events control *S. frugiperda* effectively in the United States, Canada (Buntin *et al*., 2004; Siebert *et al*., 2012; Storer *et al*., 2012; Reay-Jones *et al*., 2016) and in South American countries (Buntin, 2008; Bernardi *et al*., 2016). The pest did, however, evolve resistance to Bt maize in Puerto Rico (Matten *et al*., 2008, Storer *et al*., 2010) and in Brazil (Farias *et al*., 2014; Huang *et al*., 2014; Omoto *et al*., 2016).

1.2.5.3 Cultural control

Cultural control is defined as the manipulation of the environment in such a way that the establishment and distribution of pest populations is reduced, while maintaining the best possible conditions for high crop productivity (Gabryś and Kordan, 2013). Agro-ecological practices such as reduced soil disturbance and mulching of residues (Clark, 1993; Rivers *et al*., 2016), intercropping (Perfecto and Sediles, 1992; Midega *et al.,* 2018) and filling leaf whorls with soil, wood ashes and tobacco extracts (Kumela *et al.,* 2018) are used to reduce FAW survival, colonization, and reproduction, by promoting soil fertility, increasing biodiversity, and making the environment unfavorable for the pest (Harrison *et al.,* 2019).

1.2.5.4 Chemical control

Chemical control is the most common approach used to control FAW (Abrahams *et al.*, 2017; Sisay *et al.,* 2019). For example, in Brazil an average of five insecticidal applications per maize cycle are required for necessary control (Ribeiro *et al.,* 2014). Effective chemical control is dependent on monitoring and early detection of infestations (Abrahams *et al.,* 2017). Infestation of FAW larvae are generally discovered when larvae are in their late instars (Hardke *et al.,* 2011). Chemical application of large larvae has proven to be difficult, due to larger larvae tunnelling into the whorl, avoiding contact with insecticides (Young, 1979). Tall and high-density canopies also prevent insecticides from reaching insects deep inside the whorl, which subsequently require a larger volume of insecticide solution (Young, 1979). Challenges posed by chemical control are the potential for resistance development (Tabashnik *et al.,* 1987; Lietti *et al.,* 2005; Shad *et al.,* 2012; Saleem *et al.,* 2016), as well as environmental and human health risks (O'Brien *et al.,* 2016). Despite these constraints, chemical control remains a safe and effective method of controlling insect pests if used according to label directions (Huesing *et al*., 2018).

1.3 Resistance development

Insecticide resistance is a serious problem that influences insect control and pest management globally (Sparks and Nauen, 2015). Insecticide resistance is defined by Tabashnik *et al.* (2014) as a "genetically based decrease in susceptibility to a pesticide". Due to this decrease in susceptibility, insecticides are unable to provide effective control to pest populations when applied according to label recommendation (IRAC, 2019). Resistance to DDT was already detected in 1947 in houseflies (Pimentel and Dewey, 1950). The first paper documenting insecticide resistance dates back to more than a century ago (Melander, 1914), and since then, more than 600 different insect species have become resistant to approximately 400 different compounds (Figure 1.8) (Sparks *et al.,* 2020).

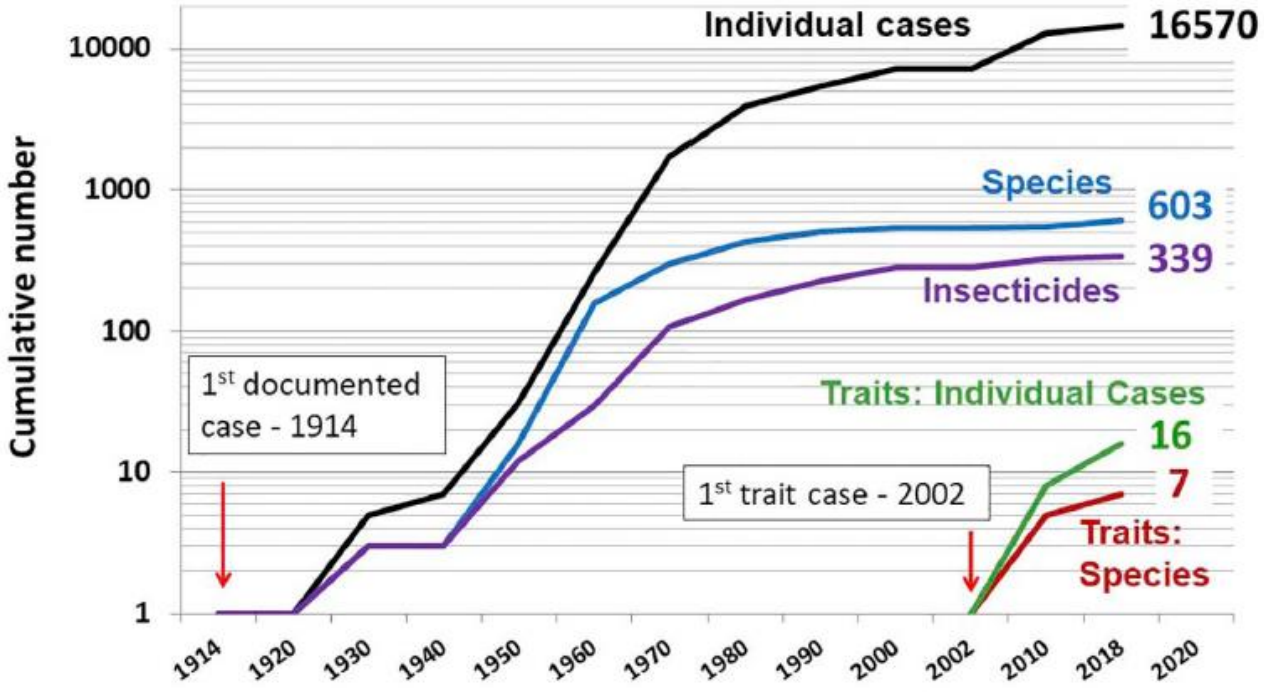

Figure 1.8: Increase over time in (a) total number of species recorded as resistant against one or more insecticides (blue line), (b) insecticides to which resistance has been recorded (purple line), and (c) amount of GMO traits to which resistance has been reported (red line) (Sparks *et al.,* 2020).

The process of resistance development in an insect population is presented in Figure 1.9. Genetic variability occurs in any population. This means that there are individuals with susceptible genotypes, as well as individuals with natural resistant genotypes,

although initially rare. When a specific insecticide is applied, susceptible genotypes are killed, while resistant genotypes survive and reproduce, increasing the frequency of resistant genes (Alyokhin *et al*., 2013). When insecticides with the same mode of action (MoA) are used on a continuous basis, it will lead to the selection of resistant individuals of the specific insect pest, and subsequently to insecticide control failure (Georghiou, 1972).

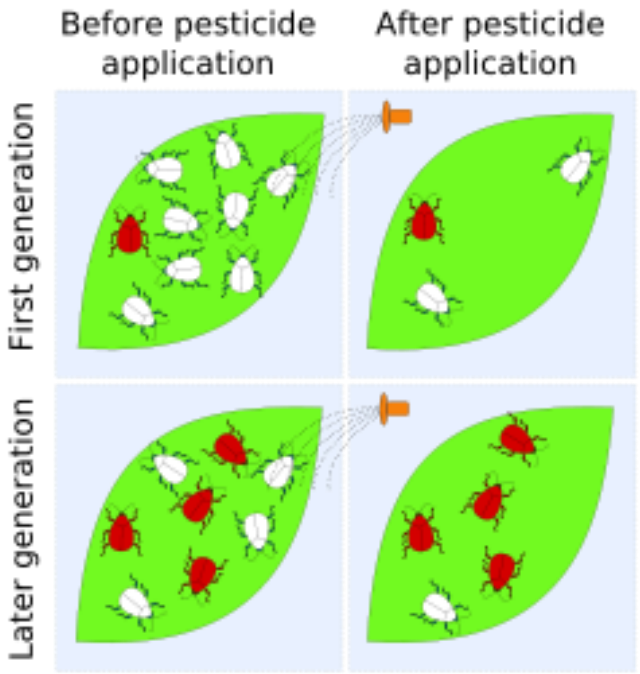

Figure 1.9: Resistance development in insect population (Karaağaç, 2002).

Continuous high dose insecticide applications to FAW populations have led to the development of resistance to many insecticide classes (Yu, 1991; Yu, 1992; Do Nascimento *et al.,* 2016; Okuma *et al.,* 2018; Gutiérrez-Moreno *et al.*, 2019). For example, resistance to organophosphates, carbamates, pyrethroids, spinosyns, benzoylureas and diamides in FAW have already been reported in Mexico and Puerto Rico (Carvalho *et al.,* 2013; Do Nascimento *et al.,* 2016; Okuma *et al.,* 2018; Bolzan *et al.,* 2019; Gutiérrez-Moreno *et al.*, 2019). Currently, 146 cases of insecticide resistance have been reported globally for FAW (Mota-Sanchez and Wise, 2020: Arthropod Pesticide Resistance Database). One of the first reports of chemical control failure to FAW was in 1978 in Alabama, USA, where synthetic pyrethroids could not provide effective control to FAW with standard chemical recommendations (Bass,

1978). Resistance mechanisms to synthetic insecticides in FAW are a wide researched field (McCord and Yu, 1987; Yu, 1991; Wheeler *et al.,* 1993; Yu *et al.,* 2003; Carvalho *et al.,* 2013; Do Nascimento *et al.,* 2015; Giraudo *et al.,* 2015; Carvalho *et al.,* 2018), and is mainly mediated by metabolic resistance and target-site insensitivity (Yu, 1991; Yu, 1992; Yu *et al.,* 2003; Carvalho *et al.,* 2013).

1.3.1 Metabolic resistance

Metabolic resistance is the detoxification and/or sequestering of toxic compounds by metabolic enzymes into a form that is easily excreted (Bullangpoti *et al.,* 2012; Després *et al.,* 2007; Carvalho *et al.,* 2013; Giraudo *et al.,* 2015). Detoxification enzymes that are involved in resistance development are Cytochrome P450, Carboxylesterases, and Glutathione-S-transferase. Cytochrome P450 and carboxylesterase are described as Phase One enzymes in a detoxification process and are responsible for converting toxic compounds into more easily excretable substances by means of an oxidation, reduction and/or hydrolyses process (Satoh and Hosokawa, 2006; Carvalho *et al.,* 2013). In Phase Two, Glutathione-S-transferase mediates the process by which Phase One products bind to endogenous intermediates to be excreted (Kirby and Ottea, 1995; Després *et al*., 2007).

Phenotypic plasticity can also influence metabolic resistance in FAW. *Spodoptera frugiperda'*s diet also plays an important role in the susceptibility of insecticides. Plant substances, such as allelochemicals, can induce or inhibit the activity of Cytochrome P450 and Glutathione-S-transferase, which can either enhance or diminish tolerance to insecticides (Yu, 1982; Wheeler *et al.,* 1993; Yu and Abo-Elghar, 2000; Bullangpoti *et al.,* 2001; Yu, 2002). For example, allelochemicals such as xanthotoxin, flavonoids, monoterpenes, indoles and menthol are known to induce the activity of Cytochrome P450 and increase the tolerance to insecticide exposure (Yu and Ing, 1984; Brown *et al.,* 2005; Giraudo *et al.,* 2015).

1.3.2 Target-site resistance

Genetic mutations in target-site proteins can alter the characteristics and toxicity rate of an insecticide and/or cause insensitivity of the target receptor, which consequently causes resistance (target-site resistance) (Feyereisen, 1995; Carvalho *et al.,* 2013). Resistance in FAW attributed to gene mutations is often due to amino acid substitutions at the acetylcholinesterase (AChE) (A201S, G227A, and F290V), voltage-gated sodium channel (VGSC) (T929I, L932F, and L1014F) (Carvalho *et al.,* 2013), ryanodine receptor (RyR) (I4790M and G4946E) (Boaventura *et al.,* 2020a) and ATP-binding cassette subfamily C2 transporter (ABCC2) (Boaventura *et al*., 2020b), which targets organophosphates and carbamates, pyrethroids, diamides and Bt proteins, respectively.

1.4 Resistance management

The Insecticide Resistance Action Committee (IRAC) is an internationally associated crop protection company dedicated to the production of effective insect resistance management (IRM) (Sparks and Nauen, 2015; IRAC, 2019). Insect resistance management (IRM) refers to the action taken to prevent or delay the evolution of pest resistance in target pest populations (Sparks and Nauen, 2015). Part of the objectives of successful IRM is to regain susceptibility in already resistant pest populations (Sparks and Nauen, 2015; IRAC, 2019). To accomplish these objectives, IRM strategies seek to limit the selection pressure toward any particular pest control measure. The basis of any IRM plan is built on the biology of target pests and pest-crop interaction (CropLife, 2011).

1.4.1 Toxicity bioassays

Bioassays seek to evaluate the toxicity of insecticides with different MoAs to insects of the same species under the same test conditions (Paramasivam and Selvi, 2017). The toxic reaction of an insecticide to an insect is dose dependent (Paramasivam and Selvi, 2017). Toxicity bioassays are used as a quantitative approach to determine the relationship between the potency of a chemical agent (insecticide) and a biological

indicator (insect) (Bliss,1957; Hoskins and Craig, 1962; Zhu, 2008). This relationship is known as a dose-response relationship and is expressed on a dose-response curve as a log dose-probit (ld-p) line (Hoskins and Craig, 1962; Zhu, 2008).

A bioassay performed on a population that has not yet been exposed to the specific chemical that is being tested will yield an ld-p line called the baseline (Hoskins and Craig, 1962). The change in ld-p line of a population which is continuously exposed to the insecticide, compared to the baseline, is used to monitor the change in insecticide toxicity (Hoskins and Craig, 1962). From the ld-p line, the median lethal dosage (LD_{50}) can be determined, which is the dosage per unit weight that causes 50% mortality of the test population (Finney, 1971; Zhu, 2008). Since it is not always possible to determine the dose taken up by the insect, an LC_{50} (lethal concentration) value is used to denote the concentration of the external media required to cause 50% mortality of the test population (Paramasivam and Selvi, 2017). The LC_{50} value reflects the toxicity of an insecticide to an insect population can be referred back to in future susceptibility tests (Miller *et al.*, 2010; Paramasivam and Selvi, 2017). A change in LC_{50} value for a specific insecticide population to an insecticide demonstrates the change in susceptibility to the insecticide and is used to detect resistance development (Paramasivam and Selvi, 2017).

Toxicity bioassays can therefore reflect the susceptibility of the test population to a specific insecticide and detect any change in the efficacy of an insecticide in order to change the necessary control strategies before developing resistance (Miller *et al.*, 2010). IRAC has developed and validated several bioassay methods for different mode of action insecticides to present data that will closely correlate with field conditions (IRAC, 2019). The ideal bioassay method should be fast, efficient, replicable, consistent, and accurately reflect field conditions (Kranthi, 2005). Commonly used methods include leaf dipping-, topical application-, diet-incorporation- and diet overlay bioassays (Kranthi, 2005; Paramasivam and Selvi, 2017).

1.4.2 Bioassay methods

1.4.2.1 Leaf dipping

Leaf dipping bioassays are used to evaluate insecticides which primary route of insect toxification is through ingestion (Kranthi, 2005). In this bioassay, pieces of leaf material are dipped into different insecticide solutions of known concentrations for a definite period of time (Paramasivam and Selvi, 2017). After leaf pieces have been air dried, a known number of insects are inoculated onto the leaves and evaluated (Ffrench-Constant and Roush, 1990; Paramasivam and Selvi, 2017). This method enables the evaluation of much larger sample sizes, which compensates for any variability in results (Ffrench-Constant and Roush, 1990). Leaf dipping bioassays also simulate insecticide field exposure more accurately, facilitating resistance detection (Ffrench-Constant and Roush, 1990; Kranthi, 2005).

1.4.2.2 Topical application

This method is commonly used to evaluate the efficacy of contact insecticides (Kranthi, 2005). In this bioassay, a known concentration of the insecticide, in known amounts, is dispensed accurately directly onto a chosen location on individual insects (Ffrench-Constant and Roush, 1990; Kranthi, 2005). One advantage topical assay has over other bioassay methods is that this method gives an idea of the dose penetrating the insect, which makes it valuable for resistance monitoring (Ffrench-Constant and Roush, 1990). Other advantages include its robustness, efficiency and reproducibility, as well as being able to test many specimens in a relative short time (Paramasivam and Selvi, 2017).

1.4.2.3 Diet incorporation

This method is used to evaluate oral insecticides (Perry *et al*., 1998; Kranthi, 2005). This method requires different insecticide concentrations to be mixed into an artificial diet. A known number of insects are released on the diet and evaluated. Although it is an easy bioassay method, factors such as thermal stability, consistent bioactivity and

availability of large amounts of toxins can a determined factor in the bioassay (Kranthi, 2005).

1.4.2.4 Diet overlay

This method is similar to diet incorporation bioassay and is also used for insecticides that act as stomach poisons (Mascarenhas and Boethel, 1997). Instead of incorporating the insecticide solutions into the artificial diet, the serial insecticide solutions are applied as overlays onto the artificial diet (Mascarenhas and Boethel, 1997).

1.5 Insecticides in South Africa

With the introduction of FAW in South Africa several insecticides received emergency registrations. Currently, these registrations consist of 21 active ingredients with 9 modes of action (Table 1.1) (Labels of the respective insecticides are available at https://www.agri-intel.com/label-information/search-registration-information/). Among these active ingredients are emamectin benzoate, a mixture of spinetoram and methoxyfenozide, chlorpyrifos and pyridalyl.

Table 1.1: Insecticides currently registered in South Africa (compiled from labels available at https://www.agri-intel.com/label-information/search-registration-information/).

Active ingredient	Mode of Action	Resistance group (IRAC)	Target Site
Bacillus thuringiensis	Microbial disruptors of insect midgut membranes	11	Midgut
Beauveria bassiana	Microbial disruptors of insect midgut membranes	11	Midgut
Benfuracarb + fenvalerate	Acetylcholinesterase (AChE) inhibitors and Sodium channel modulators	1A and 3	Nerve and muscle
Beta-cypermethrin	Sodium channel modulators	3A	Nerve and muscle
Carbosulfan	Acetylcholinesterase (AChE) inhibitors	1A	Nerve and muscle
Chlorantraniliprole	Ryanodine receptor modulators	28	Nerve and muscle
Chlorantraniliprole + lambda-cyhalothrin	Sodium channel modulators and Ryanodine receptor modulators	3 and 28	Nerve and muscle
Chlorpyrifos	Acetylcholinesterase (AChE) inhibitors	1B	Nerve and muscle
Chlorpyrifos + cypermethrin	Acetylcholinesterase (AChE) inhibitors and Sodium channel modulators	1B and 3B	Nerve and muscle
Chlorpyrifos + lambda-cyhalothrin	Acetylcholinesterase (AChE) inhibitors and Sodium channel modulators	1B and 3A	Nerve and muscle
Diflubenzuron	Inhibitors of chitin biosynthesis affecting CHS1	15	Growth
Emamectin benzoate	Glutamate-gated chloride channel (GluCl) allosteric modulators	6	Nerve and muscle
Emamectin benzoate + lufenuron	Glutamate-gated chloride channel (GluCl) allosteric modulators and Inhibitors of chitin biosynthesis affecting CHS1	6 and 15	Nerve and muscle and growth
Flubendiamide	Ryanodine receptor modulators	28	Nerve and muscle
Indoxacarb	Voltage-dependent sodium channel blockers	22A	Nerve and muscle
Indoxacarb + novaluron	Voltage-dependent sodium channel blockers and Inhibitors of chitin biosynthesis affecting CHS1	22A and 15	Nerve and muscle and growth
Lufenuron	Inhibitors of chitin biosynthesis affecting CHS1	15	Growth
Mercaptothion	Acetylcholinesterase (AChE) inhibitors	1B	Nerve and muscle
Methomyl	Acetylcholinesterase (AChE) inhibitors	1A	Nerve and muscle
Pyridalyl	Compounds of unknown or uncertain MoA	UN	Unknown
Spinetoram	Nicotinic acetylcholine receptor (nAChR) allosteric modulators	5A	Nerve and muscle
Spinetoram + methoxyfenozide	Nicotinic acetylcholine receptor (nAChR) allosteric modulators and Ecdysone receptor agonists	5A and 18	Nerve and muscle and growth

1.5.1 Emamectin benzoate

Emamectin benzoate, a semi-synthetic derivative salt of an abamectin, avermectin B1, is one of the important macrocyclic lactone insecticides belonging to the avermectin group (Group 6 of IRAC classification scheme) (Jansson *et al.,* 1997). Avermectins are glutamate-gated chloride channel (GluCl) allosteric modulators. Avermectins activate chloride channels to open into nerve cells. This activation causes a production of a chloride ion flux which causes a disruption in cell function and nerve impulses (Jansson *et al.,* 1997). Intoxication with avermectins therefore results in starvation due to irreversible paralysis that causes death (Ishaaya *et al.,* 2002). The primary route of insect toxification in avermectins is through ingestion but can also be via contact (Jansson *et al.,* 1997). Emamectin benzoate was discovered in 1984 and was first registered in Japan and the U.S. in 1997 (Jansson *et al.,* 1997). Emamectin benzoate has proven to be very potent to various lepidopteran pest species, such as *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Teja *et al.,* 2019), *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) (Abdel-Baky *et al.,* 2019), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Bird *et al.,* 2017) and *S. frugiperda* (Zhao *et al*., 2020). No cases of resistance in FAW to emamectin benzoate have been reported yet.

1.5.2 Spinetoram

Spinetoram is a spinosyn insecticide and belongs to Group 5A of IRAC classification scheme. Spinetoram is a mixture of two spinosyn derivatives that originates from the soil bacterium *Saccharopolyspora spinosa* (Mertz and Yao) (Actinomycetales: Pseudonocardiaceae) (Sparks *et al.,* 2008). Spinosyns are nicotinic acetylcholine receptor (nAChR) allosteric modulators. In insects, these neurotransmitter receptors are responsible for excitatory transmission in the central nervous systems (Wonnacott *et al.,* 2018). The allosteric activation of nAChRs induced by spinetoram results in a hyperexcitivity condition in insects leading to death (Wonnacott *et al.,* 2018). This unique mode of action, along with its low toxicity to the environment and non-target organisms (Zhao *et al*., 2015; Costa *et al.,* 2020; Ruiz *et al.,* 2020), makes is an important tool for pest management (Salgado and Sparks, 2010; Dripps *et al.,* 2011).

Spinetoram was registered in 2007 in New Zeeland, making it one of the novel insecticides on the market (Dripps *et al.,* 2008). Spinetoram appears to be very effective in controlling FAW populations (Muraro *et al.,* 2019; Sisay *et al.,* 2019; Bonni *et al.,* 2020; Deshmukh *et al.,* 2020) and currently there is only one case of FAW resistance to spinetoram, which was reported from Porto Rico (Gutiérrez-Moreno *et al.*, 2019).

1.5.3 Methoxyfenozide

Methoxyfenozide is an insect growth regulator belonging to the diacylhydrazines insecticide group (Group 18). One of the main hormones responsible for the regulation of growth and development in insects, is the molting hormone ecdysterone (20-hydroxyedysone; 20E) (Dhadialla *et al.,* 1998). Although diacylhydrazines are not structurally similar to 20E, the high affinity binding to the ecdysone receptor complex enables these compounds to mimic the molting hormone (20E) (Wing, 1988; Retnakaran *et al.,* 2003). Similar as to 20E, this binding of diacylhydrazines to the complex initiates the molting process. However, the strong bond between diacylhydrazines and the complex induces a precocious molt by repressing genes involved to complete the molt (Retnakaran *et al.,* 2003). This eventually results in larval death due to desiccation and starvation (Dhadialla *et al.,* 1998; Retnakaran *et al.,* 2003). Since the discovery diacylhydrazines in the 1980s (Wing, 1998) it has been considered as one of the most important insect regulators due to their unique MoA, high selectivity and lower toxicity to the environment (Retnakaran *et al.,* 2003; Wang *et al*., 2011). Methoxyfenozide has previously been shown to have lethal and sublethal implications for FAW in terms of abnormal larval development, larval feeding cessation, abnormal pupation and abnormal formation adult wings (Zarate *et al.,* 2011).

1.5.4 Chlorpyrifos

Chlorpyrifos is an organophosphate that belongs to Group 1B of IRAC classification scheme. Organophosphates have a mode of action in which they inhibit acetylcholinesterase (AChE) enzymes in insects by phosphorylating the serine

hydroxyl group (Colombo *et al*., 2005). Acetylcholinesterase is important for normal nerve functions in insects and animals of higher order by hydrolyzing the neurotransmitter, acetylcholine (ACh), to activate cholinergic receptors in a transient manner (Ozmen *et al*., 1999). The deactivation of AChE by organophosphates results in the accumulation of ACh which consequently overstimulates cholinergic receptors (Colombo *et al*., 2005). Insects exposed to organophosphates exhibit symptoms such as hyperexcitation, quivering and paralysis (Colombo *et al*., 2005). Since its introduction into the marketplace in 1965, chlorpyrifos has been widely used to control various agricultural pest species (Eaton *et al*., 2008). Resistance to chlorpyrifos in the invasive FAW populations in South Africa is expected, since the frequency of resistance is high to this relatively old insecticide (Yu, 1991; 1992; Carvalho *et al.,* 2013; Gutiérrez-Moreno *et al.*, 2019; Boaventura *et al*., 2020c).

1.5.5 Pyridalyl

Pyridalyl belongs to the UN group of the IRAC classification scheme, which is for compounds with an unknown or uncertain MoA. Pyridalyl was developed and introduced in 2004 by a Japanese company, Sumitomo Chemical co., Ltd. (Sakamoto *et al*., 2004). Pyridalyl is described as very distinctive in its action compared to existing insecticides and is thus considered to have a novel mode of action (Sakamoto *et al*., 2012; Stojanovic, 2019). Its high selectivity enables it to be very effective against lepidopteran (Cook *et al*., 2004; Isayama *et al*., 2004; Saito *et al*., 2004; Wang *et al*., 2019a) and thysanopteran insects (Isayama *et al*., 2005; Wang *et al*., 2020), while exhibiting little to no toxicity to the environment and non-target organisms (Saito *et al*., 2004; Isayama *et al*., 2005; Sakamoto *et al*., 2012; Stojanovic, 2019). With its chemical structure unrelated to other insecticides, pyridalyl exhibits no cross-resistance to other chemical groups, such as organophosphates and pyrethroids (Saito *et al*., 2005; Moriya *et al*., 2008). Pyridalyl is thus highly effective in managing insect populations that have developed resistance to major insecticide groups (Stojanovic, 2019; Wang *et al*., 2019b). Previous studies have shown that pyridalyl has a cytotoxic effect on cultured Sf9 cells (Saito *et al.,* 2005), and that it is possible to culture pyridalyl-resistant *S. frugiperda* Sf21 cells (Powell *et al.,* 2011).

1.6 Aims and objectives

The aim of this study is to evaluate the efficacy of selected insecticides against FAW populations in South Africa and to compare different bioassay methods used to evaluate the efficacy of an insecticide with an unknown mode of action.

Specific objectives are to:

- Evaluate the susceptibility of three *S. frugiperda* populations to emamectin benzoate, chlorpyrifos and spinetoram/methoxyfenozide
- Compare four toxicity bioassays *viz*. leaf dipping, topical application, diet overlay and insecticide incorporation into artificial diet for susceptibility evaluation of FAW to pyridalyl (unknown mode of action)

The results of the study are presented in the following chapters:

- **Chapter 2:** Efficacy of emamectin benzoate, chlorpyrifos and spinetoram/methoxyfenozide against *Spodoptera frugiperda*
- **Chapter 3:** Comparison of different bioassay methods for susceptibility evaluation of *Spodoptera frugiperda* to pyridalyl
- **Chapter 4:** Conclusion and recommendations

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Chapter 2: Efficacy of emamectin benzoate, chlorpyrifos and spinetoram/methoxyfenozide against *Spodoptera frugiperda*

Abstract

The Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is one of the most destructive pests in agriculture, attacking several crops such as maize and sorghum. In 2017, FAW invaded South Africa where it poses serious risks, especially for maize production. The continuous dependency on insecticides to control *S. frugiperda* populations resulted in insecticide resistance in the Americas. No baseline insecticide susceptibility data of FAW exists in South Africa. The aim of this study was therefore to estimate baseline susceptibility of three FAW populations to emamectin benzoate, spinetoram/methoxyfenozide and chlorpyrifos using a leaf dip bioassay. All three populations exhibited high susceptibility to emamectin benzoate (LC_{50} ranged between 0.11 ppm and 0.23 ppm) and the mixture of spinetoram and methoxyfenozide (LC_{50} ranged between 0.39 ppm and 0.76 ppm). The risk of control failure in South Africa is therefore expected to be very low. However, FAW populations exhibited moderate to high levels of tolerance to chlorpyrifos (LC_{50} ranged between 536.72 ppm and 701.56 ppm), compared to the maximum recommended field rate of 600 ppm. Control failure of FAW with chlorpyrifos is expected. Without a reference population the level of resistance to insecticides in South Africa, could not be estimated. In order to reduce the likelihood of resistance development, an effective insect resistance management (IRM) strategy must be implemented. Emamectin benzoate and the spinetoram/methoxyfenozide mixture, with different MoAs, can be included and rotated in an IRM program for control of FAW in South Africa.

Key words: chemical control, IRAC, fall armyworm, toxicity bioassay

2.1 Introduction

Native to the tropical and subtropical regions of the Americas, the Fall Armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is described as one of the most destructive pests in agriculture (Luginbill 1928, Sparks, 1979; Knipling, 1980). This pest is highly polyphagous, with more than 350 larval host plants (Montezano *et al*., 2018). Various crops such as maize (*Zea mays* (L.)), sorghum (*Sorghum bicolor* (L.)), rice (*Oryxa sativa* (L.)), cotton (*Gossypium hirsutum* (L.)), and soybean (*Glycine max* (L.) Merr.) have been reported to be damaged by this pest (Luginbill, 1928; Hardke *et al.,* 2015; Montezano *et al.,* 2018).

Leaves, stems and reproductive parts of plants are usually attacked by the larval stage of *S. frugiperda* (Johnson, 1987; Goergen *et al.,* 2016; Deole and Paul, 2018). However, this may depend on the type and developmental stage of the crop, as well as the larval stage present on the plant (Goergen *et al.,* 2016; Deole and Paul, 2018). Fall Armyworm larvae can cause excessive vegetative damage which reduces the photosynthetic leaf area, thus affecting yield in some crops (Buntin, 1986). Damage to reproductive structures in maize can cause 'dead heart', that prevents any ears from developing (Abrahams *et al.,* 2017). When pest pressure is high, larger larvae also tend to feed on the stems of their host plants, which can cause the entire stem to be cut (Burkhardt, 1952; Goergen *et al.,* 2016). Fall Armyworm is considered a devastating pests due to its polyphagous nature (Montezano *et al.,* 2018), short life cycle (Walton and Luginbill, 1916), ability to produce overlapping generations (multivoltine) (Vickery, 1929), and its strong dispersal capacity (Nagoshi and Meagher, 2008; Westbrook *et al.,* 2016). The presence of the FAW in Africa was confirmed for the first time in 2016 in West- and Central African countries (Goergen *et al.,* 2016). The migratory behaviour of this pest enabled it to spread rapidly throughout Africa (Feldmann *et al.,* 2019) as well as in Asia (Ganiger *et al.,* 2018; Jing *et al.,* 2019; Perera *et al.,* 2019; Lee *et al.,* 2020) and Australia (IPPC, 2020). Fall Armyworm larvae was first detected in South Africa, during January 2017 on farms in Limpopo and North West provinces (IPPC, 2017; Jacobs *et al.,* 2018).

Spodoptera frugiperda is largely controlled by means of insecticides (Gutiérrez-Moreno *et al.*, 2019; Sisay *et al.,* 2019). However, the behaviour of the pest

complicates its control since moths often prefer to oviposit on the lower areas of the plant, where it is possible that insufficient amounts of insecticides reach the larvae (Hardke *et al*., 2011). First to third-instar larvae tend to feed on the upper parts of the host plant, while older larvae tend to move to the lower parts of the plant, reducing their exposure to insecticides (Morrill and Greene, 1973). The tolerance of FAW larvae to insecticides also increases with age and size, making it even more difficult to control larger larvae (Yu, 1983).

Insecticides have, however, been proven to successfully control *S. frugiperda* infestations (Young, 1979), but continuous high dose insecticide applications have led to resistance development to major insecticide classes (Do Nascimento *et al.,* 2016; Okuma *et al.,* 2018; Gutiérrez-Moreno *et al.*, 2019; Mota-Sanchez and Wise, 2020). Hardke *et al.* (2011) attributed the importance of this pest to the ineffective action of chemical applications. Farmers in Africa also complain that the prescribed insecticides do not provide adequate control against *S. frugiperda* larvae (Kumela *et al.,* 2018; Kuate *et al.,* 2019; Sisay *et al*., 2019).

Toxicity bioassays provide baseline data for insecticides and is therefore an important part of an insecticide resistance management program (Cook *et al*., 2004). Fall Armyworm is a recent invasive pest in South Africa, and no baseline data exist with regard to its susceptibility to insecticides. It is therefore important to develop baseline efficacy data for insecticides currently used against this pest.

Current chemical products registered against FAW in South Africa represent 21 active ingredients with nine different modes of action (MoA) groups (Agri-Intel, 2020). The aim of this study was to estimate baseline susceptibility of *S. frugiperda* populations from three maize producing localities in South Africa to emamectin benzoate, spinetoram/methoxyfenozide and chlorpyrifos. The results of this study will serve as reference data in future susceptibility monitoring.

2.2 Materials and Methods

2.2.1 *Spodoptera frugiperda* rearing

Spodoptera frugiperda populations were collected from maize fields at Groblersdal (25°11'5.798" S; 29°18'41.572" E) (Limpopo province), Nelspruit (25°26'14.186" S; 30°59'34.598" E) (Mpumalanga province), and East London (33°11' 51.565" S; 27°35'16.998" E) (Eastern Cape province) in 2019 (Figure 2.1). From each sampling site, whorls of maize plants infested with FAW larvae were collected and placed inside containers with aerated lids (40 X 30 X 17 cm). From each site, 200-300 larvae were collected (F0 generation) and transported to the laboratory at the North-West University in Potchefstroom. Larvae were in transit for the shortest possible time. Upon arrival at the laboratory, larvae were removed from the plant material and transferred to clean containers with fresh maize leaf material (cv DKC 80-10), onto which no insecticides were applied before being used. Due to the cannibalistic nature of FAW, food was provided ad libitum and ±20 larvae were reared per container. Larvae from each sampling site were kept as separate populations in a rearing room at 28 ± 1 °C, 60-65% RH, and a 14L:10D h photoperiod.

Figure 2.1: Sites where *Spodoptera frugiperda* larvae were sampled.

Fresh maize leaf material was added to the rearing containers every second day and the larvae were transferred to clean containers every sixth day. After the larvae pupated, pupae were removed from the rearing containers and their sex was determined. Each pupa was placed in a separate, small, plastic ventilated container with a steel mesh infused lid (52 mm high and 25 mm in diameter). As the adults emerged, two male and two female moths were placed into oviposition chambers, as described by Kruger *et al*. (2012), and kept in the same rearing room as the larvae and pupae. A plastic bottle (22 cm in height and 10 cm in diameter) was cut open at the top and filled with small crusher stones up to a height of 5 cm. A piece of maize stem with the whorl intact was inserted 3 - 4 cm into the crusher stones to keep it upright. Water (50 mL) was added to the stones. A 10% sugar solution provided in a small bottle closed with a cotton plug, served as food for the moths. The containers were covered with a fine gauze mesh to allow air flow (Figure 2.2).

Figure 2.2: Oviposition chamber with maize stem cutting.

Oviposition chambers were examined, and eggs were removed daily. Eggs that were deposited on the maize stem and on the side of the plastic containers were carefully removed with a fine paintbrush. Egg batches were placed into separate, small, plastic-ventilated containers (52 mm high and 25 mm in diameter) with steel mesh infused lids and kept in the same rearing room as described above. Larvae that hatched from these egg batches (F1), were reared on maize leaf tissue until they reached the third-instar (L3) for use in susceptibility evaluations.

2.2.2 Bioassays

Commercial formulations of the following insecticides, provided by the respective companies, were use in this study: emamectin benzoate (Proclaim®, Syngenta), spinetoram/methoxyfenozide (Uphold® 360SC, Corteva Agriscience) and chlorpyrifos (Chlorpyrifos® 480EC, Villa Crop Protection). Emamectin benzoate is an avermectin which belongs to Group 6 of the Insecticide Resistance Action Committee (IRAC) International Mode of Action Classification Scheme (IRAC, 2019). Spinetoram is a spinosyn (Group 5A), while the insect growth regulator, methoxyfenozide, belongs to the diacylhydrazines insecticide group (Group 18) (IRAC, 2019). Chlorpyrifos is an organophosphate belonging to Group 1B.

Since insecticidal activity occurs after ingestion of these active ingredients (Carlson *et al.,* 2001; Shimokawatoko *et al.,* 2012, Bengochea *et al.,* 2014; Plata-Rueda *et al.,* 2020), evaluations were done according to the IRAC susceptibility test method no. 007 (leaf-dip bioassay) under laboratory conditions (www.irac-online.org). Rangefinder assays were conducted for each insecticide prior to the final susceptibility evaluations. These rangefinder assays provided a range of concentrations sufficient to provide different degrees of mortality (at least 6 points between 20 and 100%) on a regression line. Triton X-100 was used as a non-ionic wetting agent and was added to each solution (0.25 ml/L), including the control treatment (water). The concentrations used for emamectin benzoate ranged from 0.07 to 0.54 parts per million (ppm), while the concentrations for spinetoram/methoxyfenozide ranged from 0.05 to 1.8 ppm, and 200 to 1300 ppm for chlorpyrifos.

Each insecticide susceptibility test per population consisted of seven serial concentrations, which included a control. Each evaluation was replicated three times. All assays were performed in 32-cell bioassay trays (Frontier: Scientific services). A 1% (28 g/L) agar-agar solution was prepared, and 5 mL was poured onto the bases of each cell of the bioassay trays (to assist in keeping the leaf pieces fresh for the duration of the test). For each concentration, twenty-four pieces of maize leaf material (3.5 x 3.5 cm) were immersed individually for five seconds in the solution. Treated maize leaf pieces were placed, with the adaxial surface upwards, on a plastic wired net at room temperature and allowed to air dry. Treated maize leaf pieces were placed individually per cell unit of the bioassay tray, onto the agar. One, L3 larva was carefully removed from a rearing container by means of a fine forceps and placed on the treated leaf piece inside each cell of the rearing trays. Bioassay trays were firmly sealed with transparent ventilated adhesive lids (Frontier: Scientific services) to prevent larvae from escaping. All bioassay trays were kept in an incubator at 26 ± 2 °C, 60-65% RH and a 16L:8D photoperiod.

Mortality of the larvae was assessed after 48 hours. Larvae were considered either dead or alive. Larvae that were unable to move or make coordinated movements when exposed to an external stimulus probing the insect with a fine forceps), were also considered dead.

2.2.3 Data analysis

Abbott's formula was used to correct for control mortality (Abbott, 1925). Corrected mortality data from the respective dose-response bioassays were subjected to probit analysis using PoloSuite® software (version 1.8) (LeOra Software). This program tests the linearity of the relationship between concentrations and mortality responses and provides lethal concentration (LC) estimates and the slope. LC_{50} values are mainly used to compare the susceptibility between populations, while the slope gives an indication of the sensitivity of the test population to the insecticide concentration (Robertson *et al*., 2017). Measures to assure that the data fit the assumptions of the probit model were applied for each data set. The t-ratio of the slope was examined to determine whether a data set should be included for analysis. The significance of the

slope of the respective regression lines was determined by this t-ratio. If the t-ratio for a slope was less than 1.96, the regression was not significant, and the data set was excluded from the analysis.

The χ^2 goodness-of-fit test compared the actual values observed in the bioassay with the predicted values of the model to measure how well the data fit the assumptions of the model (Robertson *et al*., 2017). Residuals were used to identify responses that caused lack of fit of the model, which was indicated by the chi-square (χ^2) value, as well as the heterogeneity factor (χ^2/df). In cases where the heterogeneity factor was greater than one, data sets were examined to identify sources that caused a lack of fit to linear regression. Points outside the bounds of -2 and 2, when standardized residuals were plotted against concentrations, were regarded as outliers and were discarded from the series.

Lethal concentrations (LCs) with the corresponding 95% confidence limits (CLs) for each relationship were also calculated. Responses from two evaluations were considered significantly different when the 95% CLs of the 50% lethal concentration ratios did not include the value 1 (Robertson *et al*., 2017).

No reference population which is susceptible to insecticides in South Africa was identified prior to this study. The most susceptible population identified for each insecticide evaluated, was used to calculate relative potency ratios (PR) by dividing each LC_{50} value with the LC_{50} of the most susceptible population per insecticide. LC_{80} and LC_{95} values were compared with the recommended label rate of each insecticide to evaluate the susceptibility of each population.

The estimated percentage mortality of each population tested at the maximum recommended label rate was also calculated based on the work of Silva *et* al. (2011) and Roditakis *et al.* (2013) to evaluate the potential for insecticide control failure. Mortality of 80% was considered as the minimum acceptable level of insecticide control success. The mortality due to the recommended label rate was considered significantly lower than 80% when the recommended label rate was lower than the lower 95% confidence limit of the LC80 value of the insecticide tested for each

population. The maximum recommended label rates for the insecticides were 75 ppm for emamectin benzoate, 480 ppm for spinetoram/methoxyfenozide, and 600 ppm for chlorpyrifos.

2.3 Results

Responses in all bioassays yielded t-ratios higher than 1.96, indicating significant slopes. The χ^2 values were also significant, which confirmed that the responses fitted the dose-mortality regression model. Therefore, responses of the three populations to all three insecticides fitted the log(dose)/probit(mortality) model, and linearity of data was obtained. Results obtained from probit analyses of the respective leaf dip bioassays are summarized in Table 2.1.

2.3.1 Emamectin benzoate

Slopes of the response lines for emamectin benzoate of all three *S. frugiperda* populations were high and ranged between 2.67 and 3.74 (Table 2.1). This potentially suggests homogeneity of the populations in susceptibility to emamectin benzoate. The LC_{50} values for the three populations ranged between 0.11 (Nelspruit) and 0.23 ppm (Groblersdal) (Table 2.1). The LC_{50} from the Nelspruit population for emamectin benzoate was significantly lower compared to the LC_{50}s of the Groblersdal and East London populations, which did not differ significantly ($P < 0.05$). The LC_{95} was well below the recommended label rate (75 ppm) for all three populations. Hence, larval mortality (≤L3) caused by the recommended label rate of emamectin benzoate is therefore estimated at 100% (Table 2.2).

2.3.2 Spinetoram/methoxyfenozide

Slopes of the response lines for spinetoram/methoxyfenozide of all three populations were also high and ranged between 3.28 and 5.49 (Table 2.1). This also suggests homogeneity of the populations in susceptibility to spinetoram/methoxyfenozide. The LC_{50} values ranged between 0.39 (East London) and 0.76 ppm (Groblersdal) (Table 2.1). The LC_{50}s of the Groblersdal and Nelspruit populations differed significantly from,

and increased two-fold, compared to the East London population. With the LC_{95} values 200 – 600 times smaller than the recommended label rate (480 ppm), 100% mortality of larvae (≤ L3) is estimated when the recommended rate is applied (Table 2.2).

2.3.3 Chlorpyrifos

The LC_{50} values ranged between 536.72 and 701.56 ppm (Table 2.1). The tested populations exhibited significant variations in slopes. The slopes of the regression lines for chlorpyriphos of the Nelspruit and East London populations, were high (>1) (Table 2.1). The slope for the Groblersdal population, was significantly lower (0.003) compared to the other two populations (2.78 and 4.53) (Table 2.1). The LC_{50} of the Groblersdal population also differed significantly from the other two populations, while the LC_{50} of the Nelspruit and East-London populations did not differ significantly at $P < 0.05$. The LC_{50}, LC_{80}, and LC_{95} were always higher than the recommended label rate (600 ppm), except for the LC_{50} of the Groblersdal population which was lower than 600 ppm. The percentage mortality of third-instar larvae, when controlled at the recommended label rate of chlorpyrifos, is estimated to be two-thirds of the expected mortality for the Groblersdal population, and only about half for the Nelspruit and East London populations (Table 2.2).

Table 2.1: Susceptibility of *Spodoptera frugiperda* to emamectin benzoate, spinetoram/methoxyfenozide and chlorpyrifos.

Population	*n*	LC_{50}	CL (95%)	LC_{80}	CL (95%)	LC_{95}	Slope	SE	X^2	df	PR
Emamectin benzoate											
Groblersdal	504	0.23	0.193-0.258	0.47	0.402-0.572	0.93	2.67	0.29	2.27	4	2.1
Nelspruit	432	0.11	0.091-0.130	0.19	0.157-0.238	0.31	3.74	0.39	2.70	3	1.0
East London	504	0.19	0.178-0.211	0.38	0.348-0.433	0.73	2.85	0.31	0.97	4	1.7
Spinetoram/methoxyfenozide											
Groblersdal	336	0.76	0.583-0.902	1.33	1.120-1.708	2.29	3.42	0.51	2.90	4	1.9
Nelspruit	336	0.61	0.512-0.697	1.10	0.965-1.308	1.93	3.28	0.46	1.67	4	1.6
East London	240	0.39	0.314-0.454	0.56	0.479-0.678	0.78	5.49	0.83	1.82	3	1.0
Chlorpyrifos											
Groblersdal	360	536.72	361.6-696.3	843.71	686.1-1177.9	1481.24	0.003	0.0002	2.36	2	1.0
Nelspruit	288	617.38	572.1-657.1	946.67	894.1-1011.8	1423.60	4.53	0.67	0.36	3	1.2
East London	114	701.56	637.5-790.5	1408.65	1184.0-1801.7	2739.88	2.78	0.79	0.13	3	1.3

n = total larvae tested; LC = lethal concentration; CL = confidence limits; SE = standard error; df = degrees of freedom; PR = relative potency ratio

Table 2.2: Estimated percentage mortality of *Spodoptera frugiperda* larvae from three localities in South Africa, when controlled with the maximum recommended label rate.

Insecticide	Population		
	Groblersdal	Nelspruit	East London
Emamectin benzoate	100	100	100
Spinetoram/methoxyfenozide	100	100	100
Chlorpyrifos	67	49	50

2.4 Discussion

Baseline susceptibility data of *S. frugiperda* populations from three maize producing areas in South Africa, *viz*. Groblersdal, Nelspruit and East London, to emamectin benzoate, spinetoram/methoxyfenozide and chlorpyrifos were estimated in this study. This is the first published toxicological data for *S. frugiperda* populations to these insecticides in South Africa.

Insecticides are an essential part of controlling arthropod pests in the agricultural industry (Slater *et al.,* 2017). However, the evolution of insecticide resistance threatens the sustainability of insecticides, which can consequently affect the productivity of an agricultural system (Sparks and Nauen, 2015; Slater *et al.,* 2017). Insect resistance management (IRM) is therefore important for efficacy of these products. Baseline toxicity and susceptibility monitoring are key aspects of resistance management in which early stages of resistance can be detected to proactively implement measures to suppress and delay resistance evolution (Cook *et al.,* 2004).

With the arrival of *S. frugiperda* in South Africa in 2017, 50 synthetic insecticides were registered as an emergency control measure (Department of Agriculture, Forestry and Fisheries, 2017). Since insecticide application is the main method for controlling this pest, the potential for resistance evolution is high. *Spodoptera frugiperda* developed resistance to various insecticides since the 1970s, mainly attributed to the misuse and overuse of synthetic insecticides in the USA (Bass, 1978; Young and McMillian, 1979). Baseline toxicity and susceptibility monitoring has been successfully used to detect

and manage resistance in FAW populations to major chemical classes (Adamczyk *et al.,* 1999; Cook *et al.,* 2004; Hardke *et al.,* 2011; Gutiérrez-Moreno *et al.*, 2019).

Results from this study indicate high susceptibility in South African FAW populations to emamectin benzoate, with no risk of control failure currently. Emamectin benzoate causes a disruption in the nervous and muscular system and has proven to be more effective than its homologue, abamectin (Mrozik *et al*., 1989; Jansson *et al.,* 1997; Jueping *et al.,* 2003), and is still very effective against various lepidopteran pests, such as *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Bird *et al.,* 2017), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and *Spodoptera litura* (Fabricius) (Karuppaiah *et al*., 2017) (Lepidoptera: Noctuidae) (Ramzan *et al*., 2019). The rapid photodegradation of emamectin benzoate is one of the important factors for its selectivity, due to the lower contact possibility between the chemical and natural enemies on the leaf surface. (Prabhu *et al.,* 1991; Lopez *et al*., 2011). Despite this, emamectin benzoate has a comparatively longer residual effect through its fast penetration rate and translaminar action, controlling pests that feed on plant tissue, while maintaining a low risk to non-target organisms (Ishaaya *et al*., 2002; Lopez *et al*., 2011; Bengochea *et al*., 2014). Due to its efficiency in controlling an extensive range of pests with low application rates, emamectin benzoate gained popularity in agricultural production (Zhou *et al.,* 2016; Vaneva-Gancheva, 2018; Abdel-Baky *et al.,* 2019).

Results from this study shows a 300-fold increase in the LC_{50} for emamectin benzoate compared to a laboratory susceptible population from Tennessee, as reported by Gutiérrez-Moreno *et al.* (2019), (0.177 ppm vs. 0.0006 ppm). Although emamectin benzoate is still very effective against FAW field populations in South Africa, early stages of resistance development can be anticipated. Low levels of resistance to emamectin benzoate have already been reported for other lepidopteran pests, *viz*. *S. litura* (Ahmad *et al.,* 2008) and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Roditakis *et al.,* 2018). Since the LC values are still very low and effective control of FAW is achieved at the recommended field rates, the importance of IRM should be highlighted in the farming industry in order to delay resistance development.

Fall Armyworm larvae also exhibited high susceptibility to spinetoram/methoxyfenozide. Spinosyns are composed of two active ingredients: the older spinosad and the more recent spinetoram (Crouse *et al.,* 2001; Dripps *et al.,* 2008). The frequent use of spinosyns, due to their high efficiency, has caused resistance to spinosad in various lepidopterous pests (Moulton *et al.,* 2000; Ahmad *et al*., 2003; Zhao *et al.,* 2006; Okuma *et al.,* 2018). Regardless of resistance to spinosad, spinetoram, until recently, still exhibited high efficacy to a broad range of pest species, such as the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Torticidae) (Cormier *et al.,* 2013), diamondback moth, *P. xylostella* (Li *et al.,* 2015), corn earworm, *H. zea* (Boddie) (Lepidoptera: Noctuidae), and *H. armigera* (Da Silva *et al*., 2020). However, due to the same mode of action, spinosad resistance caused cross resistance to spinetoram in species such as *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae) (Tiwari *et al.,* 2011), *Choristoneura rosaceana* (Harris) (Lepidoptera: Torticidae) (Sial *et al*., 2010), *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) (Watson *et al.,* 2010), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Wang *et al.,* 2016a), and *P. xylostella* (Neto *et al.,* 2016).

In recent studies conducted in India (Deshmukh *et al.,* 2020) and China (Zhao *et al.,* 2020), FAW larvae exhibited high susceptibility to spinosyn insecticides which are still regarded as a key component in FAW management in those regions. The only case of FAW field resistance to spinetoram was reported from Puerto Rico (Gutiérrez-Moreno *et al.*, 2019). The dependency on spinosyns for control of *S. frugiperda* populations increases the selection pressure which favours resistance development (Lira *et al*., 2020). Cross resistance between spinosad and spinetoram in FAW is also possible (Lira *et al.,* 2020). Effective resistance management strategies must therefore be implemented to preserve the effectiveness of this unique mode of action against FAW populations.

One proactive measure to prevent the loss of compounds to resistance is combining two active ingredients into a mixture (Roush, 1989; Slater *et al.,* 2017). The simultaneous exposure to both active ingredients can cause mortality in individuals even though they are resistant to one of the active ingredients. In this way the heritability of resistance is reduced (Slater *et al.,* 2017). For this measure to be effective the two compounds in a mixture should not have the same mode of action,

both must be effective towards the target species, and resistance alleles should be initially rare (Curtis, 1985; Mani, 1985; Comins, 1986). Methoxyfenozide combined with spinetoram is an example of such a mixture registered against FAW in South Africa (Agri-Intel, 2020).

Methoxyfenozide is an insect growth regulator and a member of the diacylhydrazines class (Carlson *et al.,* 2001). Insect growth regulators are selective and exhibit high insecticidal activity against target species (Carlson *et al.,* 2001). Insect growth regulators, considered as a type of biological control approach, are important tools in mixtures, due to their high target specificity and low environmental impact (Sun *et al.,* 2015). Methoxyfenozide is effective against a wide range of lepidopteran pests (Carlson *et al.,* 2001; Enríquez *et al.,* 2010) and is often used in combination with other insecticides, which is attributed to the potential to effectively synergized the toxicity of other active ingredients, such as lufenuron and abamectin (Sun and You, 2014; Chen *et al.,* 2019). Recently Wang *et al.* (2020) reported a method in which the synergistic effect of spinetoram and methoxyfenozide, in a ratio of between 1:1 and 1:10 (spinetoram: methoxyfenozide), provide effective control against the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae). The unique mode of action and low toxicity to non-target organisms of spinetoram (Shimokawatoko *et al.,* 2012), in combination with the high selectivity of methoxyfenozide (Dhadialla *et al*., 1998), makes this combination an important tool in pest management.

The LC values observed for chlorpyrifos in this study are similar to and much higher than the recommended label rate, which shows that chlorpyrifos is less toxic to South African FAW populations, compared to emamectin benzoate and spinetoram/methoxyfenozide. Control failure of *S. frugiperda* populations in South Africa is expected with chlorpyrifos. Although chlorpyrifos has proven to be very effective against FAW populations in field evaluations in the past (Bass, 1978; Van Huis, 1981; Monzon and De Llana, 1999), low efficacy of FAW control has also been reported in the Americas (Yu, 1991; 1992), Brazil (Carvalho *et al.,* 2013; Gutiérrez-Moreno *et al.*, 2019) and China (Zhang *et al*., 2020). Since chlorpyrifos is one of the older insecticides on the market, commercialized in 1965, and still one of the most popular commercial insecticides for FAW control (Wang *et al.,* 2016b; Tambo *et al*., 2020), this low performance may likely be due to resistance in FAW populations.

The slopes of the dose response lines for chlorpyrifos varied between the respective populations in this study. Slopes in a dose-response regression represents the magnitude of the response of an organism per unit change in dose (Lei and Sun, 2018). It is often assumed that the slope of a dose-mortality line is directly associated with the genetic diversity of a population. Hoskins and Gordon (1956) hypothesized that the slope value is higher with less genetic diversity in a population and it is at its lowest at genetic heterogenicity. Therefore, a homozygous susceptible population will have a steep dose-response slope. As the population develop resistance, heterogenicity will increase and consequently lower the slope. A continuous selection pressure will cause a reduction in the frequency of susceptible genes in a pest population and reduce heterogenicity, which will lead to an increase the slope of the response line. According to these assumptions, the Groblersdal population is heterogeneous and may be in a process of resistance development. This would be consistent with the data obtained, as the Groblersdal population had the lowest LC values and still exhibits high levels of tolerance. Although this data seems to support these assumptions, Chilcutt and Tabashnik (1995) differ and reported no predictable relationship between the slope of the response line and genetic diversity within a population.

Since several cases of resistance of FAW to insecticides have been reported in the past and for chlorpyrifos in this study, measures have to be employed to delay resistance. Integrated pest management (IPM) programs with appropriate IRM strategies are essential for FAW management (Day *et* al., 2017; Bateman *et al.*, 2018; Gutiérrez-Moreno *et al.*, 2019). Strategies such as routine pest scouting and alternation of insecticides with different modes of action is important to ensure insecticide efficacy (Slater *et al.,* 2017). Other insecticides, also registered in South Africa for FAW control, such as chlorantraniliprole, spinosad (Sisay *et al*., 2019) and indoxacarb (Worku and Ebabuye, 2019), have been reported to exhibit high toxicity to FAW in Ethiopia and could be included in a rotation program. One of the difficulties in any resistance management strategy is to ensure compliance (Slater *et al.,* 2017). It is therefore important that IPM strategies are developed to manage this pest in South Africa and that chemical control is applied judiciously, based on economic threshold levels.

2.5 Conclusion

The evolution of insecticide resistance in FAW populations remains a threat to its management. Susceptibility monitoring of FAW populations is therefore an important factor in managing this pest. In this study, baseline susceptibility data for emamectin benzoate, spinetoram/methoxyfenozide, and chlorpyrifos were established. All three populations were susceptible to emamectin benzoate and the mixture of spinetoram and methoxyfenozide and currently no risk of control failure is expected with these insecticides against FAW in South Africa. Control failure can, however, be expected with chlorpyrifos.

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Chapter 3: Comparison of different bioassay methods for susceptibility evaluation of *Spodoptera frugiperda* to pyridalyl

Abstract

Chemical control is the main method for controlling Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). In South Africa, several insecticides are registered against this pest, among which one chemical, pyridalyl, has an unknown mode of action. No specific method is prescribed for susceptibility evaluation of *S. frugiperda* to this insecticide. The aim of this study was to evaluate and compare different bioassay methods, *viz*. leaf dipping, topical application, diet incorporation and diet overlay, to find the most suitable method to study the toxicity of pyridalyl to *S. frugiperda* larvae. Although all the bioassay methods provided a linear relationship in the dose/response regressions, only the topical application and diet incorporation bioassays fitted the probit model assumptions. With all the statistical parameters taken into account, the topical application bioassay was determined to be most suitable for evaluating FAW susceptibility to pyridalyl.

Key words: pyridalyl, chemical control, fall armyworm, toxicity bioassay

3.1 Introduction

Effective control measures against insect pests are of utmost importance since pest infestations often result in significant reductions in the quality and quantity of crops (Oerke, 2006). Since the commercialisation of insecticides, chemical control has been the cornerstone of crop protection against insect pests (Dent, 2000). If applied correctly, insecticides can be very effective due to the wide variety of pests it can control and the rapid rate at which certain insecticides act on the pests (Dent, 2000). However, chemical control can also have some adverse effects since the over-use and misuse of insecticides can lead to destruction of natural enemy populations (Fritz *et al.,* 2013; Monzo *et al.,* 2014; Regan *et al.,* 2017), environmental contamination (Herrero-Hernández *et al.,* 2013; O'Brien *et al.,* 2016), increased secondary pest populations (Eveleens *et al.,* 1973; Hill *et al.,* 2017), and adverse effects on human

health (McCarthy *et al.,* 2006). One of the most concerning adverse effects of misusing insecticides is the development of resistant insect populations (Sparks and Nauen, 2015; Abrahams *et al.*, 2017). Dent (2000) also stated that, over the long run, the adverse effects of chemical control will outweigh the benefits it can provide. Although these concerns are well known, insecticides are still in high demand by farmers (Alavanja, 2009; Fausti *et al.,* 2012; Douglas and Tooker, 2015; Fausti *et al.,* 2018), given its economic benefits (compared to potential loss) and fast acting mechanisms (Dent, 2000).

In 1984, the Insecticide Resistance Action Committee (IRAC) was established with one of its main objectives to improve insecticide resistance management strategies (Sparks and Nauen, 2015; IRAC, 2019). Insecticide resistance is defined by IRAC (2019) as "a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species". Insecticide resistance has long been a global problem affecting insect pest control (Sparks *et al*., 2020). With more than 16 000 reports of insecticide resistance documented, the need for effective resistance management is of utmost importance (Sparks *et al*., 2020). IRAC developed a classification scheme based on insecticides' MoA, which provided guidelines to stakeholders on the use of insecticides in an effective and sustainable manner (Sparks and Nauen, 2015; IRAC, 2019). Currently, 30 MoA groups are listed in the IRAC classification scheme. These MoA groups can be used in rotation- and alternation programs against insect pests (IRAC, 2019).

Another key factor in the management of resistance is the monitoring of insecticide susceptibility among pest populations using toxicity bioassay methods (Kranthi, 2005; Miller *et al.*, 2010). Toxicity bioassays are used to detect any change in the efficacy of an insecticide in order to adapt the necessary control strategies before resistance develops (Paramasivam and Selvi, 2017). Several toxicity bioassays have been developed by IRAC for use in susceptibility testing of field populations of insect pests to insecticides (Kranthi, 2005; Paramasivam and Selvi, 2017). Bioassay methods are developed in accordance with the method of insecticide application in the field (Ffrench-Constant and Roush, 1990), and should be easy to perform and provide consistent and reliable results (Kranthi, 2005). Commonly used bioassay methods are

topical application, leaf dipping and insecticide incorporation into artificial diets (Ffrench-Constant and Roush, 1990).

The Fall Armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is one of the most important lepidopterous pests of maize. It is native to the Americas (Luginbill 1928, Sparks, 1979; Knipling, 1980), where it primarily attacks maize, cotton, sugarcane, soybean and grasses (Adamczyk *et al*., 1999; Blanco *et al*., 2010; Montezano *et al*., 2018). Fall Armyworm is polyphagous and can feed on more than 350 plant species (Montezano *et al*., 2018), threatening the African grain industry (Abrahams *et al*., 2017; IPPC, 2017). Although the excessive use of insecticides has resulted in many FAW populations being resistant to several commonly used pesticides (Yu, 1992; Yu and McCord, 2007, Do Nascimento *et al*., 2016; Gutiérrez-Moreno *et al*., 2019; Mota-Sanchez and Wise, 2020), chemical control remains the main control method (Abrahams *et al*., 2017; Sisay *et al.,* 2019).

Upon arrival of FAW in South Africa, 50 emergency registrations for agrochemicals were made for its control (Department of Agriculture, Forestry and Fisheries, 2017). The currently approved insecticides consist of 21 active ingredients with nine modes of action (Labels of the respective insecticides are available at https://www.agri-intel.com/label-information/search-registration-information/), among which one chemical, i.e. pyridalyl, has an unknown mode of action. Pyridalyl was developed and introduced in 2004 by a Japanese company, Sumitomo Chemical co., Ltd. (Sakamoto *et al*., 2004), and has since been used globally (Powell *et al*., 2011, Sakamoto *et al*., 2012). Its high selectivity enables it to be very effective against lepidopteran (Cook *et al*., 2004; Isayama *et al*., 2004; Saito *et al*., 2004; Wang *et al*., 2019b) and thysanopteran insects (Isayama *et al*., 2005; Wang *et al*., 2020), while exhibiting little to no toxicity to the environment and non-target organisms (Saito *et al*., 2004; Isayama *et al*., 2005; Sakamoto *et al*., 2012; Stojanovic, 2019). Showing no cross-resistance to other chemical groups, such as organophosphates and pyrethroids (Saito *et al*., 2005; Moriya *et al*., 2008), pyridalyl is considered to have a novel mode of action (Sakamoto *et al*., 2012; Stojanovic, 2019). Pyridalyl is thus highly effective in managing insect populations that have developed resistance to major insecticide groups (Stojanovic, 2019; Wang *et al*., 2019b).

The intensive use of pyridalyl resulted in development of pyridalyl-tolerant field populations of *Spodoptera exigua* (Hübner) (Lepidoptera, Noctuidae) and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in China (Wang *et al*., 2019a; Yin *et al*., 2019). However, since the MoA of this chemical is unknown, it is difficult to monitor resistance levels in pest populations since no specific method is prescribed by IRAC for susceptibility evaluation.

In this study several bioassay methods, *viz*. leaf dipping, topical application, diet incorporation and diet overlay, were evaluated and compared to find the most suitable method to evaluate FAW susceptibility to pyridalyl.

3.2 Materials and Methods

3.2.1 *Spodoptera frugiperda* populations and rearing

Fall Armyworm populations were sampled from maize fields of subsistence farmers in the Limpopo province of South Africa, in the areas surrounding Thohoyandou (22°47'54.6" S; 30°32'25.6" E). Larvae were sampled from infested maize plants by hand picking. Approximately 50 larvae were collected (F0 population) and transported to the North-West University in Potchefstroom. Larvae were kept with maize plant material in containers with aerated lids (40 X 30 X 17 cm). Larvae were in transit for the shortest possible time period. Upon arrival at the laboratory, larvae were removed and transferred from the containers into clean containers with fresh maize leaf material (cv DKC 80-10) that has not been treated with any pesticides. Due to the cannibalistic nature of FAW larvae, food was provided ad libitum and only ±20 larvae were reared per container. Once a week, all the maize leaf material was removed from the containers and replaced with fresh maize leaves. Larvae were kept in a rearing room at 28 ± 1 °C, 60-65% RH, and a 14L:10D h photoperiod.

Upon pupation, pupae were separated according to their sex, placing each pupa in a small, plastic ventilated container with a steel mesh infused lid (52 mm high and 25 mm in diameter). As adults emerged, two of each sex were placed into oviposition chambers as described by Kruger *et al*. (2012). A plastic bottle with an open top (22

cm high and 10 cm in diameter) was filled with small crusher stones, to the 5 cm mark, and 50 mL of water was added. A piece of maize stem with the whorl intact was inserted into the crusher stones. A small bottle containing a 10% sugar solution with a cotton plug served as food source for the moths. Containers were covered with a fine gauze mesh and kept in the same rearing room as the larvae and pupae. Adults were allowed to oviposit until the adults died.

Eggs were removed daily from the oviposition chambers by means of a fine paintbrush. Egg batches were placed separately into small plastic bottles with steel mesh infused lids (52 mm high and 25 mm in diameter) and kept at the same rearing conditions as described above.

The larvae that hatched (F1 population) were divided into two groups and reared either on maize leaf material as described above, or on Stonefly *Heliothis* premix diet (Ward's Natural Science Establishment, LLC). The premix diet was mixed with water in a 1:4 ratio (diet: water) to obtain a smooth paste. Twenty larvae were kept per rearing bucket (14 X 10 X 6 cm). The rearing containers with maize stems and premix diet were kept in a rearing room at 28 ± 1 °C, 60-65% RH, and a 14L:10D h photoperiod. When larvae were in the third-instar (L3), they were used in the bioassays.

3.2.2 Bioassay Methods

The commercial formulation of pyridalyl (Sumipleo® 50EC, Philagro) was used in all the bioassays. For each bioassay method, rangefinder assays were conducted beforehand to determine the range of concentrations that should be used in bioassays. In order to compare the different bioassay methods, the same series of concentrations were used in all four methods. Six serial concentrations (excluding the control) which resulted in 20 – 100% mortality in each of the bioassay methods, were determined. These concentrations ranged from 20 to 370 parts per million (ppm). For every replicate, the six concentrations were prepared from a stock solution with a concentration of 500 ppm. For the topical application bioassay, the stock solution was prepared in a solution of 95% acetone and 5% distilled water. All bioassays were performed in 32-cell bioassay trays (Frontier: Scientific services) containing a 5 ml

Agar-Agar solution (28 g/L) to prevent leaf/diet desiccation. Each bioassay method was replicated three times. Twenty-four larvae per replicate were used for each bioassay.

3.2.2.1 Leaf dipping bioassay

The leaf dipping bioassay was performed according to IRAC susceptibility test method no. 007. The six insecticide dilutions were prepared in 250 ml beakers. Distilled water was used as the untreated control. To each dilution, as well as the control, a non-ionic surfactant was added (Triton X-100; 0.25 ml/L) and dissolved with the use of a magnetic stirrer. The surfactant was added to provide optimal leaf coverage. For each replicate, 24 maize leaf pieces (4 x 4 cm) were dipped individually into the respective concentrations for five seconds, ensuring that the entire surface was covered (Figure 3.1). Treated maize leaves were kept at room temperature and allowed to air dry on a plastic wire net with the adaxial surfaces of leaves facing upwards (Figure 3.1).

The pieces of treated maize leaves were each placed individually into a cell unit of a bioassay tray. Individual L3 *S. frugiperda* larvae (reared on maize leaf material) were also carefully transferred from the rearing containers onto the treated leaf material in each cell. Care was taken to use only L3 larvae of similar size, since *S. frugiperda* larvae from different instars, are known to have different tolerance levels to insecticides (Yu, 1983). Bioassay trays were firmly sealed with transparent ventilated adhesive lids (Frontier: Scientific services) to prevent any larvae from escaping. All trays were kept in an incubator at 26 ± 2 °C, 60-65% RH and a 16L:8D photoperiod.

Figure 3.1: Maize leaf pieces dipped into a pyridalyl solution and allowed to air dry on a plastic wire net.

3.2.2.2 Topical application bioassay

Fall armyworm used in this bioassay were reared on maize leaf material. The six insecticide concentrations were prepared with a 95% acetone and 5% water solution, whilst the control was a solution of 95% acetone and 5% water. Third-instar larvae were individually transferred from rearing containers to marked cell units (one larva per cell) containing a 4 x 4 cm maize leaf which served as a food source. Using a micropipette, one drop (2 µl) of the insecticide solution was applied to the dorsal thorax of each larva (Figure 3.2). A single pipette point was used per concentration to avoid contamination between concentrations. Bioassay trays were sealed with transparent ventilated adhesive lids (Frontier: Scientific services) and kept at the conditions described above.

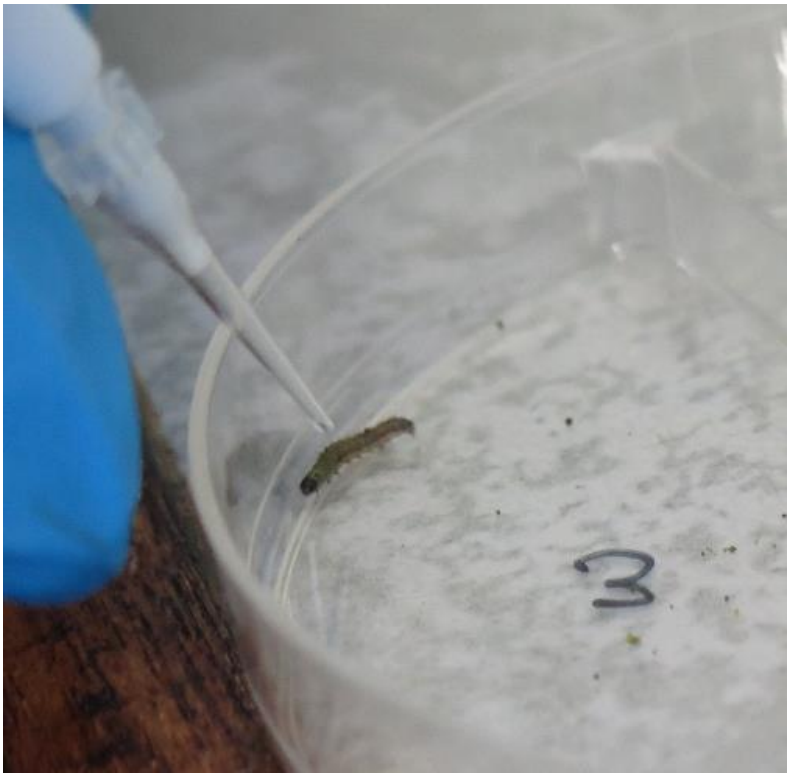

Figure 3.2: Insecticide solution applied onto the dorsal thorax of a third-instar *Spodoptera frugiperda* larva.

3.2.2.4 Diet-incorporated bioassay

The diet-incorporated bioassay was performed according to IRAC susceptibility test method no. 020. In this bioassay, the respective concentrations were incorporated into the Stonefly artificial diet. However, to ensure that the correct concentration was presented in the diet, each insecticide concentration was prepared at 1.25x the final dilution rate required as per IRAC protocol. For each concentration per replicate, 80 ml of the solution were thoroughly mixed with 20 g Stonefly *Heliothis* premix diet. Distilled water was used as mixing agent for the untreated control. Using a large syringe, 2 ml of diet were dispensed into each of the 24 cell units (Figure 3.3). One L3 *S. frugiperda* larva, reared on the Stonefly *Heliothis* diet, was inoculated onto the insecticide incorporated diet in each cell. All cell units were tightly sealed with transparent ventilated adhesive lids to prevent any larvae from escaping. The bioassay trays were incubated at the conditions described above.

Figure 3.3: Insecticide incorporated diet being dispensed into bioassay cell units.

3.2.2.3 Diet overlay bioassay

This bioassay method is similar to the diet-incorporated bioassay, however, in this bioassay method, the insecticide solution was not incorporated into the artificial diet, but rather applied as an overlay onto the diet. Six insecticide dilutions were prepared while water was used as the untreated control. Stonefly *Heliothis* diet was thoroughly mixed with water in a 1:4 ratio to obtain a smooth paste. A syringe was used to apply 2 ml of the paste into a ball-shape in the centre of each cell. For each concentration, 140 µl per concentration was pipetted onto the surface of the diet (Figure 3.4). Water was used as the untreated control. Each tray was waggled to ensure a uniform spread of the insecticide solution over the whole diet surface. Trays were left open at room temperature to allow the diet to air dry for approximately one hour. One, L3 larva, reared on Stonefly *Heliothis* diet, was inoculated onto the treated diet in each well. Bioassay trays were sealed with transparent ventilated adhesive lids and incubated at the conditions described above.

Figure 3.4: Insecticide mixture being pipetted onto artificial diet.

3.2.3 Mortality assessment

Mortality of L3 *S. frugiperda* larvae were assessed 24, 48 and 72 hrs after exposure. Larvae were either classified as dead or alive. Larvae that were unable to make movements by itself or make coordinated movements when exposed to an external stimulus (e.g. probing the insect with a fine forceps), were also classified as dead.

3.2.4 Statistical analyses

Mortality values obtained from bioassays were corrected by using Abbott's formula (Abbott, 1925). Corrected mortality data from the dose-response bioassays were subjected to probit analysis using PoloSuite® software (version 1.8) (LeOra Software). Bioassay methods were evaluated according to the criteria that would fit the assumptions of the probit model according to Robertson *et al*. (2017).

Linearity of the respective dose/response regressions was determined by the t-ratios. If the t-ratio for a slope is less than 1.96, the regression is not significant, and the data set cannot be included in an analysis. The chi-square (χ^2) goodness-of-fit test measures how well the data fit the model assumptions by comparing the actual values of the bioassay with the predicted values of the model. If these values differ significantly at $P = 0.05$, the data does not fit the model. For the model to fit, the χ^2 value must be less than the tabular value for the appropriate degrees of freedom at $P = 0.05$. The lack of fit is indicated by the χ^2 value and the heterogeneity factor (χ^2/df). If chi-square is not significant and the heterogeneity factor greater than 1, standardized residuals are plotted to identify responses that causes a lack of fit. Responses outside the bounds of -2 and 2 are regarded as outliers and can be discarded.

Lethal concentrations (LCs) with the corresponding 95% confidence limits (CLs) for each bioassay were determined. Lethal concentration ratios were used to determine whether the lethal concentrations from different bioassay methods differ significantly from each other. If the 95% CLs ratio of the 50% lethal concentration from different responses did not include the value 1, they were considered to be significantly different ($P = 0.05$). The likelihood ratio (LR) test of equality and parallelism was used to test whether the slopes and intercepts of the different regression lines were significantly the same.

3.3 Results

The slopes of the regression lines for all bioassay methods yielded a t-ratio higher than 1.96, indicating the existence of a linear relationship between the responses and the dosages used (Table 3.1). Slopes of the response lines were relatively constant (ranging between 1.80 and 2.73), which could potentially suggest homogeneity of the population in susceptibility to pyridalyl (Table 3.1).

The χ^2 values for the topical application and diet-incorporation bioassays were significant and fitted the dose-mortality regression model. Since the χ^2 values for the leaf dipping and diet overlay bioassays were higher than the tabular value of χ^2 at

P = 0.05 for 2 and 4 degrees of freedom (5.991 and 9.488 respectively), and their heterogeneity factor being higher than 1, the data sets for these bioassays did not fit the assumptions of the probit model (Table 3.1). The χ^2 value for the topical application bioassay was the lowest compared to the other bioassays and therefore fitted the probit model the best.

The estimated LC_{50} values for these bioassays ranged between 30.19 and 181.28, with the LC_{50} of the topical application bioassay the lowest, followed by leaf dipping, diet-incorporation and diet overlay bioassays (Table 3.1). The 95% confidence limits for the LC_{50} value was the shortest for the topical application bioassay, followed by the diet-incorporation, leaf dipping and diet overlay bioassays (Table 3.1). According to the probit model, the estimated LC_{50} values for all the bioassays differed significantly from each other ($P < 0.05$). The likelihood ratio (LR) test of equality and parallelism rejected the test for equality while accepting the test for parallelism between regressions when all four bioassays were analysed simultaneously. This indicated that the slopes of these regressions did not differ significantly even though their intercepts differed significantly.

The estimated LC_{80} values were always below the recommended label rate for pyridalyl (300 ppm), except in the diet overlay bioassay.

Table 3.1: Susceptibility of *Spodoptera frugiperda* to pyridalyl with different bioassay methods.

Bioassay	***n***	**LC_{50}**	**CL (95%)**	**LC_{80}**	**Slope**	**SE**	**t-ratio**	**X^2**	**df**	**h**
Leaf dipping	480	43.18	0.19-102.50	126.37	1.80	0.19	9.61	7.89	2	3.95
Topical application	432	30.19	25.08-35.37	65.11	2.52	0.26	9.84	0.60	3	0.20
Diet-incorporation	504	104.29	88.74-118.79	261.24	2.11	0.28	7.64	0.74	4	0.18
Diet overlay	504	181.28	1862.33-299.25	368.44	2.73	0.49	5.53	19.33	4	4.83

n = total larvae tested; LC = lethal concentration; CL = confidence limits; SE = standard error; X^2 = chi-square; df = degrees of freedom; h = heterogeneity factor

3.4 Discussion

Suitable bioassay methods which can be used in susceptibility evaluations against an unknown MoA insecticide, pyridalyl, have been determined in this study. One of the key management strategies for insect resistance is the continuous susceptibility monitoring of insect populations by means of toxicity bioassays (Kranthi, 2005; Miller *et al.*, 2010; Roditakis *et al*., 2018). With advances in this research field, many bioassay techniques have been developed over the years that can accurately determine the resistance of a pest population in the field (Durmuşoğlu *et al*., 2015). However, since different bioassay methods may result in a variation in results, it is important to develop and standardize a specific bioassay method for comparison between insecticide resistance studies (Durmuşoğlu *et al*., 2015). In a laboratory study done by Leibee and Savage (1992), a great difference in relative toxicity were seen in *P. xylostella* populations evaluated for their susceptibility to fenvalerate in two different bioassay methods (topical application and leaf residue). This phenomenon was also recorded by Dennehy *et al.* (1983) in evaluation of spider mites, where a lack of correlation between leaf residue and slide dip bioassays were found. It is thus of utmost importance to know the method of toxification in order to use the correct bioassay method.

Since the MoA of pyridalyl is unknown and considered as novel (Sakamoto *et al*., 2012; Stojanovic, 2019), no exact bioassay method is prescribed to study the toxicity of this product to insect pest populations. The goal of this study was therefore to attempt to determine a standard bioassay method by which the susceptibility of *S. frugiperda* populations can be evaluated. During this study, some unique symptoms in treated larvae were observed. In the topical application bioassay, unique burn scars were formed on the epidermis of the larvae at and around the treatment area. These cytotoxic symptoms have previously been observed in *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) larvae (Saito *et al*., 2004), *Bombyx mori* (L.) (Lepidoptera: Bombycidae) cell lines (BM36) (Powell *et al*., 2011) and *S. frugiperda* sell lines (Sf9) (Saito *et al*., 2005). Powell *et al.* (2011) hypothesised that this phenomenon is due to the metabolism of pyridalyl by cytochrome P450 which activates the formation of reactive oxygen species (ROS) and consequently causes necrotic cell death. Other symptoms observed in larvae treated with pyridalyl also include flaccid paralysis and

a loss of mobility and body elasticity (Saito *et* al., 2004; Stojanovic, 2019). Insecticidal activity by pyridalyl can therefore occur through ingestion of the active ingredient as well as by dermal application (Nishimura *et al*., 2007).

One of the more common techniques used in toxicity bioassays for Lepidoptera is the leaf dipping bioassay (Ahmad *et al.,* 2003, 2008; Da Silva Galdino *et al*., 2011; Li *et al.,* 2015; Deshmukh *et al.,* 2020), in which the chemical solution is evenly distributed on the leaf surface. However, the results of this study indicated that this method is not suitable for use in susceptibility studies with FAW to pyridalyl. Although there was a linear relationship between the dosages used and their response values, the high χ^2 value together with a low degrees of freedom indicated that this method did not fit the probit model assumptions. The same applies to the diet overlay bioassay in which the data did not fit the model assumptions and cannot be used.

Only the topical application and diet incorporation bioassay methods provided the required linearity and fitted the assumptions of the probit model, however with vastly different toxicity results. Various explanations could be provided for the great difference in LC_{50} values obtained with these two bioassay methods (30.19 ppm vs. 104.29 ppm). Firstly, it may be that the toxicity of pyridalyl depends on the route of uptake. In this case, pyridalyl appeared to be more toxic to *S. frugiperda* larvae if administered by contact rather than by consumption. This phenomenon has also been observed by Bengochea *et al.* (2014), where emamectin benzoate appeared to be more toxic through consumption rather than by contact, due to the polar nature of emamectin benzoate which limits contact toxicity. Secondly, symptoms such as a decrease in mobility, vigor and body elasticity cause an anti-feeding activity in treated larvae (Nishimura *et al*., 2007), which reduces the exposure of larvae to the toxicant in the diet incorporation bioassay. This may mean that larvae in the topical application bioassay have a higher mortality rate due to the continual exposure to the toxicant. Other factors that may potentially influence the suitability of a bioassay include the type of diet used and the chemical properties of pyridalyl, such as water solubility and polarity.

Different toxicity bioassay methods, which include leaf dipping, topical application, diet overlay, foliar spray, body immersion and adult vial tests, have previously been used to evaluate insecticidal activity of pyridalyl to various lepidopterous pests, such as *S. litura*, *S. exigua*, *P. xylostella, Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), *H. zea* (Boddie) (Lepidoptera: Noctuidae) and *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) (Cook *et al.,* 2005; Sakamoto *et al*., 2004; Cook *et al*., 2005; Isayama *et al*., 2005). All these studies emphasised the effectiveness of pyridalyl in the control of lepidopteran pests. In this study the LC_{80} values were lower than the recommended label rate for FAW control (300 ppm) in the topical application and diet incorporation bioassays, further emphasising the efficacy of pyridalyl against FAW.

This study indicated that topical application and insecticide incorporation bioassays were sufficient for evaluating the susceptibility of *S*. *frugiperda* larvae to pyridalyl. However, with shorter 95% confidence limits for the LC_{50} value, the topical application method seems to be the best method for evaluating FAW susceptibility to pyridalyl. Although the leaf dip bioassay did not fit the model assumptions in this study, it will not be omitted from use in toxicity studies. One of the main reasons for it not fitting the probit model assumptions was due to the low number of concentrations used during this evaluation. A higher number of concentrations will increase the degrees of freedom and consequently lower the heterogeneity factor (Robertson *et al*., 2017). Thus, future studies are recommended in which more concentrations are used to evaluate the effectiveness of leaf dip bioassays with pyridalyl to FAW.

3.5 Conclusion

With insecticide resistance being a global problem in agriculture, accurate susceptibility monitoring remains an important factor in insecticide resistance management strategies. In this study, topical application and diet incorporation bioassays were found to be sufficient to evaluate the susceptibility of FAW larvae to pyridalyl. Pyridalyl has also been shown to be a useful candidate for the protection of crops against *S. frugiperda* larvae in South Africa. The results of this study will assist in fast and easy determination of the susceptibility status of FAW to pyridalyl.

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Chapter 4: Conclusion and Recommendations

With more than 350 larval host plants reported (Montezano *et al*., 2018), the Fall Armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a voracious agricultural pest able of causing much damage in especially grain crops such as maize (*Zea mays* (L.)) and sorghum (*Sorghum bicolor* (L.) Moench) (Luginbill, 1928; Murúa *et al.,* 2015). The arrival of this pest in Africa threatens food security of many people and holds serious economic risks for the agricultural sector (Abrahams *et al*., 2017). In invaded areas with favourable climatic conditions and a diverse host plant range, FAW is expected to be a consistent threat to crops (Goergen *et al*., 2016; Midega *et al*., 2018).

One of the main methods for controlling FAW larvae is chemical control (Abrahams *et al.*, 2017; Sisay *et al.,* 2019). However, effective chemical control depends on early detection of infestation (Abrahams *et al.,* 2017), as larger larvae can tunnel into the whorl avoiding contact with insecticides (Young, 1979). Another constraint of chemical control includes resistance development to important chemical classes such as organophosphates, carbamates, pyrethroids and benzoylureas, due to continuous high dose applications (Carvalho *et al.,* 2013; Nascimento *et al.,* 2016; Gutiérrez-Moreno *et al.*, 2019). The use of genetically modified crops is another control strategy used against FAW populations, and has been proven to be effective and reliable in the Americas (Siebert *et al*., 2012; Storer *et al*., 2012; Reay-Jones *et al*., 2016). However, a total dependence on biotechnology to manage this pest in South Africa is not recommended. Integrated pest management (IPM) programs with appropriate insect resistance management (IRM) will ensure high crop productivity and insecticide efficacy.

The main goal of IRM is to prevent or delay the evolution of pest resistance in target pest populations (Sparks and Nauen, 2015), as well as to regain susceptibility in already resistant pest populations (Sparks and Nauen, 2015; IRAC, 2019). One of the strategies described by IRAC for achieving this is the alteration of MoAs. This rotation approach is based on using different MoA insecticides within a specific “treatment window”, which can either be defined by the generation length of a pest or the growth

stages of a crop (Sparks and Nauen, 2015). This is done to ensure that successive generations are not exposed to the same MoA. IRAC has developed a MoA classification scheme that categorises insecticides into different groups based on their MoA, thus providing a means to select insecticides for a rotation program, minimizing the risk for cross-resistance (Sparks *et al*., 2020).

Due to the short life cycle of FAW, it is suggested that the treatment window in a spray program should be approximately 30 days apart, to ensure that the follow-up generation of the pest is exposed to a different MoA (IRAC, 2019) (Figure 4.1). The four treatment windows for control of any pest on maize are 1) pre-plant, 2) early vegetative stage (VE-V6), 3) late vegetative stage (V7 to VT), and 4) reproductive stage (VT to R6). Since no seed treatment is registered for control of FAW during the early vegetative plant growth stages, control relies on applications of insecticide sprays onto plant leaves and ears. As Figure 4.1 illustrates, foliar application within the first window may be required. This treatment will however not provide effective control for the entire window period. If a second application is needed during this period, it should be of a different MoA than the first spray within this window. In the next window period (late vegetative stage), the target of an insecticide application will be larvae that were not controlled with the MoA of the insecticide sprayed earlier, and larvae of a possible follow-up infestation from outside of the particular maize field. Insecticides applied during this window should therefore be of another MoA.

In this study emamectin benzoate (Group 6) and spinetoram/methoxyfenozide (Group 5A and 18) (Chapter 1, Table 1.1) exhibited high toxicity to FAW, while chlorpyrifos (Group 1B) was less toxic. With baseline susceptibility values showing high levels of susceptibility to emamectin benzoate and the mixture of spinetoram and methoxyfenozide, these two insecticides can be used for control of FAW and no control failures are expected. Since emamectin benzoate and spinetoram/methoxyfenozide belongs to two different resistance groups they can be used in an IRM spray program against FAW in South Africa.

Pyridalyl (unknown MoA) was also found to be highly effective against FAW. Since pyridalyl shows no cross-resistance to other chemical groups, such as organophosphates and pyrethroids (Saito *et al*., 2005; Moriya *et al*., 2008), and is

considered to have a novel mode of action (Sakamoto *et al*., 2012; Stojanovic, 2019), it may be effectively used in managing insect populations that have developed resistance to major insecticide groups (Stojanovic, 2019; Wang *et al*., 2019). Susceptibility monitoring remains an important factor in insecticide resistance management, and it is of utmost importance that FAW populations are monitored for early detection in resistance development. The suitable bioassay method, *viz*. topical application, determined for susceptibility testing of FAW to pyridalyl in this study, can be used for susceptibility testing of FAW to this insecticides, if it is used in spray programs.

For further studies, it is recommended that baseline studies for all the chemicals registered against the FAW in South Africa be conducted with pest populations from different regions in South Africa. It is also recommended that the evaluation of the leaf dip bioassay for FAW susceptibility evaluations to pyridalyl be repeated in future studies.

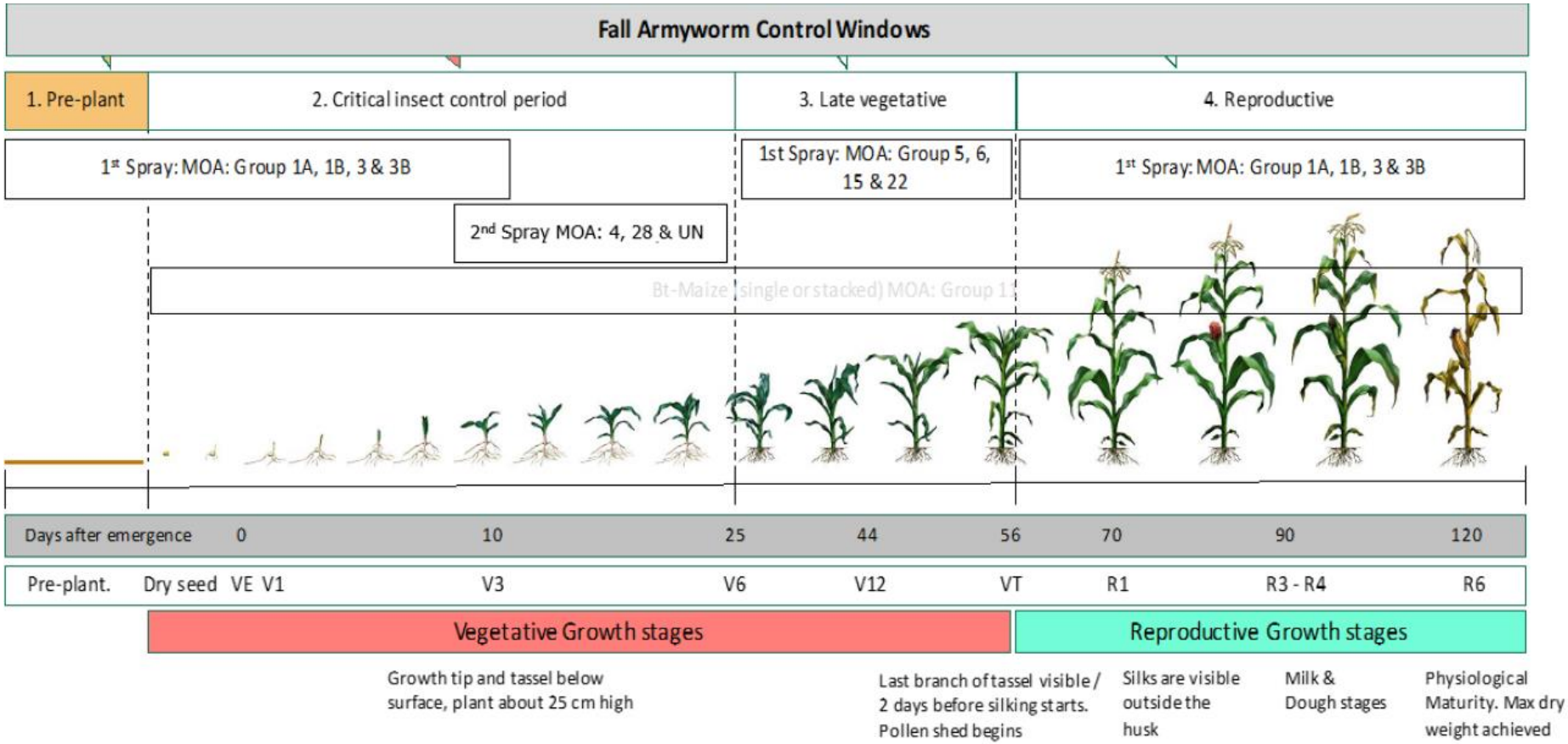

Figure 4.1: Example of spray program for *S. frugiperda* (IRAC, 2019).

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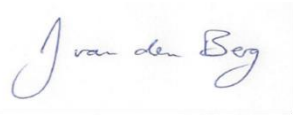
Appendix A: Declaration of language editing

Language editing statement

To whom this may concern,

I, Prof. J van den Berg, hereby declare that the thesis titled: “Toxicity of four insecticides to *Spodoptera frugiperda* in South Africa” by D.C. van Niekerk has been edited for language correctness and spelling by the supervisors. No changes were made to the academic content or structure of this work.



Prof. J van den Berg Date: 03/12/2020

Appendix B: Ethics letter

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ETHICS APPROVAL LETTER OF STUDY

Based on approval by the Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC), the Faculty of Natural and Agricultural Sciences Ethics Committee hereby approves your study as indicated below. This implies that the North-West University Senate Committee for Research Ethics (NWU-SCRE) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Efficacy of four insecticides for control of Spodoptera frugiperda in South Africa

Study Leader/Supervisor: Prof MJ Du Plessis
Student: DC Van Niekerk

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Ethics number:															
	Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application type: Single **Risk Category:** **Minimal**

Commencement date: 01/02/2020
Expiry date: 01/04/2021

Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special in process conditions of the research for approval (if applicable):

- The following documentation are archived by FNASREC and should be complete and kept up to date:
  - Research proposal
  - Signed approval from the scientific committee indicating the proposed risk category
- All researchers involved in the study should submit signed NWU code of conduct statements annually.
- All researchers of low risk studies should submit proof of relevant ethics training every two years.
- All researchers that take part in activities that pose a safety and security threat to the researchers or the environment should submit a risk assessment form annually.
- All research involving human interaction should follow best ethical practise and keep documents as proof. This includes informed consent, questionnaires, incorporation of risk-benefit, and responsible data management.
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the FNASREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the FNASREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the FNASREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and FNASREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or mon- itor the conduct of your research or the informed consent process;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected;*
    - *it becomes apparent that any relevant information was withheld from the FNASREC or that information has been false or misrepresented;*
    - *submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or*
    - *new institutional rules, national legislation or international conventions deem it nec- essary.*
- *FNAS-REC can be contacted for further information or any report templates via Roelof.Burger@nwu.ac.za 018 299 4269*

The FNASREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the FNASREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely,

Prof Roelof Burger
Chairperson Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)