Susceptibility of a *Spodoptera frugiperda* (Lepidoptera: Noctuidae) reference population to insecticides and entomopathogenic fungi in South Africa

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ABSTRACT

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is regarded as the most important pest of maize (*Zea mays* L.) (Poaceae) in the world. The recent invasion of *S. frugiperda* in South Africa (SA) during 2017 therefore poses a risk, particularly for future maize production. Management of *S. frugiperda* mainly relies on chemical control strategies. It is, however, known for its rapid evolution of resistance to insecticides. The objective of this study was therefore to estimate the susceptibility of the first confirmed *S. frugiperda* population (Mk-0117) that invaded SA in 2017, to active ingredients from four mode of action groups. The susceptibility of a second *S. frugiperda* population (Mb-22), sampled in Mbombela in 2022, was also estimated to these same active ingredients, since this population occurred at a research station, with an overwintering site that is heavily controlled with insecticides. Chlorantraniliprole, indoxacarb, pyridalyl dichloropropene-derivitive and spinetoram were used since these are the only active ingredients currently being registered for control of *S. frugiperda* in 2024. The susceptibility data from the 2017 population was then compared to the 2022 population. Larvae from both populations were highly susceptible to the respective insecticides, with their respective LC_{80}-values well below the maximum field recommended label rate. This indicates that no control failure is currently expected. However, responses in susceptibility of *S. frugiperda* between 2017 and 2022 to all the respective insecticides, were considered significantly different. This indicates a risk of potential resistance evolution. In order to detect the evolution of resistance it is therefore necessary to conduct follow-up assessments of susceptibility levels of *S. frugiperda* populations in SA. *Spodoptera frugiperda* is expected to remain an agricultural pest in SA, especially in areas where it persists. Biological control using entomopathogenic fungi (EPF) has been identified as an alternative control tool to synthetic insecticides. However, there are no EPF bioinsecticides registered in SA against *S. frugiperda*. The second objective of this study was therefore to assess the potential of two *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Clavicipitaceae) and two *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) based commercial biopesticides registered in SA for their efficacy against *S. frugiperda*. *Spodoptera frugiperda* larvae were not susceptible to the biopesticides, and adult emergence as well as fecundity and longevity was not significantly affected. Prepupae were, however, susceptible to the *Metarhizium* formulations used, with *M. anisopliae* ICIPE 78 (Mt 78), causing the highest prepupal mortality of 56.67%. *Metarhizium anisopliae* ICIPE 78 can therefore be presented as a potential candidate for management of *S. frugiperda* when applied as a soil drench in an IPM program.

Keywords: entomopathogenic fungi, fall armyworm, insecticides, maize, resistance

DECLARATIONS

DECLARATION BY THE CANDIDATE

I, S. Louw, declare that the work presented in this MSc thesis is my own work, that it has 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: 27/11/2024

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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1.1 General introduction

Sufficient food supply for the future world population is a global concern (Van Dijk *et al.,* 2021; Daszkiewicz, 2022). Given that the world population is expected to reach 9.7 billion people by 2050 (UN, 2022), global agricultural production is required to increase by 70% by 2050 to maintain food security (Carvajal-Yepes *et al.,* 2019). It is, however, estimated that 40% of the global crop production loss can annually be ascribed to pests (FAO, 2021).

Challenges that pose a threat to sustainable agriculture include pests and diseases (Souto *et al.,* 2021). An example of such a pest species is *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), commonly known as the fall armyworm (FAW) (Sparks, 1979). *Spodoptera frugiperda* is native to the tropical-subtropical regions of the Western hemisphere (Luginbill, 1928; Kenis *et al.,* 2023a). This pest was first reported in west and central Africa in early 2016 where it caused major damage to maize (*Zea mays* L.) (Poaceae) crops (Goergen *et al.,* 2016). Since then, *S. frugiperda* rapidly spread throughout Sub-Saharan Africa (SSA) (Cock *et al.,* 2017) and arrived in South Africa (SA) in early 2017 (DAFF, 2017). *Spodoptera frugiperda* is a polyphagous herbivore with 353 larval host plant species recorded to date (Montezano *et al.,* 2018). Examples of larval host plant species include alfalfa (*Medicago sativa* L.) (Fabaceae), cotton (*Gossypium hirsutum* L.) (Malvaceae), grain sorghum (*Sorghum bicolor* L. Moench subsp. *bicolor*) (Poaceae), potato (*Solanum tuberosum* L.) (Solanaceae) and soybean (*Glycine max* L. Merr.) (Fabaceae) (Montezano *et al.,* 2018). Maize is the staple crop of over 300 million smallholder families in SSA, as well as the most preferred host plant of *S. frugiperda* (Huesing *et al.,* 2018). This pest has been reported to cause significant yield losses (Day *et al.,* 2017; Van den Berg *et al.,* 2021a), and it is now regarded as the most important pest of maize in the world (Van den Berg & Du Plessis, 2022).

Control of *S. frugiperda,* particularly in SSA, largely depends on chemical control with synthetic insecticides (Kumela *et al.,* 2019; Kansiime *et al.,* 2019; Tambo *et al.,* 2020; Sanou *et al.,* 2023). It is, however, known for its rapid evolution of resistance (Young

& McMillan, 1979; Yu *et al.,* 2003; Gutiérrez-Moreno *et al.,* 2019; Ahissou *et al.,* 2021a; Chen *et al.,* 2023). Several factors influence their susceptibility to insecticides (Van den Berg & Du Plessis, 2022). However, the off-label use, including misuse and overuse of insecticides, is the primary driver of the evolution of resistance (Carvalho *et al.,* 2013; Paredes-Sánchez *et al.,* 2021; Van den Berg & Du Plessis, 2022). Additionally, *S. frugiperda* is a migratory species of which resistance alleles may spread when new areas are invaded (Arias *et al.,* 2019; Yainna *et al.,* 2021; Nguyen *et al.,* 2022).

After its introduction into SSA, significant damage was caused to maize crops (Stokstad, 2017; Huesing *et al.,* 2018). This led to synthetic insecticide-based emergency responses in many African countries (Stokstad, 2017). Unfortunately, due to the lack of effective management strategies, most of these responses were ineffective (Stokstad, 2017). Knowledge on the susceptibility status to insecticides, of an initial *S. frugiperda* population that invaded SA in 2017, is important to serve as a baseline for future monitoring of possible resistance evolution. This susceptibility data is therefore important for effective insecticide resistance management (IRM) of *S. frugiperda* in SA. It will ultimately form part of a larger, widespread integrated pest management (IPM) approach (Gutiérrez-Moreno *et al.,* 2019; Zhang *et al.,* 2021). Consequently, there is a need to develop an effective and coordinated IPM approach, to manage this pest (Huesing *et al.,* 2018), that may also be suitable for maize production areas across SSA (Kenis *et al.,* 2023b). Biological control with entomopathogenic fungi (EPF), such as *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Clavicipitaceae) and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae), is an alternative control tool to synthetic insecticides for control of *S. frugiperda* (Cruz-Avalos *et al.,* 2018; Akutse *et al.,* 2019; Fakeer *et al.,* 2024; Jeon & Kim, 2024).

1.2 Biology of *Spodoptera frugiperda*

1.2.1 Life cycle and morphology

Spodoptera frugiperda is a multivoltine insect, with a holometabolic life cycle (Walton & Luginbill, 1916). Temperature has a significant influence on the duration of the life cycle of *S. frugiperda* (Du Plessis *et al.,* 2020a). Depending on environmental conditions, it may complete its life cycle in about 30 days (Dew, 1913; Capinera, 2020).

This is under favourable conditions during summer (Capinera, 2020), with temperatures ranging between 26 and 30 °C (Du Plessis *et al.,* 2020a). Its life cycle may extend up to 60 days in spring and autumn, and 80 to 90 days during the winter, when temperatures are low (Capinera, 2020). *Spodoptera frugiperda* does not undergo diapause, and only have resident populations in tropical-subtropical regions (Luginbill, 1928; Sparks, 1979; Timilsena *et al.,* 2022), where infestations occur year-round (Harrison *et al.,* 2019). *Spodoptera frugiperda* generations overlap, and all life stages can therefore be found in a single field at the same time (Harrison *et al.,* 2019).

Moths prefer to lay their eggs on the abaxial surface of leaves, generally near the base of the plant, close to the junction of the leaf and the stem (Sparks, 1979). However, when population density is high, females may oviposit eggs on all plant structures (Sparks, 1979). The eggs are laid in clusters of a few (50) to several hundred (600) at night (Dew, 1913) and a female can produce up to 1700 eggs during her lifespan (Vickery, 1929). Eggs are deposited in a single layer, but also up to four layers (Dew, 1913; Vickery, 1929). These eggs are oblate-spheroidal with a diameter of approximately 0.47 mm and a height of 0.39 mm (Luginbill, 1928). After oviposition, the female covers the egg mass with downy material (silken threads and scales) from her body (Dew, 1913; Sparks, 1979). Newly deposited eggs are white to green in colour and gradually change to brown and then black over time (Figure 1.1) (Luginbill, 1928). However, some newly deposited eggs have been documented to be brown in colour (Visser, 2017).

Figure 1.1. Newly deposited egg mass of *Spodoptera frugiperda.*

Egg eclosion usually occurs after two to four days under favourable conditions (Dew, 1913; Sparks, 1979) but eclosion has been observed to occur as long as 11 days after oviposition (Luginbill, 1928). Newly hatched neonates feed on their own egg shells and surrounding plant material (Dew, 1913; Walton & Luginbill, 1916; Sparks 1979). These young larvae rapidly disperse to neighbouring plants by either crawling, ballooning on silk threads, or by being blown a distance by wind (Luginbill, 1928; Pannuti *et al.,* 2016), to avoid intraspecific competition and cannibalism (Bentivenha *et al.,* 2017). The larval stage consists of six instars (Dew, 1913; Sparks, 1979). Depending on environmental conditions, the development period for larvae, from first-instar to prepupa, range from 10 to 34 days (Du Plessis *et al.,* 2020a). Larvae in the first-instar are white to light green with a black head capsule (Walton & Luginbill, 1918) and their bodies have small black spots from which primary setae protrude (Oliver, 1981). As the larvae grow, they darken in colour (Walton & Luginbill, 1918; Oliver, 1981). The second- and third-instars are similar in colour to first-instars immediately after moulting, and darken prior to moulting to the following instar (Hardke *et al.,* 2015). The fourth- to sixth-instars are dark in colour, with varying colour patterns depending on factors such as their diet (Hardke *et al.,* 2015). They may range from light green to brown or sometimes black in colour, and they also lack primary setae and are generally smooth (Oliver, 1981). These late-instar larvae (Figure 1.2) display a prominent inverted “Y” on their head capsule (Dew, 1913). Larvae also have a distinct pattern of four spots, arranged in a square, on the second-last abdominal segment (Hardke *et al.,* 2015). Other distinctive markings on the larvae may include white lines along the mid-dorsal, sub-dorsal and sub-spiracular areas (Oliver, 1981). Larvae may range in length from around 1.68 mm in the first-instar to 34.15 mm in the sixth-instar (Hardke *et al.,* 2015). The larvae are most active in the early morning and late evening (Luginbill, 1928).

Figure 1.2. *Spodoptera frugiperda* fifth-instar larva. Characteristic markings are indicated by the red circles.

Final-instar larvae leave the plant and burrow into the soil to pupate (Dew, 1913), often at a depth of 20 to 80 mm, depending on soil texture, moisture and temperature (Sparks, 1979). Pupation may also take place on the host plant or on hard soil where loose leaf debris and silk are utilised to construct a pupal cell (Luginbill, 1928; Capinera, 2020). Initially, pupae are light green and darken to a reddish-brown colour over time (Figure 1.3) (Hardke *et al.,* 2015). Pupae may range from 14 to 18 mm in length and are about 4.5 mm in width (Capinera, 2020). Depending on environmental conditions the pupal period lasts between 8 and 30 days (Du Plessis *et al.,* 2020a). However, moth eclosion has also been observed after 45 days (Luginbill, 1928).

Figure 1.3. *Spodoptera frugiperda* pupa.

There is a clear differentiation between sexes in the adult phase (Figure 1.4) (Luginbill, 1928). Moth wingspan ranges from 32 to 40 mm (Capinera, 2020). The forewings of male moths are mottled dark grey to brown in colour, with a gold distal cell near the center (Rwomushana, 2019), and a prominent white triangular spot towards the dorsal tip of the wing (Walton & Luginbill, 1916; Capinera, 2020). Females have a more uniform grey to brown forewing that is less prominently marked (Capinera, 2020). Hind wings of both sexes are silver to white with a narrow brown border (Walton & Luginbill, 1916). The body of both sexes are light grey to brown (Walton & Luginbill, 1916). The lifespan of an adult ranges from 10 to 21 days (Capinera, 2020). *Spodoptera frugiperda* moths are nocturnal (Vickery, 1929; Sparks, 1979), and move to host plants suitable for feeding, mating and oviposition early in the evening (Sparks, 1979). Moths feed on the nectar of various plant species, for example, on the buds of cowpea (*Vigna unguiculata* L. Walp. subsp. *unguiculata*) (Fabaceae) plants (Luginbill, 1928; Montezano *et al.,* 2018). Oviposition normally occurs within the first two to three days of a female moth's life (Vickery, 1929). However, oviposition has been observed after 17 days (Johnson, 1987).

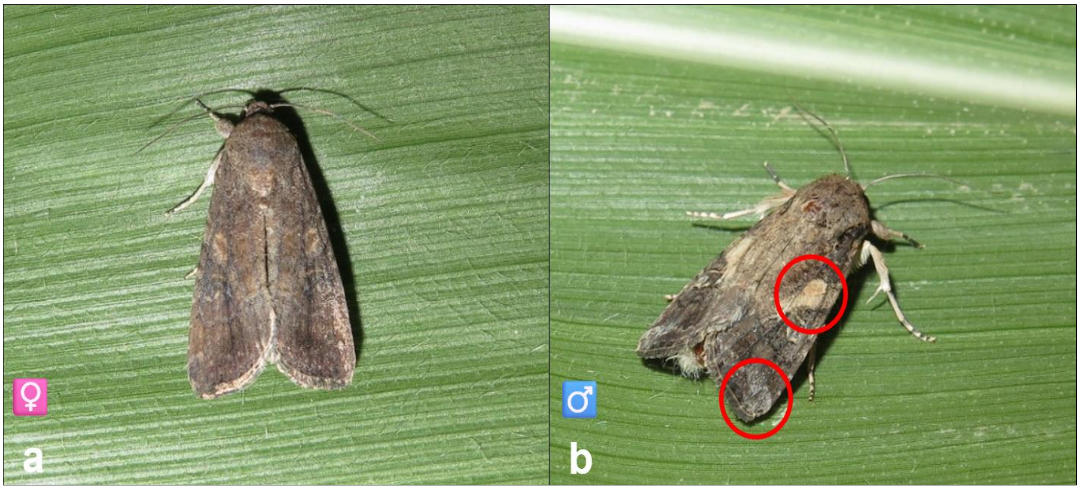

Figure 1.4. **a -** Female and **b -** male *Spodoptera frugiperda* moths. Characteristic markings on male moths are indicated by the red circles.

1.2.2 Distribution and dispersal ability

Spodoptera frugiperda is native to the tropical-subtropical areas of the Western hemisphere (Luginbill, 1928; Kenis *et al.,* 2023a). This pest was first reported in west and central Africa in early January 2016 in Nigeria, Benin, Togo as well as São Tomé and Príncipe where it caused major damage to maize crops (Goergen *et al.,* 2016). Since then, *S. frugiperda* rapidly spread throughout SSA (Cock *et al.,* 2017) and arrived in SA in early 2017 (DAFF 2017).

The introduction of *S. frugiperda* from the Americas into Africa may have been in the form off eggs, larvae, pupae and/or moths (Cock *et al*., 2017). Six possible introduction pathways were discussed by Cock *et al*. (2017) from the framework presented by Hulme *et al.* (2008). However, only three of these pathways are applicable to this situation. These are unaided dispersal, contamination of a commodity and stowaways on a vector (Cock *et al*., 2017). Since prevailing winds are generally west, the unaided dispersal by flight is an unlikely pathway of entry into Africa (Early *et al.,* 2018). Tay *et al*. (2022) also supports the eastward expansion hypothesis, which is corroborated by the chronological detection across the Old World. It is also unlikely that *S. frugiperda* entered as a contaminant of a commodity, since the importation of fresh produce from the Americas into Africa is limited (Cock *et al*., 2017). Cock *et al*. (2017) postulated the most likely way of introduction was transportation on direct commercial flights between the Americas and Africa. The countries of the initial outbreaks in west Africa (Nigeria, Benin, Togo and São Tomé and Príncipe) are those which have major air transportation hubs and climate conditions that are similar to the regions in the Americas from where large volumes of agricultural produce is imported (Early *et al.,* 2018). Data obtained from molecular analyses suggest that the *S. frugiperda* introduction from the Americas into Africa originates from the east coast of the United States (US) as well as the Caribbean region (Nagoshi *et al.,* 2017). *Spodoptera frugiperda* now has a world-wide occurrence (Van den Berg & Du Plessis, 2022; Lowry *et al.,* 2022; GBIF, 2023; Kenis *et al.,* 2023a).

The spread within SSA may also be attributed to the three introduction pathways discussed by Cock *et al.* (2017). However, the most likely way that *S. frugiperda* rapidly spread throughout SSA was through unaided dispersal (Sparks; 1979; Early *et al.,* 2018). Moths have the capacity to migrate long distances within short periods of time. It is estimated that a moth can fly up to 100 km per night (FAO, 2022), however, depending on the wind pattern, moths may fly much longer distances (Huesing *et al.,* 2018). For example, a flight of 1600 km in the US from southern Mississippi to

Southern Canada in 30 hours has been recorded (Rose *et al.,* 1975). Because of their fast dispersal rate (Cock *et al.,* 2017; FAO, 2022) and widespread distribution (Van den Berg & Du Plessis, 2022; Lowry *et al.,* 2022; GBIF, 2023; Kenis *et al.,* 2023a), there may be little that can be done to stop its sporadic spread into new areas (Du Plessis *et al.,* 2018).

The first detections of *S. frugiperda* in SA were reported in January 2017 from various locations in the Limpopo and Gauteng provinces (DAFF, 2017; IPPC, 2017). Currently, this pest occurs sporadically in all nine provinces of SA (GBIF, 2023), where it causes damage to maize crops (Makgoba *et al.,* 2021). Considering that maize is the most important crop in SA, this pest poses a serious economic risk to maize producers in the country (Makgoba *et al.,* 2021).

Population dynamics of species are greatly influenced by environmental factors (Solbreck *et al.,* 2022). Two major climatic conditions which influence the distribution of *S. frugiperda* are temperature and rainfall (Early *et al*., 2018), with temperature having the greatest influence (Damos & Soulopoulou, 2015; Solbreck *et al.,* 2022). If global temperatures increase as predicted, multivoltine migratory insects such as *S. frugiperda,* may increase their number of generations per year (Ramirez-Cabral *et al.,* 2020) and expand their distribution to areas of higher latitudes and elevations (Timilsena *et al.,* 2022). Du Plessis *et al*. (2018) predicted the potential distribution of *S. frugiperda* in Africa (Figure 1.5) using a CLIMEX model. Areas suitable for resident (permanent) populations, are indicated in yellow to red, while areas where seasonal/sporadic (temporary) infestations may occur are indicated in green (Du Plessis *et al*., 2018). Field observations in SA confirmed resident populations of *S. frugiperda*, in the Lowveld region, Northern parts of Limpopo, as well as the coastal areas of KwaZulu-Natal and the Eastern Cape (Du Plessis *et al.,* 2020b). Infestations in other areas will therefore be as a result of migrating moths, and they will be seasonal/sporadic (Du Plessis *et al.,* 2020b). Their year-round distribution is restricted to tropical-subtropical regions (Luginbill, 1928; Sparks, 1979; Timilsena *et al.,* 2022) consisting of warm, humid areas (Luginbill, 1928; Sparks, 1979; Early *et al*., 2018; Timilsena *et al.,* 2022). In SA these resident populations occur outside of the main maize producing areas (Du Plessis *et al*., 2018; Van den Berg *et al*., 2021b). Furthermore, moths may migrate long distances into temperate regions during warm summers, causing seasonal/sporadic infestations (Sparks, 1979; Timilsena *et al.,* 2022). Because they lack any diapause mechanism (Sparks, 1979; Timilsena *et al.,* 2022), they are not able to survive in areas with prolonged freezing temperatures, and they therefore establish as transient populations in these temperate regions (Timilsena *et al.,* 2022). In SA these sporadic infestations may occur inside the main maize producing areas (Du Plessis *et al*., 2018; Van den Berg *et al*., 2021b).

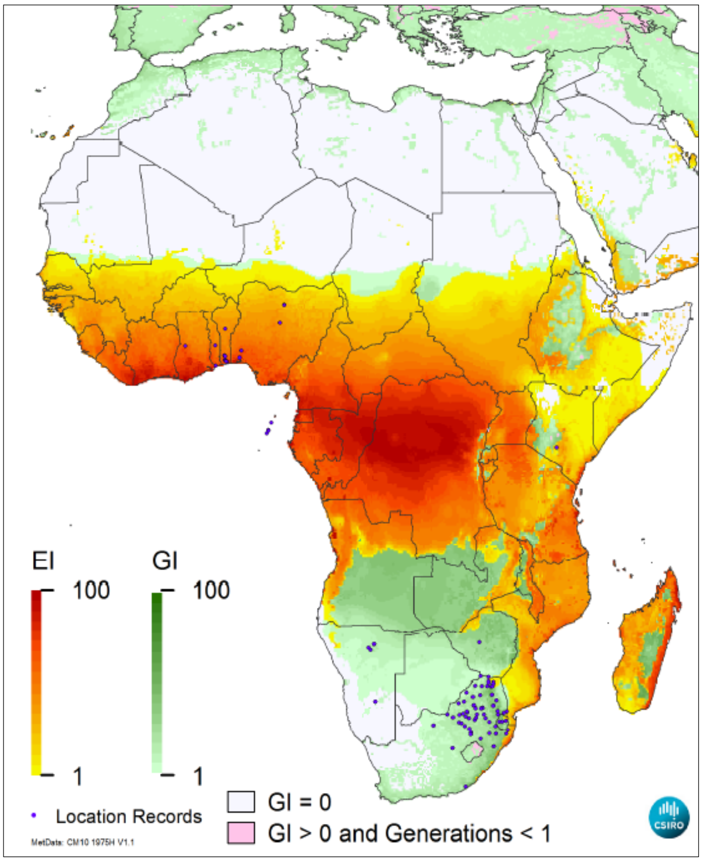

Figure 1.5. Distribution map of *Spodoptera frugiperda* in Africa according to climate suitability (Du Plessis *et al.,* 2018). The Ecoclimatic Index (EI) indicates areas that have potential suitability for persistence, while the Growth index (GI) indicates areas potentially suitable for population growth.

1.2.3 Maize damage

Spodoptera frugiperda is highly polyphagous, with larval host plant species in 76 plant families, which include over 80 crop species (Montezano *et al.,* 2018). However, *S. frugiperda* is subdivided genetically into two strains that also differ regarding larval host plant preference (Pashley *et al.,* 1985; Pashley, 1986; 1988). The “corn strain” feeds primarily on maize, cotton and grain sorghum whereas the “rice strain” feeds primarily on rice (*Oryza sativa* L.) (Poaceae) and various grasses including Bermuda grass (*Cynodon dactylon* [L.] Pers.) (Poaceae) (Pashley, 1988). Although these strains are morphologically identical (Nagoshi & Meagher, 2004), there is, however, some evidence that they may be differentiated by their wing morphometrics (Cañas-Hoyos *et al.,* 2014). Studies on the genetic identity of the *S. frugiperda* introduced from the Americas into Africa indicated that most of the genetic group consists of heterogeneous hybrids from both the “corn” and “rice strain” (Nagoshi *et al.,* 2019). However, these introduced *S. frugiperda* mostly infests maize crops (Acharya *et al.,* 2021).

Spodoptera frugiperda larvae are most active in the early morning and in the late evening (Luginbill, 1928; Hardke *et al.,* 2015). Larvae hide in plant whorls during the day while most of the damage is done at night (Luginbill, 1928). *Spodoptera frugiperda* infests all maize plant parts depending on growth stages (Goergen *et al.,* 2016). Damage results in both quantitative (direct and indirect) (Kasoma *et al.,* 2021) and qualitative (Chisonga *et al.,* 2023) losses. Direct yield losses occur when larvae feed on ears or silk, thereby directly reducing yields (Harrison, 1984). Indirect yield losses occur when larvae defoliate plants, which may in turn reduce grain production due to a decrease in photosynthetic area (Cruz & Turpin, 1983; Buntin, 1986). Quality losses caused by *S. frugiperda* occur when larval feeding introduces a fungus, such as *Aspergillus* spp. P. Micheli (Eurotiales: Aspergillaceae) which could lead to aflatoxin contamination of the grain (Abbas *et al.,* 2009; Pruter *et al.,* 2019).

When feeding on maize, first-instar larvae feed from one side of the leaf while the opposing epidermal layer remains intact (Makgoba *et al.,* 2021). This causes the characteristic ‘window-paning’ (Figure 1.6a) (McGrath *et al.,* 2018). The second- and third-instar larvae, which feed from the edge of the leaf inward (Luginbill, 1928), are responsible for creating holes in the leaves (Makgoba *et al.,* 2021). These larvae usually feed on folded leaves, creating horizontal rows of three to four small to large holes that are observed across the leaf at the time when leaves grow out (Figure 1.6b) (McGrath *et al.,* 2018; Makgoba *et al.,* 2021). Farmers are not always able to observe damage caused by first- to third-instar larvae, hence no control measures are applied

(Hardke *et al.,* 2011). However, it is recommended that farmers target smaller larvae since larger larvae are difficult to control (Ghidiu & Andaloro, 1993). Late-instar larvae tend to remain inside the maize whorl where they are protected from insecticide applications (Day *et al.,* 2017). Here they create a frass plug, which makes control difficult (Figure 1.6c) (Goergen *et al.,* 2016). Direct injury to developing kernels may result when larvae enter the ear through the husk (Morrill & Greene, 1973; Capinera, 2020) (Figure 1.6d). Final-instar larvae may cause significant defoliation of maize leaves, often leaving plants with a frayed, torn appearance (Capinera, 2020) (Figure 1.6e).

Figure 1.6. Damage caused by *Spodoptera frugiperda* to maize: **a -** window-paning from first-instar larvae; **b -** rows of pinhole damage from second- and third-instar larvae; **c -** frass plug from later-instar larvae; **d -** damage to a maize husk/grain from later-instar larvae; **e -** leaf defoliation from final-instar larvae.

1.2.3.1 Yield loss

Data on maize yield losses caused by *S. frugiperda* in Africa vary. Van den Berg *et al.* (2021a) reported yield losses ranging from 11 to 100%, while 20 and 50% yield loss was reported by Early *et al.* (2018). Furthermore, it has also been predicted that, in the absence of control measures, *S. frugiperda* may be able to cause between 8.3 and 20.6 million tonnes of yield loss per year in only 12 maize producing countries in Africa (Day *et al.,* 2017). These losses represent 21 to 53% of the annual production of maize in these countries (Day *et al.,* 2017).

Most of the maize yield loss data in Africa were obtained from farmer surveys. *Spodoptera frugiperda* has been reported to cause estimated yield losses of between 11.6 (Baudron *et al.,* 2019) and 58% (Chimweta *et al.,* 2018) in Zimbabwe, 22-67% in Ghana and 25-50% in Zambia (Day *et al.,* 2017). The estimated yield loss in Ethiopia was at 32% (Kumela *et al.,* 2019), and between 33 - 47.3% in Kenya (Kumela *et al.,* 2019; De Groote *et al.,* 2020). There is currently no survey data available on the estimated maize yield losses in SA, however data are available based on field trials (Van den Berg *et al.,* 2021a). Crop losses obtained from farmer surveys are usually overestimated (Baudron *et al.,* 2019; Overton *et al.,* 2021; Van den Berg & Du Plessis, 2022), often more than double that derived in experimental trials (Overton *et al.,* 2021). Overton *et al.* (2021) identified at least three factors that explain the variability between yield losses obtained through farmer surveys and experimentally derived yield losses. Firstly, farmers may associate the yield loss with that caused by infestations by other pests, which may increase the perceived yield losses (Willocquet *et al.,* 2004). Secondly, there is a possibility of over-reporting of crop losses due to loss-aversion (Heong *et al.,* 2021). Thirdly, experimentally derived yield losses may be higher since it is always attempted to establish the maximum level of yield loss that may occur as a result of the pest (Overton *et al.,* 2021).

1.3 Integrated management of *Spodoptera frugiperda*

Spodoptera frugiperda is difficult to control since it multiplies fast, has a short life cycle, lacks diapause, migrates easily, is a trans-boundary pest and has a polyphagous feeding characteristic (Matova *et al.,* 2020). Although chemical control is the most common control method for *S. frugiperda* in SSA (Kumela *et al.,* 2019; Kansiime *et al.,* 2019; Tambo *et al.,* 2020; Sanou *et al.,* 2023), it is not a long-term and sustainable solution (Young & McMillan, 1979; Yu *et al.,* 2003; Stokstad, 2017; Gutiérrez-Moreno *et al.,* 2019; Ahissou *et al.,* 2021a; Chen *et al.,* 2023).

An IPM approach provides a useful framework to achieve effective management of *S. frugiperda* (Huesing *et al.,* 2018). Integrated pest management may be defined as a holistic approach to "a decision-based process involving coordinated use of multiple tactics for optimizing the control of all classes of pests (insects, pathogens, weeds, vertebrates) in an ecologically and economically sound manner" (Prokopy, 2003). A variety of integrated approaches, which consists of cultural control, host plant resistance, biological control and chemical control, is needed (Huesing *et al.,* 2018; Matova *et al.,* 2020). It is crucial to keep in mind that IPM focuses first on preventing pest outbreaks while making use of the least harmful strategy first (Green *et al.,* 2020). Chemical control should therefore be used as a last resort, and only introduced when necessary (Dara, 2019; Green *et al.,* 2020).

1.4 Control measures for *Spodoptera frugiperda*

1.4.1 Cultural control

Cultural control includes any strategy that involves the modification of the environment in such a way as to make it less favourable for a pest (Dent, 2000). These strategies interfere with, and prevent a pest's ability to colonize, survive, establish, find mating partners and reproduce (Dent, 2000). Several cultural control methods have been adopted in SSA to manage *S. frugiperda.* Early interventions include the push-pull method (Midega *et al.,* 2018), early planting with the first rains (Harrison *et al.,* 2019; Kansiime *et al.,* 2019; Njuguna *et al.,* 2021), crop rotation and intercropping (Kansiime *et al.,* 2019; Njuguna *et al.,* 2021) and frequent handpicking and crushing of *S. frugiperda* eggs or larvae found on maize plants (Harrison *et al.,* 2019; Kansiime *et al.,* 2019; Njuguna *et al.,* 2021). When larvae are present in the whorls, methods applied include application of water into maize whorls (Njuguna *et al.,* 2021), tobacco and neem extracts (Kansiime *et al.,* 2019), ash, soil (Njuguna *et al.,* 2021; Harrison *et al.,* 2019 Kansiime *et al.,* 2019; Maphumulo *et al.,* 2023), sawdust, (Njuguna *et al.,* 2021) or liquid detergent (Kansiime *et al.,* 2019). Spray applications used by smallholder farmers in SSA include hot pepper solutions (Kansiime *et al.,* 2019; Njuguna *et al.,* 2021), mixtures of lime, soap, salt and oil (Njuguna *et al.,* 2021) and sugar solutions or fish soup (Harrison *et al.,* 2019; Njuguna *et al.,* 2021).

1.4.2 Host plant resistance

Plants have coevolved with herbivorous insects for millennia, and as a result through natural selection, evolved a wide range of physiological and morphological traits that serve as a basis for defence against herbivory (Cutler, 2020). Human intervention allowed the breeding of crop cultivars with defensive traits that reduce their susceptibility to herbivores (Dent, 2000; Cutler, 2020). Host plant resistance may be defined as those traits "which enable a plant to avoid, tolerate or recover from the attacks of insects under conditions that would cause greater injury to other plants of the same species" (Dent, 2000). There are three primary ways in which plants might have reduced susceptibility to *S. frugiperda* (Cutler, 2020). Firstly, resistant host plants may fail to provide attractive stimuli (antixenosis or non-preference). Secondly, adverse biological effects may be caused from feeding on resistant plants (antibiosis), and thirdly, resistant host plants may withstand or recover from injury (tolerance) (Smith & Clement, 2012). An example of host plant resistance in maize, is transgenic maize.

Sub-Saharan Africa may potentially benefit from using transgenic maize that has been genetically modified to contain genes from the soil bacterium *Bacillus thuringiensis* (Bt) Berliner (Caryophanales: Bacillaceae) (Prasanna *et al.,* 2018). These transgenic maize plants express insecticidal toxins that kill target species such as *S. frugiperda* (Prasanna *et al.,* 2018; Botha *et al.,* 2019; Van den Berg *et al.,* 2021b). South Africa is considered the leading country, regarding biotechnology in Africa, since it is the only country where Bt maize is cultivated (Clement, 2022; ISAAA, 2023). In SA, two Bt maize products provide resistance against *S. frugiperda* (Prasanna *et al.,* 2018). Firstly, MON810 maize expressing the Cry1Ab toxin, which is intended for stem borer control (Prasanna *et al.,* 2018; Botha *et al.,* 2019) but confers partial resistance to *S. frugiperda* (Prasanna *et al.,* 2018). Secondly, MON89034 expressing the Cry1A.105 + Cry2Ab2 toxins, which is intended for control of both stem borer and *S. frugiperda* (Prasanna *et al.,* 2018; Botha *et al.,* 2019). In SA, *S. frugiperda* has been included as a target pest of MON89034 maize, since November 2018 (Botha *et al.,* 2019). Agricultural research institutions are currently testing the performance of Bt maize under the TELA$^{®}$ maize project, for possible commercialization in Ethiopia, Kenya, Nigeria, Mozambique, Tanzania and Uganda (AATF, 2023). However, resistance to Bt maize by *S. frugiperda* has already been documented in many countries outside of

SSA. For example, *S. frugiperda* was found to be resistant to Bt maize expressing Cry1F in Puerto Rico (Storer *et al.,* 2010), Brazil (Farias *et al.,* 2014) and in the southern states of the US (Huang *et al.,* 2014), and also to Cry1Ab in Brazil (Farias *et al.,* 2014; Omoto *et al.,* 2016). The rapid evolution of resistance to Bt maize demonstrates that reliance cannot be placed on biotechnology only and that there is a need to develop alternative control strategies for implementation in an IPM strategy.

1.4.3 Biological control

Biological control includes any strategy which use natural enemies to suppress pest numbers (Cutler, 2020). In SSA three types of biological control strategies against *S. frugiperda* are feasible (Cutler, 2020). Firstly, natural enemies may be imported from the native area of *S. frugiperda* and released to permanently establish in invaded areas where the pest has become established (Kenis *et al.,* 2019). These natural enemies usually target introduced/exotic pests, which arrive in a region without the natural enemies that controlled their populations in their native area (classical biological control) (Kenis *et al.,* 2019, 2023b; Cutler, 2020). Secondly, natural enemies occur naturally in crop fields but are unable to survive or persist at sufficient numbers to effectively suppress pest populations (Cutler, 2020). However, it may be possible to mass rear and continually release these natural enemies for temporary control (augmentative biological control) (Kenis *et al.,* 2019; Cutler, 2020). Thirdly, the survival and/or effectiveness of natural enemies that are already present in a crop field may be enhanced by conservation biocontrol practices such as minimizing insecticide use and providing natural enemies with habitats that support their populations (conservation biological control) (Kenis *et al.,* 2019; Cutler, 2020).

1.4.3.1 Pathogens

Pathogens are disease causing microorganisms (Federici, 2009), namely bacteria, fungi, viruses, nematodes and protozoa (Dent, 2000). Pathogens that infect and kill insects are known as entomopathogens (Deka *et al.,* 2021). These entomopathogens are often formulated as bioinsecticides which are utilised as alternatives to synthetic insecticides (Waage, 1997). The use of pathogens may be considered as a component of biological control, however, when dealing with commercial products, the control may be referred to as chemical (Perry *et al.,* 1988). Although the development and implementation of bioinsecticides in SSA is still in its early stages (Ndolo *et al.,* 2019), there are several institutions investigating potential bioinsecticide options for *S. frugiperda* control (Akutse *et al.,* 2019; Ndolo *et al.,* 2019).

Sub-Saharan Africa may potentially benefit from using EPFs such as *B. bassiana* and *M. anisopliae*, for control of *S. frugiperda* (Cruz-Avalos *et al.,* 2018; Akutse *et al.,* 2019; Fakeer *et al.,* 2024; Jeon & Kim, 2024). Entomopathogenic fungi are not yet registered in SA for use against *S. frugiperda* (Agri-Intel, 2024). These EPF's has however, been successfully used in several other countries (Cruz-Avalos *et al.,* 2018; Akutse *et al.,* 2019; Fakeer *et al.,* 2024; Jeon & Kim, 2024), demonstrating its potential to be commercialized on maize against *S. frugiperda*.

The general mode of infection for all EPFs is the same (Figure 1.7) (Vega *et al.,* 2012; Lacey *et al.,* 2015). Genetic studies indicate that different EPF genera may be differentiated by physiological characteristics (i.e., toxins produced during the infection process) (Xiao *et al.,* 2012; Sbaraini *et al.,* 2016). Infection by an EPF consists of six stages *viz*. adhesion, germination, formation of infective structures, penetration, colonization of hemocoel, extrusion and sporulation (Aw & Hue, 2017). Infection starts when the host cuticle comes into contact with an EPF conidium/spore (Hajek & St Leger, 1994; Vega *et al.,* 2012; Ortiz-Urquiza & Keyhani, 2013). This conidium adheres to the cuticle by means of hydrophobins and adhesins, which are proteins, located on their surface (Mora *et al.,* 2017). After a conidium attaches to the cuticle it germinates, producing several specialized structures which allow penetration into the host. This includes a germ tube, appressorium, infection peg, penetrant hypha and a hyphal body/blastospore (Hajek & St Leger, 1994). For penetration to occur, the combined action of mechanical pressure and the production of cuticle-degrading enzymes is needed (Gillespie & Claydon, 1989; Hajek & St Leger, 1994; Bava *et al.,* 2022). Once the EPF has invaded the hemocoel, the host may die 3-14 days after conidium application, as a result of a combination of physical tissue damage produced by fungal growth, dehydration of cells due to loss of fluid, nutrient exhaustion and toxicosis (Gillespie & Claydon, 1989). A variety of toxins have been reported to cause symptoms such as slowness, reduced responsiveness to external stimuli and paralysis (Bava *et al.,* 2022). After host death, hyphae/mycelia grow out of the cadaver and sporulate to produce conidia (Shah & Pell, 2003; Litwin *et al.,* 2020). Several environmental factors, especially, temperature and humidity as well as biological factors, such as the host life stage, behaviour and immune responses play a role in the successful infection of a host (Vega *et al.,* 2012).

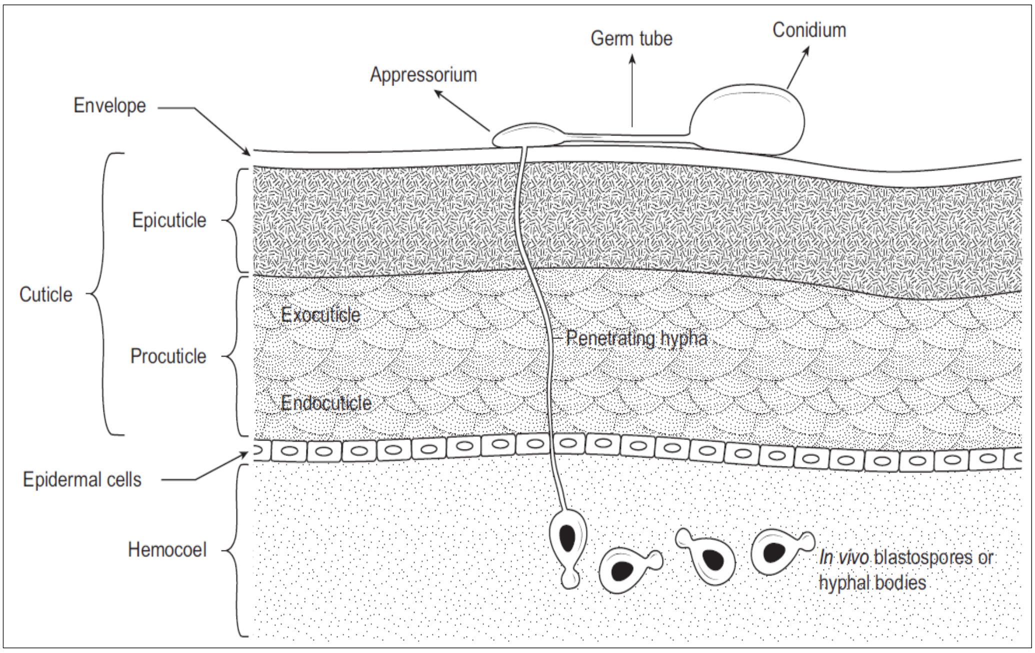

Figure 1.7. The general mode of infection of entomopathogenic fungi infecting a host (Vega *et al.,* 2012).

1.4.3.1.1 Entomopathogenic fungi

1.4.3.1.1.1 *Beauveria bassiana*

Beauveria bassiana was first identified approximately 180 years ago (Zimmermann, 2007a). Colonies of *B. bassiana* are characterised by white, cottony aerial mycelia (Sharma *et al.,* 2020). The most common toxins produced during the infection process include bassianin, beauvericin, bassianolide, beauverolides and tenellin (Strasser *et al.,* 2010).

1.4.3.1.1.2 *Metarhizium anisopliae*

Metarhizium anisopliae was first identified over 130 years ago (Zimmermann, 2007b). Colonies of *M. anisopliae are* characterised by white, aerial mycelia, which turns to green at later stages (Sharma *et al.,* 2020). The most common toxins produced during the infection process include destruxins, swainsinone and cytochalasin C (Strasser *et al.,* 2010).

1.4.3.2 Parasitoids and predators

Parasitoids refer to insects whose immature stages develop either within or attached to the outside of their hosts (Raupp *et al.,* 2023). These parasitoids inevitably cause the death of their host (Dent, 2000). Parasitoids associated with *S. frugiperda* are wasps (Hymenoptera) and flies (Diptera) (Kenis *et al.,* 2023b). Predators associated with *S. frugiperda* are earwigs (Dermaptera), ladybird beetles (Coleoptera), ground beetles (Coleoptera), assassin and flower bugs (Hemiptera), eusocial, solitary and other predatory wasps (Hymenoptera), spiders (Arachnida), ants (Hymenoptera) as well as birds and bats (FAO, 2018).

Some of the most common native parasitoid and predator species of *S. frugiperda* that occurs in SSA, are listed in table 1.1. Introducing natural enemies for control of *S. frugiperda* is challenging (Kenis *et al.,* 2023b). Two main criteria are used to select parasitoids and predators for use in classical biocontrol programs. These are the ability of natural enemies to control pests in native or invaded areas, and host specificity to prevent non-target effects (Kenis *et al.,* 2023b). Parasitoids are the most common natural enemy of *S. frugiperda* (FAO, 2018). Around 150 parasitoid species are associated with *S. frugiperda* in their native range (FAO, 2018). Based on these two criteria, six parasitoid species from their native range in the Western hemisphere, have been identified as possible biological control agents for introduction in invasion areas, including SSA. These are *Aleiodes laphygmae* (Viereck) (Hymenoptera: Braconidae), *Archytas marmoratus* (Townsend) (Diptera: Tachinidae), *Campoletis* spp. Förster (Hymenoptera: Ichneumonidae), *Chelonus insularis* Cresson (Hymenoptera: Braconidae), *Cotesia marginiventri* (Cresson) (Hymenoptera: Braconidae) and *Eiphosoma laphygmae* Costa Lima (Hymenoptera: Ichneumonidae) (Kenis *et al.,* 2023a).

Table 1.1. Most common native parasitoid and predator species of *Spodoptera frugiperda* that occurs in Sub-Saharan Africa.

Order: Family	**Species**	**Source**
Parasitoids		
Hymenoptera: Platygastridae	*Telenomus remus* Nixon	Cruz *et al.,* 2018; Kenis *et al.,* 2019; Agboyi *et al.,* 2020; Abang *et al.,* 2021; Otim *et al.,* 2021
Hymenoptera: Braconidae	*Chelonus bifoveolatus* Szépligeti	Agboyi *et al.,* 2020; Koffi *et al.,* 2020; Ahissou *et al.,* 2021b; Otim *et al.,* 2021
	Coccygidium luteum (Brullé)	FAO, 2018; Sisay *et al.,* 2018; Agboyi *et al.,* 2020; Koffi *et al.,* 2020; *Abang et al.,* 2021; Ahissou *et al.,* 2021b; Otim *et al.,* 2021
	Cotesia icipe Fernández-Triana & Fiaboe	Cruz *et al.,* 2018; FAO, 2018; Sisay *et al.,* 2018; Agboyi *et al.,* 2020; Koffi *et al.,* 2020; Abang *et al.,* 2021; Otim *et al.,* 2021
Diptera: Chloropidae	*Drino quadrizonula* (Thomson)	Agboyi *et al.,* 2020; Otim *et al.,* 2021
Predators		
Hymenoptera: Formicidae	*Pheidole megacephala* (Fabricius)	Koffi *et al.,* 2020; Ahissou *et al.,* 2021b; Dassou *et al.,* 2021
Dermaptera: Carcinophoridae	*Euborellia annulipes* (Lucas)	Cruz *et al.,* 2018; FAO 2018

1.4.4 Chemical control

Chemical control refers to the use of any substance with an active ingredient "intended for preventing, destroying, repelling or mitigating any pest" (NPIC, 2021). Resistance evolution will always remain a problem if insecticides are used in pest management programs (Tabashnik *et al.*, 2014). At the Insecticide Resistance Action Committee (IRAC), the crop protection industry addresses the need for improved insect resistance management (IRM) (IRAC, 2024a). Insect resistance management aims to prevent or delay the evolution of resistance to insecticides or to recover the susceptibility of an insect pest/mite population in which resistance has already evolved (Nauen *et al.,* 2012; Sparks & Nauen, 2015). One of the main tools is the mode of action (MoA) classification system (Sparks & Nauen, 2015), which provides guidelines on the selection of insecticides to be used in a rotation-based IRM program (Nauen *et al.,* 2012; Sparks & Nauen, 2015). The Insecticide Resistance Action Committee categorizes insecticides into groups based on their MoA, which refers to the different target sites where insecticides act on, for example, the functions of nerves and muscles, growth, respiration and midgut as well as insecticides with an unknown or non-specific function (IRAC, 2024a). The IRAC MoA classification system recommends that insecticides from different MoA groups or combinations of compounds from MoA groups should be rotated in spraying programs (Nauen, 2019). Rotation of insecticides in the same group, but in different subgroups/classes (e.g., carbamates 1A and organophosphates 1B) are not recommended unless there are no other insecticides available that are in different groups (Sparks & Nauen, 2015). Insecticides within the same group, but in different subgroups/classes, share a common MoA/target site within a pest (Nauen *et al.,* 2012). When resistance evolves to an insecticide it often also confers cross-resistance to related insecticides within the same group (Nauen *et al.,* 2012). The rotation of insecticides in an IRM program should therefore be based on a window or block application strategy (Figure 1.8) (Sparks & Nauen, 2015; IRAC, 2021; IRAC 2024b). The latter strategy is based on the length of a pest generation or crop growth stages (IRAC, 2024b), which ensures that successive generations of the pest are not treated with insecticides from the same MoA group (Sparks & Nauen, 2015; Nauen *et al.,* 2012).

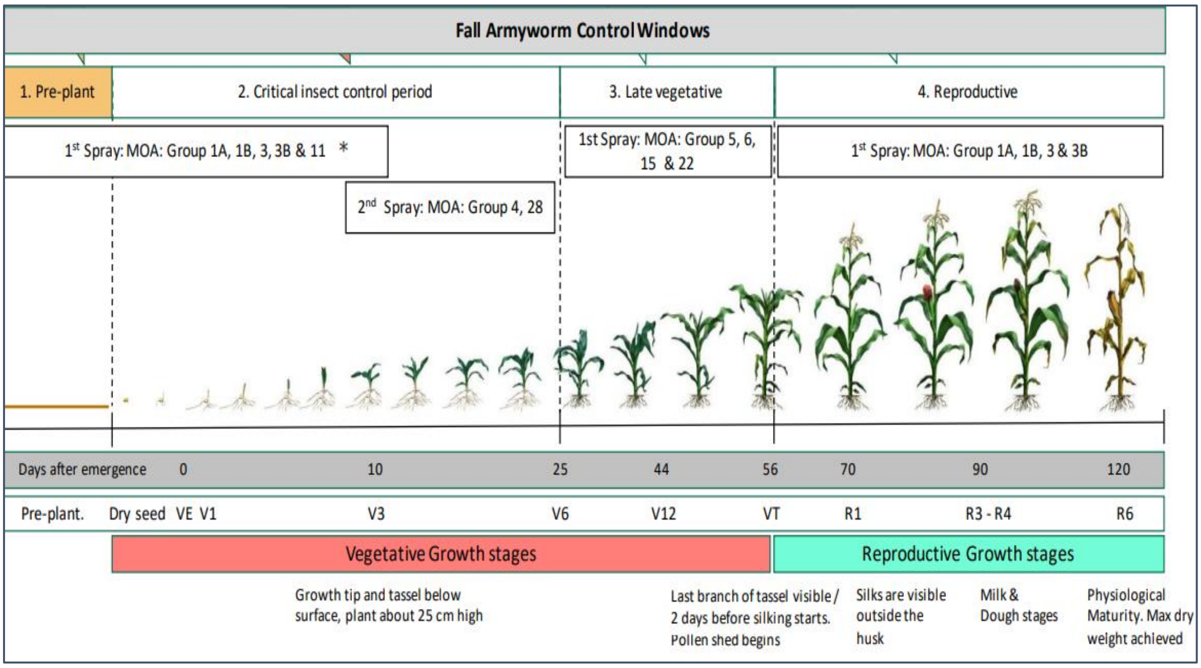

Figure 1.8. Example of a window application strategy for *Spodoptera frugiperda* (IRAC, 2021).

In the initial response to *S. frugiperda* in SSA, governments haphazardly purchased and distributed insecticides (Stokstad, 2017). At the time, many SSA countries had no insecticide formulations registered for use against *S. frugiperda* (Sisay *et al.,* 2019, Suguiyama *et al.,* 2020), regardless of the Pesticide Emergency Use Authorization (PEUA) regulatory tool that exists in several countries (Suguiyama *et al.,* 2020). Without these emergency registrations, farmers had limited alternative control options other than off-label application of older and, in many cases, more toxic insecticides (Sisay *et al.,* 2019; Suguiyama *et al.,* 2020). In only two SSA countries (SA and Kenya) emergency registrations of insecticides were done (Suguiyama *et al.,* 2020). When *S. frugiperda* first invaded SA 2017, 22 different active ingredients (excluding Bt and *B. bassiana*), belonging to 10 MoA groups were registered for its control (Agri-Intel, 2017; DALRRD, 2017). However, after the emergency registrations of these active ingredients lapsed, several were not re-registered, resulting in only four active ingredients, belonging to four MoA groups currently being registered for *S. frugiperda* control. The insecticides that received emergency registrations in 2017 in SA are provided in table 1.2.

Table 1.2. Insecticides that received emergency registrations for control of *Spodoptera frugiperda* on maize in South Africa (Agri-Intel, 2017; DALRRD, 2017).

Active ingredient	**Chemical class**	**IRAC mode of action**
Carbosulfan	Carbamate	1A
Chlorantraniliprole*	Diamides	28
Chlorpyrifos	Organophosphate	1B
Emamectin benzoate	Avermectin	6
Flubendiamide	Diamides	28
Indoxacarb*	Oxadiazine	22A
Lufenuron	Benzoylureas	15
Mercaptothion	Organophosphates	1B
Methomyl	Carbamate	1A
Profenofos	Organophosphates	1B
Pyridalyl dichloropropene-derivative*	Pyridalyl	UN
Spinetoram*	Spinosyns	5
Benfuracarb/Fenvalerate	Carbamate /Pyrethroid	1A/3A
Chlorantraniliprole/Lamda-cyhalothrin	Diamides/Pyrethroid	28/3A
Chlorpyrifos/Cypermethrin	Organophosphate/Pyrethroid	1B/3A
Novaluron/Indoxacarb	Benzoylureas/Oxadiazine	15/22A
Spinetoram/Methoxyfenozide	Spinosyns/Diacylhydrazines	5/18

*currently registered

1.4.4.1 Insecticides used in this study

1.4.4.1.1 Chlorantraniliprole

Chlorantraniliprole acts as ryanodine receptor modulators and belongs to group 28 (diamides) of the IRAC classification system (IRAC, 2024a). Ryanodine receptors are named after ryanodine, a plant alkaloid obtained from the plant *Ryania speciosa* Vahl (Salicaceae) (Sattelle *et al.,* 2008), which is known for its use as a natural insecticide (Nauen, 2006). Ryanodine receptors are intracellular calcium release channels (Ebbinghaus-Kintscher *et al.,* 2006; Teixeira & Andaloro, 2013) necessary for excitation-contraction coupling in muscles (Ebbinghaus-Kintscher *et al.,* 2006).

Diamides bind to ryanodine receptors causing calcium channels to remain open, resulting in an uncontrolled release of calcium stores (Ebbinghaus-Kintscher *et al.,* 2006; Teixeira & Andaloro, 2013). This calcium store depletion impairs regulation of muscle contraction (Bentley *et al.,* 2010), resulting in symptoms such as lethargy (Bentley *et al.,* 2010; Teixeira & Andaloro, 2013), muscle paralysis (Bentley *et al.,* 2010; Du & Fu, 2023) and feeding cessation (Teixeira & Andaloro, 2013), which eventually leads to death (Bentley *et al.,* 2010; Teixeira & Andaloro, 2013). Chlorantraniliprole is commercially produced since 2007 (Troczka *et al.,* 2017) and it is the most widely used diamide insecticide (Du & Fu, 2023). Thirteen cases of resistance to chlorantraniliprole in *S. frugiperda* have been reported to date (Mota-Sanchez & Wise, 2024).

1.4.4.1.2 Indoxacarb

Indoxacarb belongs to group 22A (oxadiazines) of the IRAC classification system, and acts as a voltage-dependent sodium channel blocker (IRAC, 2024a). Voltage-gated sodium channels are responsible for the generation of action potentials across the membranes of excitable cells (Song *et al.,* 2006; Dong *et al.,* 2007). Oxadiazines are activated by means of enzymes into metabolites (Wing *et al.,* 1998). Oxadiazines block voltage-dependent sodium channels in insect motor nerves (Wing *et al.,* 1998; 2000; Lapied *et al.,* 2001), causing hyperpolarization of cells (Lapied *et al.,* 2001). This leads to symptoms such as feeding cessation, convulsions and paralysis which is eventually followed by death (Wing *et al.,* 1998). After oxadiazines were first discovered in the 1970s (McCann *et al.,* 2001), it led to the discovery of indoxacarb that was first commercialised in 2000 (EPA, 2000). Twelve cases of resistance to indoxacarb in *S. frugiperda* has been reported (Mota-Sanchez & Wise, 2024).

1.4.4.1.3 Pyridalyl dichloropropene-derivitive

Pyridalyl dichloropropene-derivitive belongs to group UN of the IRAC classification system, which implies that its MoA is unknown (IRAC, 2024a). It is described as distinctive in its action compared to existing insecticides and is thus considered to have a novel MoA (Isayama *et al.,* 2005; Saito *et al.,* 2004; Nishimura *et al.,* 2007; Sakamoto *et al.,* 2012). Although its MoA is unknown, the hypothesis is that pyridalyl dichloropropene-derivitive exhibits insecticidal traits, by means of cytochrome P450 activation (Powell *et al.,* 2011). This causes damage and degradation to cellular macromolecules (e.g., proteins) leading to necrotic cell death (Powell *et al.,* 2011). Similar studies confirm this hypothesis. For example, burn-like scars appeared in lepidopteran larvae treated with sub-lethal concentrations of pyridalyl dichloropropene-derivitive, although this symptom only appeared in larvae that survived treatment (Saito *et al.,* 2004; Sakamoto *et al.,* 2012). Larvae that die within several hours of treatment with lethal concentrations do not exhibit these symptoms (Saito *et al.,* 2004; Sakamoto *et al.,* 2012). This indicates that symptoms produced by both lethal and sub-lethal concentrations of pyridalyl dichloropropene-derivitive may be related to degeneration of cells in larvae (Sakamoto *et al.,* 2012). Other symptoms such as a decrease in vigour, mobility and body elasticity, which leads to feeding inhibition (Nishimura *et al.,* 2007), abortive moult as well as abnormal pupal development and failure to emerge have also been documented (Saito *et al.,* 2004). Previous studies have shown that pyridalyl dichloropropene-derivitives has a cytotoxic effect on cultured Sf9 cells (Isayama *et al.,* 2005; Nishimura *et al.,* 2007; Sakamoto *et al.,* 2012). After the pyridalyl dichloropropene-derivitive was first launched in 2004, it has been commercialized globally (Sakamoto *et al.,* 2012). No cases of resistance to pyridalyl dichloropropene-derivitive in *S. frugiperda* has been reported to date (Mota-Sanchez & Wise, 2024).

1.4.4.1.4 Spinetoram

Spinetoram belongs to group 5 (spinosyns) of the IRAC classification system, acting as allosteric modulators of the nicotinic acetylcholine receptor (nAChR) (IRAC, 2024a). Spinosyns are a fermentation derived product from the soil microorganism *Saccharopolyspora spinosa* Mertz and Yao (Thompson & Sparks, 2002; Galm & Sparks, 2016). Spinetoram is a semi-synthetic mixture of two spinosyn derivatives, spinosyn-J and-L (Kirst, 2010; Saglam *et al.,* 2013; Galm & Sparks, 2016). Nicotinic acetylcholine receptors are ligand-gated ion channels that mediate a fast synaptic transmission of the neurotransmitter acetylcholine (ACh) (Bacci *et al.,* 2016), which ensures that the activation of cholinergic receptors is transient (Colombo *et al.,* 2005). Spinosyns act as agonists (allosteric activators) of both the nAChR and gamma-aminobutyric acid (GABA) receptors (Thompson & Sparks, 2002; Bacci *et al.,* 2016). This disrupts neuronal activity by exciting motor neurons (Salgado, 1998; Thompson & Sparks, 2002; Bacci *et al.,* 2016) causing symptoms such as feeding cessation (Cisneros *et al.,* 2002), involuntary muscle contractions/tremors and paralysis

(Salgado, 1998; Cisneros *et al.,* 2002; Thompson & Sparks, 2002; Bacci *et al.,* 2016) eventually leading to death (Cisneros *et al.,* 2002; Bacci *et al.,* 2016). The first spinosysns were registered in the 1990s (Thompson *et al.,* 2002; Galm & Sparks, 2016). However, it was not until 2007 that spinetoram gained its first registration (Sparks *et al.,* 2008; Bacci *et al.,* 2016; Galm & Sparks, 2016). Six cases of resistance to spinetoram in *S. frugiperda* has been reported (Mota-Sanchez & Wise, 2024).

1.5 Resistance evolution

Resistance is defined as a "genetically based decrease in susceptibility to a pesticide" (Tabashnik *et al.*, 2014). This decrease in susceptibility may be observed as the repeated failure of an insecticidal product to achieve the intended level of control when used according to label directions for that pest species (IRAC, 2024b).

Although *S. frugiperda* is known for its rapid evolution of resistance (Young & McMillan, 1979; Yu *et al.,* 2003; Gutiérrez-Moreno *et al.,* 2019; Ahissou *et al.,* 2021a; Chen *et al.,* 2023), control is still largely dependent on the use of synthetic insecticides (Kumela *et al.,* 2019; Kansiime *et al.,* 2019; Tambo *et al.,* 2020; Sanou *et al.,* 2023). Four major factors contribute to the increased risk of resistance evolution of *S. frugiperda* to insecticides (Van den Berg & Du Plessis, 2022). These are: policy issues, pest management practices, pest biology and invasion patterns as well as insecticide application methods that are not effective. However, the indiscriminate use of insecticides is the primary driver of the evolution of resistance (Carvalho *et al.,* 2013; Paredes-Sánchez *et al.,* 2021; Van den Berg & Du Plessis, 2022). For example, in Puerto Rico insecticides are sprayed thrice per week during the peak season and up to 25 sprays have been reported in one maize crop cycle, resulting in the continuous exposure of *S. frugiperda* to insecticides (Belay *et al.,* 2012). Additionally, *S. frugiperda* is a migratory species in which resistance alleles may spread when new areas are invaded (Arias *et al.,* 2019; Yainna *et al.,* 2021; Nguyen *et al.,* 2022).

There is genetic variation in any insect pest population (Figure 1.9) (Georghiou, 1972; Gut *et al.,* 2002). This implies that there exist individuals with inherent resistance (initially uncommon) (red), as well as well as abundant susceptible individuals (green) (Georghiou, 1972; Gut *et al.,* 2002). Susceptible biotypes are killed when a certain insecticide is used, whereas resistant biotypes survive and reproduce, increasing the prevalence of resistance genes in the population (Georghiou, 1972; Gut *et al.,* 2002). Selection for resistance happens when insecticides with the same MoA are used on a continuous basis (Sparks & Nauen, 2015), or when insecticides are not sprayed according to label recommendations (IRAC, 2024b; Gut *et al.,* 2002). When insects continue to evolve resistance to insecticides, new insecticides have to be introduced on a regular basis (Kogan & Bajwa, 1999; Sparks & Nauen, 2015).

The evolution of insecticide resistance is a natural response of a pest population, and resistance will therefore remain a problem as long as insecticides are used (Tabashnik *et al.*, 2014). Despite these constraints, chemical control, if used appropriately and according to label recommendations, remains a safe and effective method of controlling insect pests (McGrath *et al*., 2018).

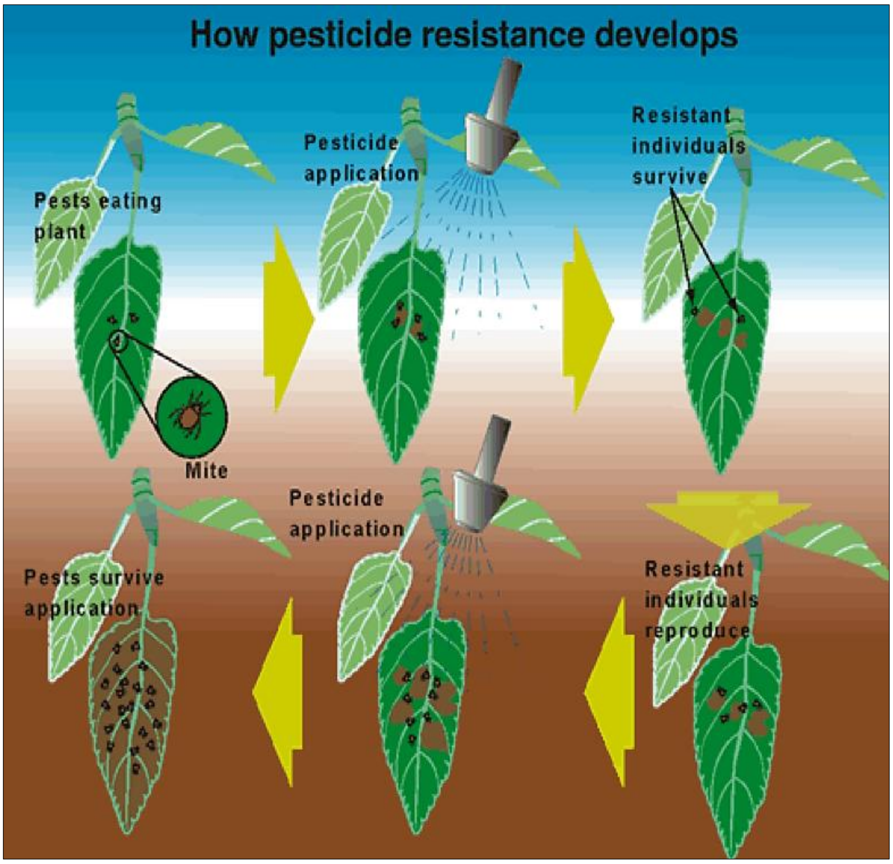

Figure 1.9. A graphical presentation of the process of selection for resistant individuals in a pest population (Gut *et al.,* 2002).

1.6 Economic and action thresholds

The economic threshold level (ETL), action threshold (AT) and economic injury level (EIL) are important concepts in pest control (Figure 1.10). A control measure is only justifiable once a pest population reaches a particular density level (Seiter, 2018). The ETL is defined as the pest population density at which a control measure should be implemented to prevent an increasing pest population from reaching the EIL (Stern *et al.,* 1959). The two concepts of ETL and AT are often used interchangeably, although the AT is defined as the optimal (exact) time at which control measures should be implemented (Pedigo *et al.,* 1986). The EIL is defined as the lowest pest population density that will cause economic harm (Stern *et al.,* 1959). The EIL is considered as the break-even point, where the economic loss from the pest justifies the costs of managing the pest (Hunt *et al.,* 2009). Contrary to the EIL, ATs are estimated based on professional opinion and experience along with field scouting assessments (Huesing *et al.,* 2018). Conversely, the EIL takes into account the pest management costs, market value of crop and expected yield loss (Huesing *et al.,* 2018). It is not cost-effective to implement control measures when the pest density level is below the EIL, since the cost involved will exceed the cost of yield loss due to pest damage (NCSU, 2023). However, it is not always desirable to delay taking control measures until a pest population reaches the breakeven point at the EIL (NCSU, 2023). The pest density level of the EIL is somewhat higher than that of the ETL. This is because there is usually a lag time (this offers a buffer period) between the implementation of a control measure and its effect on the pest population. It is therefore always desirable to initiate control measures before the infestation level reaches the EIL (NCSU, 2023).

Action thresholds have been identified for the control of *S. frugiperda* in Africa (Table 1.3). The use of these thresholds relies on regular scouting throughout the season (McGrath *et al.,* 2018; Lowry *et al.,* 2022).

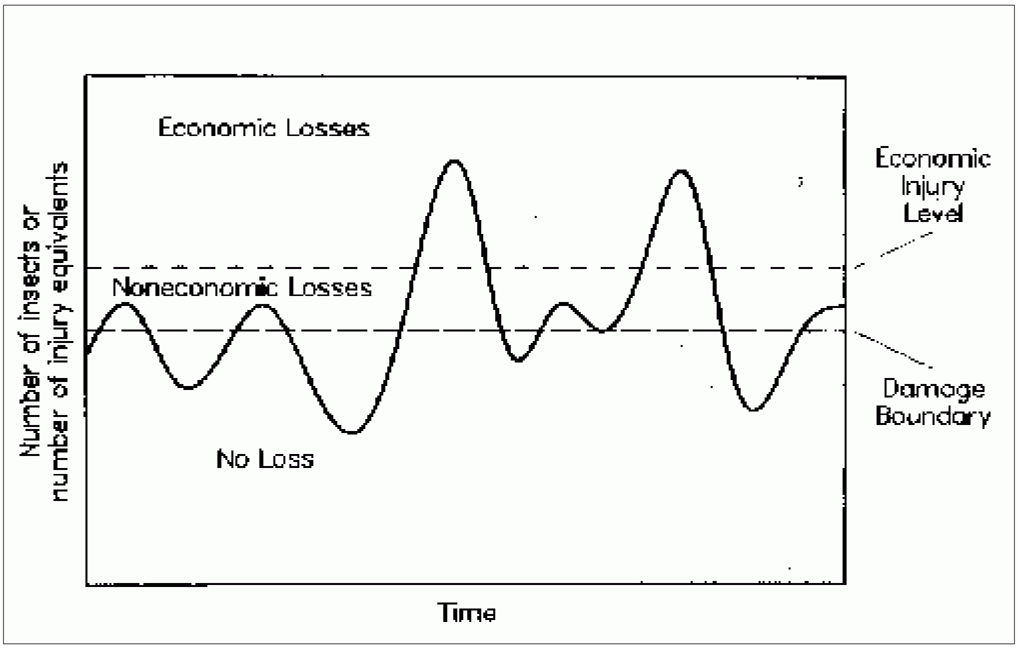

Figure 1.10. A graphical presentation of the relationship between economic threshold level, action threshold and economic injury level (Pedigo, 2024).

Table 1.3. Action thresholds for *Spodoptera frugiperda* in maize in Sub-Saharan Africa (McGrath *et al.,* 2018).

Farming scale	**Maize growth stage**	**Action threshold**
Smallholder farmers	Early whorl stage	Damage reaches 20% (10-30%)
	Late whorl stage	Damage reaches 40% (30-50%)
	Tassel and silk stage	No treatment, unless insecticide has low toxicity and/or supports conservation biological control
Village level/larger scale farmers	Early whorl stage	Damage reaches 20% (10-30%)
	Late whorl stage	Damage reaches 40% (30-50%)
	Tassel and silk stage	Damage reaches 20% (10-30%)

1.7 Aims and objectives of this study

The aims of this study were to:

- estimate the baseline susceptibility of the first confirmed *S. frugiperda* population that invaded SA in 2017, to selected insecticides registered for its control in SA.
- estimate the susceptibility of a *S. frugiperda* population, sampled from an overwintering area in 2022 where an intensive chemical control program was followed, to selected insecticides registered for its control in SA.
- estimate the susceptibility of *S. frugiperda* to selected EPFs.

The objectives were to:

- estimate the baseline susceptibility of a *S. frugiperda* population sampled in 2017 (Mk-0117) to chlorantraniliprole, indoxacarb, pyridalyl dichloropropene-derivitive and spinetoram.
- estimate the susceptibility of a *S. frugiperda* population, sampled in 2022 (Mb-22) to chlorantraniliprole, indoxacarb, pyridalyl dichloropropene-derivitive and spinetoram.
- compare the baseline susceptibility of the 2017 population to the susceptibility of a *S. frugiperda* population sampled in 2022.
- estimate the susceptibility of *S. frugiperda* to *B. bassiana* strain PPRI 5339, *B. bassiana* strain R444, *M. anisopliae* strain ICIPE 69 and *M. anisopliae* strain ICIPE 78.

The results of this study are presented in the form of chapters with the following titles:

Chapter 2: Susceptibility of *Spodoptera frugiperda* populations to insecticides in South Africa

Chapter 3: Susceptibility of *Spodoptera frugiperda* to entomopathogenic fungi in South Africa

Chapter 4: Conclusion and recommendations

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CHAPTER 2: Susceptibility of *Spodoptera frugiperda* populations to insecticides in South Africa

Abstract

Management of the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) mainly relies on the use of pesticides. This pest is, however, known for its rapid evolution of resistance to insecticides. It has already evolved resistance to at least 46 insecticidal active ingredients, globally. Estimation of baseline susceptibility data for populations in newly invaded areas is therefore important for use as a future reference to monitor for insecticide resistance. The susceptibility of *S. frugiperda* larvae from two populations was estimated to active ingredients from four mode of action groups. One of these populations was the first confirmed population that invaded South Africa (SA) in 2017. It was collected at Makoppa, Thabazimbi (Mk-0117) in 2017. The other population (Mb-22) was collected in 2022 at a research station in Mbombela, where an intensive chemical control program was followed. The insecticides used were chlorantraniliprole, indoxacarb, pyridalyl dichloropropene-derivitive and spinetoram. Two Insecticide Resistance Action Committee (IRAC) susceptibility test methods were used for susceptibility testing. Larvae from both these *S. frugiperda* populations were highly susceptible to all four insecticides, with their respective LC_{80}-values well below the maximum field recommended label rate. No control failure with the application of any of the four active ingredients registered for control of *S. frugiperda* is therefore currently expected. The resistance ratios of all four active ingredients were significantly higher for the Mb-22 population indicating a risk of potential resistance evolution. Monitoring of the susceptibility of *S. frugiperda* populations in SA, using the baselines for the insecticides estimated in this study, could provide data to guide insecticide application programs to prevent evolution of resistance.

Keywords: chemical control, fall armyworm, maize, mode of action, resistance

2.1 Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is native to the tropical-subtropical regions of the Western hemisphere

(Luginbill, 1928; Kenis *et al.,* 2023). It rapidly expanded eastward since 2016 (Goergen *et al.,* 2016; Cock *et al.,* 2017) and was first reported in South Africa (SA) in early 2017 (DAFF, 2017). Chemical control of *S. frugiperda* relies heavily on synthetic insecticides (Kumela *et al.,* 2017; Kansiime *et al.,* 2019; Tambo *et al.,* 2020; Sanou *et al.*, 2023), despite its ability to rapidly evolve resistance (Young & McMillan, 1979; Yu *et al.,* 2003; Gutiérrez-Moreno *et al.,* 2019; Ahissou *et al.,* 2021; Chen *et al.,* 2023). Resistance to at least 46 active ingredients and 255 cases of resistance in *S. frugiperda* has been reported to date (Mota-Sanchez & Wise, 2024). Maize (*Zea mays* L.) (Poaceae) is the preferred host plant of *S. frugiperda* (Huesing *et al.,* 2018), and its cultivation is therefore threatened by infestations of this pest.

Insecticide resistance management (IRM) is an important aspect of sustaining the efficacy of insecticides (Nauen *et al.,* 2012). The aim of IRM is to prevent or delay the evolution of resistance to insecticides or to recover the susceptibility of an insect pest/mite population in which resistance has already evolved (Sparks & Nauen, 2015; Nauen *et al.,* 2012). In an IRM program, insecticides with different modes of action (MoA) or combinations of compounds from different MoA groups, should be altered or rotated in application sequences (Nauen *et al.,* 2019). The risk of resistance evolution is then spread over all available classes of insecticides (Phillips *et al.,* 1989). Once an insect population has evolved resistance to a particular group of insecticides, these insecticides are no longer able to control the target pest, even when used according to label directions (IRAC, 2024).

In total, 22 different active ingredients [excluding *Bacillus thuringiensis* Berliner (Caryophanales: Bacillaceae) and *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Clavicipitaceae)], belonging to 10 MoA groups received emergency registration for control of *S frugiperda* in South Africa (SA) (Agri-Intel, 2017; DALRRD, 2017). However, after the emergency registrations of these active ingredients lapsed, several were not registered for control, resulting in only four active ingredients, belonging to four MoA groups currently (2024) being registered for control of *S. frugiperda.*

Control failure of an insecticide, due to resistance, is based on the significant reduction in efficacy of the (commercial) product (i.e., an insecticide formulation) when used at its recommended label rate but not reaching expected control level (Guedes, 2017; Guedes *et al.,* 2017). To estimate the likelihood of control failure by an insecticide, the recommended label rate of the insecticide and the minimum efficacy threshold for insecticide registration (e.g., 80%) are used (Guedes, 2017). The label rate is used as the discriminating concentration and the mortality achieved in the bioassay is used for comparison with the minimum efficacy threshold (Guedes, 2017). To recognise if populations are likely to undergo control failure with the tested insecticide, the estimated lethal concentration 80 (LC_{80}) (i.e., LC for 80% of the insect population) is compared with the recommended label rate of the insecticide (Guedes, 2017). If the estimated LC_{80} is higher than the label rate of the commercial formulation, control failure would likely take place (Guedes, 2017).

Susceptibility data of the first confirmed *S. frugiperda* population that invaded SA in 2017 is critical for its effective IRM. This baseline data can be used to monitor subsequent resistance evolution (Cook *et al.,* 2004). It can be expressed as a resistance ratio (RR) of a pest population, which indicates the extent of resistance to an insecticide, i.e., a quantitative measure of how much more resistant a test population is compared to a susceptible reference population (Mota-Sanchez *et al.,* 2008; Tabashnik *et al.,* 2014).

It is, therefore, essential to do follow-up estimates of susceptibility levels of *S. frugiperda* populations in SA, using the available baselines to detect the evolution of resistance. The objective of this study was to estimate the susceptibility of an initial *S. frugiperda* population that invaded SA in 2017, to chlorantraniliprole, indoxacarb, pyridalyl dichloropropene-derivitive and spinetoram, and to compare it with the susceptibility data of a population sampled in 2022.

2.2 Materials and methods

2.2.1 *Spodoptera frugiperda* populations

Two *S. frugiperda* populations were used in this study. The population that served as a reference population was collected by research personnel from Corteva Seeds, in 2017, soon after *S. frugiperda* invaded SA. Sampling was done in a maize field at Makoppa, Thabazimbi in the Limpopo province (24° 32' 44.268'' S; 27° 11' 5.564'' E) (designated Mk-0117). Five hundred larvae (F0 generation) were sampled soon after its invasion into SA, before they were exposed to any insecticide treatment. This population was reared for 80 consecutive generations before it was donated to the North-West University (NWU) in Potchefstroom to serve as a reference population for future research. They were mass reared and larvae from the 90th generation were used for susceptibility testing. The population was rejuvenated once in the 88th generation with 100 larvae collected in Venda (23° 1' 36.472" S; 30° 26' 24.061" E), in small-scale farmer's fields where no insecticides are used.

Larvae from the other population were collected at Mbombela (25° 26' 14.186" S; 30° 59' 34.598" E) (designated Mb-22) in 2022. One thousand larvae (F0) were collected from a maize field at a research station where an intensive chemical control program for *S*. *frugiperda* was followed. These larvae were transported to the NWU insect rearing facility. Larvae of the F1-generation of this field-collected population were used for susceptibility testing of this population.

2.2.2 Rearing of *Spodoptera frugiperda*

Larvae were reared individually in small plastic containers with aerated plastic mesh-infused lids (55 mm in length × 25 mm in diameter), due to their cannibalistic nature. Stonefly *Heliothis* premix diet (Ward's Natural Science Establishment, LLC, USA) was prepared with distilled water added to the premix powder in a 4:1 ratio and provided to larvae as food. The containers were kept in a rearing room at 28 ± 1 °C, 60-65% RH and a 14L:10D photoperiod. Larvae were transferred to clean containers with fresh food every third day until pupation. Pupae were removed from the rearing containers and placed in Petri dishes (150 mm in height × 100 mm in diameter) inside a clean oviposition container aerated with a mesh-infused lid (400 mm in length × 300 mm in width × 170 mm in height). As the pupae eclosed, the male and female moths were free to mate. Three small bottles closed with a cotton plug, containing a 10% sucrose solution, served as a food source for the moths. These bottles were replaced trice weekly. Three wax paper sheets (300 mm in length × 300 mm in width) were placed into the container for oviposition. Oviposition containers were examined daily, the wax paper sheets were replaced, and the eggs adhered to the wax paper were removed. Eggs laid on the side and lid of the plastic containers were carefully removed with a fine paintbrush. Egg batches were placed separately into transparent plastic containers aerated with steel mesh-infused lids (55 mm in height × 25 mm in diameter) and kept in the same rearing room as described above. Larvae that hatched from these egg batches were placed into plastic containers similar to those used for oviposition. When the larvae reached the third instar (L3), they were individually transferred to small plastic containers with aerated plastic mesh-infused lids (55 mm in length × 25 mm in diameter), for rearing until pupation. The pupae were removed and kept as described above for eclosion and oviposition.

2.2.3 Insecticides used in this study

Commercial formulations of the insecticides used in this study, their IRAC MoA group, application rate per hectare [ml, g and ppm (parts per million per hectare)] as well as the recommended spray volume are provided in table 2.1. The concentration of the maximum field recommended label rates for the respective insecticides was calculated using the following formula: recommended label rate (ppm/ha) = [recommended amount of product (ml or g) × product concentration (ml/l or g/l or g/kg)]/recommended tank water volume (ml/ha).

Table 2.1. Maximum field recommended application rates of insecticides currently registered in South Africa on maize for protection against *Spodoptera frugiperda*.

Commercial name and manufacturer	Active ingredient	IRAC mode of action group	Application rate/ha	Spray volume (l/ha)	Rate (ppm/ha)
Prevathon 5 SC®, FMC Chemicals (Pty) Ltd©	Chlorantraniliprole	28	600 ml	450	68
Steward 150 EC®, FMC Chemicals (Pty) Ltd©	Indoxacarb	22A	300 ml	450	100
Sumipleo®, Philagro SA (Pty) Ltd©	Pyridalyl dichloropropene-derivitive	UN	300 ml	200	750
Delegate 250 WG®, Dow AgroSciences SA (Pty) Ltd©	Spinetoram	5	120 g	300	100

ppm = parts per million

2.2.4 Preparation for bioassays

Susceptibility testing was conducted according to the IRAC Susceptibility Test Methods Series. The diamide, chlorantraniliprole, was tested by means of an insecticide incorporated into artificial diet bioassay, IRAC test method no. 020 (IRAC, 2011). Susceptibility testing of larvae to the remaining active ingredients was done by means of a leaf dipping bioassays, IRAC test method no. 007 (IRAC, 2014).

A stock solution was prepared for each insecticide, taking into account the formulation of the respective insecticides. A series of 10-12 dilutions were prepared from each stock solution and used as the different insecticide concentration treatments in the respective bioassays (Figure 2.1). A fresh stock solution was prepared for each replicate of each bioassay. The stock solution was used immediately after it was prepared. Each bioassay consisted of three replicates, and 32 larvae were tested per dilution/replicate, totalling 96 larvae being tested at each dilution in the serial dilution range. For these bioassays, 32-cell bioassay trays (Frontier: Scientific services) were used. Preliminary susceptibility testing (range finding) was done to determine the serial dilutions in the range used for each insecticide. These preliminary range findings were done to ensure that the range included at least six serial concentrations (excluding the control), which resulted in 20-100% mortality.

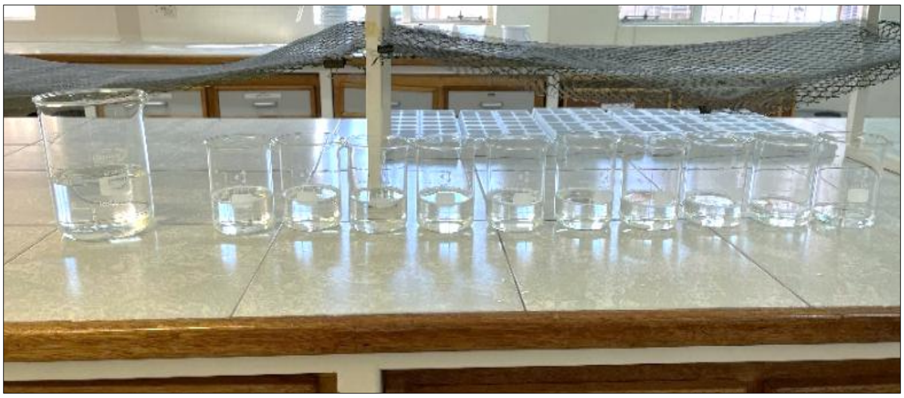

Figure 2.1. Insecticide dilution series were prepared from a stock solution.

2.2.4.1 Insecticide-incorporated artificial diet bioassay

The insecticide-incorporated diet was prepared by adding 55 g of the Stonefly *Heliothis* artificial diet to 220 ml of each insecticide dilution, after which it was thoroughly mixed into a paste. The control treatment consisted of artificial diet prepared with distilled water. A spoon was used to place 2 ml of the diet per well of a bioassay tray. One *S. frugiperda* larva (L2) was inoculated onto the diet in each well of the bioassay trays (Figure 2.2a). These bioassay trays were covered with transparent ventilated adhesive lids (Frontier: Scientific services). The trays were placed in an incubator at 26 ± 2 °C, 60-65% RH and 16L:8D photoperiod (Figure 2.3).

2.2.4.2 Insecticide leaf dipping bioassay

The bioassay trays for the insecticide leaf dipping bioassay were prepared prior to testing by pouring 5 ml agar-agar (3% solution) into each cell of the trays. This ensured that the leaf pieces remained turgid for the duration of the test. Triton X-100 (0.1%) was added to each insecticide dilution as well as to the distilled water used as the control treatment. This ensured optimal leaf coverage by the insecticides. For each dilution, as well as the control, 30 mm x 30 mm maize leaf tissue pieces (Hybrid: DKC 80-10) were immersed individually for five seconds ensuring that the entire surface was covered. Treated maize leaf pieces were placed, with the adaxial surface upwards, on a plastic wired net at room temperature and allowed to air dry. One leaf piece was placed per well of the bioassay trays. One *S. frugiperda* larva (L2) was inoculated onto each leaf piece (Figure 2.2b) after which the trays were firmly sealed with transparent ventilated adhesive lids. The trays were placed in an incubator at 26 ± 2 °C, 60-65% RH and 16L:8D photoperiod (Figure 2.3).

Figure 2.2 One second-instar *Spodoptera frugiperda* larva was inoculated onto **a,** the diet, and **b,** the leaf tissue in each well.

Figure 2.3 Bioassay trays kept in an incubator.

2.3 Data collection

An assessment of larval mortality in the chlorantraniliprole bioassay was done after 168 hours, and for the active ingredients tested with the leaf dip method, after 72 hours. The larvae were lightly probed with a fine paintbrush, and those that were unable to move or make coordinated movements were considered dead.

2.4 Data analysis

Abbott's formula was used to correct for control mortality where necessary (Abbott, 1925). The corrected mortality data from the respective dose-response bioassays was subjected to probit analysis using PoloSuite software (version 1.8) (LeOra Software). The parameters of these analyses were evaluated according to Robertson *et al.* (2017) to determine whether a linear relationship existed between the probits and the dose. In this regard, the t-ratio of the slope of the respective regression lines was used, which determined the significance of the slope of the respective regression lines. If the t-ratio for a slope was less than 1.96, the regression parameters were not significant, and the data set was not included in the analysis. The goodness of fit of the regression line was assessed by means of the chi-square (χ^2) goodness-of-fit test. This test compared the actual values measured in the bioassay with the predicted values of the model as a measurement of how well the data fitted the assumptions of the model. In cases where χ^2 was not significant, and the heterogeneity factor (χ^2/df) was greater than 1, the data did not fit the model. Residuals were used to identify the response(s) causing the lack of fit, by plotting them against doses. Those responses were regarded as outliers outside the bounds of -2 to 2 and were discarded from the respective concentration series for analyses.

For this study, the minimum acceptable level of control success was decided at 80% mortality. The estimated LC_{80} value for each insecticide was therefore compared with their recommended label rate to indicate possible control failure. Where the estimated LC_{80} value was higher than the label rate of the insecticide, control failure may be expected (mortality achieved by the label rate is considered higher than 80% when the label rate was higher than the LC_{80} value) (Guedes, 2017).

The RR was calculated by dividing the LC_{50} for the Mb-22 population by the LC_{50} of the Mk-0117 population according to Tabashnik *et al.* (2014). Its 95% confidence limit (CL) was calculated using PoloSuite software (version 1.8) (LeOra Software). In instances where the 95% confidence interval did not include the value 1, the LC of the RR was considered significantly different (Robertson *et al.*, 2016).

2.5 Results

The estimated LC_{50} and LC_{80}-values for the respective insecticides are provided in table 2.2. Responses in all bioassays in both years, yielded t-ratios higher than 1.96, indicating significant slopes. The slopes of the regression lines in response to exposure to the respective insecticides ranged from 1.37 - 2.16 in 2017 and from 1.09 - 4.63 in 2022. The $\chi 2$ values were also significant and the heterogeneity values were smaller than one, which confirmed that the mortality data of *S. frugiperda* larvae fitted the Probit model for all insecticides. The LC_{80} for all insecticides, were lower than their maximum field recommended label rates (Table 2.1) for both these populations.

The LC_{50} RRs and 95% CL, for the respective insecticides for Mk-0117 and Mb-2022 are provided in table 2.3. The response in susceptibility of *S. frugiperda* for all four insecticides, differed significantly between 2017 and 2022. The RRs for the four insecticides varied from 1.8 to 12.5 times.

Table 2.2 Log dose Probit mortality data for two *Spodoptera frugiperda* populations for selected insecticides.

Insecticide	Population	N	LC_{50}	CL95%	LC_{80}	CL95%	Slope	SE	χ^2	df
Chlorantraniliprole	Mk-0117	1152	0.02	0.016-0.024	0.09	0.076-0.106	2.16	0.19	5.21	9
	Mb-22	960	0.07	0.067-0.076	0.11	0.102-0.116	4.63	0.29	7.00	8
Indoxacarb	Mk-0117	864	1.06	0.919-1.201	4.34	3.749-5.144	1.37	0.11	2.82	7
	Mb-22	672	13.24	10.738-15.884	50.81	41.729-64.454	1.44	0.12	2.94	5
Pyridalyl dichlopropene-derivitive	Mk-0117	864	0.25	0.202-0.300	0.88	0.718-1.113	1.54	0.10	6.87	7
	Mb-22	864	1.96	1.540-2.490	11.67	8.360-18.040	1.09	0.08	7.10	7
Spinetoram	Mk-0117	960	0.05	0.039-0.055	0.18	0.145-0.233	1.45	0.11	7.38	8
	Mb-22	576	0.09	0.061-0.119	0.24	0.166-0.394	1.93	0.14	8.90	4

n = number of larvae used in calculation; LC = lethal concentration; CL = confidence limits; SE = standard error; χ^2 = chi-square value; df = degrees of freedom

Table 2.3. Relative potency ratio of *Spodoptera frugiperda* populations for the Mk-0117 and Mb-22 populations, respectively.

Insecticide	LC_{50} RR	CL95%
Chlorantraniliprole	3.5	2.8 - 4.6*
Indoxacarb	12.5	12.3 - 17.9*
Pyridalyl dichlopropene-derivitive	7.9	6.1 - 10.2*
Spinetoram	1.8	1.5 - 2.3*

RR = Resistance ratio; LC = lethal concentration; CL = confidence limits

*Significant difference in responses between the 2017 and 2022 populations.

2.6 Discussion

This study provides baseline susceptibility data for *S. frugiperda* in SA to insecticides from four MoA groups. These are chlorantraniliprole, indoxacarb, pyridalyl dichloropropene-derivative and spinetoram. Larvae from the Mk-0117 population were highly susceptible to insecticides. Their susceptibility levels were compared to the susceptibility levels of a field collected population (Mb-22), sampled five years later at Mbombela. This field collection site is in an area which is known to be an overwintering site for *S. frugiperda,* and where it has become a permanent resident pest since its invasion. Since the collection site was at a research station, the pest was intensively controlled with insecticides since 2017, and selection pressure for resistance evolution was high.

Larvae from the Mb-22 population were also highly susceptible to insecticides. Control failure is therefore not currently expected in SA with any of the four insecticides. However, the RRs for the respective insecticides changed over the past five years. A RR of ≥10 can be used as a threshold to categorize cases of resistance (Mota-Sanchez *et al.,* 2008). This ratio is, however, discretional since RRs tenfold or greater may or may not indicate the evolution of practical resistance to insecticides in the field (Mota-Sanchez *et al.,* 2008; Tabashnik *et al.,* 2014). It is evident in the present study, where a RR of ≥10 was recorded for indoxacarb, that no practical consequences (control failure) for its control is expected.

The practical applicability of laboratory bioassays to field conditions is often criticised. Bioassays may have limitations in predicting field outcomes with absolute certainty, due to external factors, such as pest density and frequency of resistance alleles in a pest population (Daly & Murray, 1988). This can be seen in instances where, for example, control failure may not occur even if a high frequency of resistant alleles is present at low pest densities, in contrast, control failure may occur even if a low frequency of resistant alleles is present at high pest densities (Ffrench-Constant & Roush, 2012). These bioassays, nevertheless, continue to provide crucial data which aid in the decision making of IRM strategies (Daly & Murray, 1988). Frequent monitoring of susceptibility data in pest populations, by means of laboratory bioassays, allows for the early detection of resistance evolution enabling the implementation of preventative strategies (Ffrench-Constant & Roush, 2012).

This underscores the importance of insecticide susceptibility data obtained from laboratory bioassays, which serves as a baseline for future monitoring of possible resistance evolution. It further shows the importance of including it in IRM programs as a preventative measure, rather than as a reactive response to control failure by insecticides, to detect any changes in insecticide efficacy while also providing an early warning to modify control strategies (Forrester & Cahill, 1987; Miller *et al.,* 2010). By modifying control strategies, the efficacy of an insecticide may be extended, which in turn, is crucial for the production of a sufficient food supply for the world population (Miller *et al.,* 2010). It is, therefore, essential to do follow-up estimates of susceptibility levels of *S. frugiperda* populations in SA, using the available baselines to detect the evolution of resistance.

The population dynamics and ecology of *S. frugiperda* may have an effect on the evolution of resistance. The susceptibility of *S. frugiperda* in a particular region, to a certain insecticide, is influenced by the extent that moths can migrate from overwintering regions, where they may be subjected to high selection pressure, into regions that are annually invaded (Pitre 1986). In the first case, migrating moths may contribute to sustaining susceptible populations which may delay resistance evolution (Arias *et al.,* 2019; Yainna *et al.,* 2021; Nguyen *et al.,* 2022). Helps *et al*. (2017) indicated that resistance in a pest population can be suppressed with high immigration. To ensure that resistance does not increase over time, there must be an influx of untreated (susceptible) individuals into the treated (not susceptible) population, and no emigration back into the untreated population (Downes & Mahon, 2012). Suppression is then attained by diluting the treated population with untreated individuals, resulting in a reversion to a susceptible population. Downes & Mahon (2012) described the crucial part that migratory populations, which carry susceptible alleles, can play in delaying resistance evolution for *Helicoverpa punctigera* (Wallengren) (Lepidoptera: Noctuidae) This pest is still susceptible to insecticides in Australian cotton (*Gossypium hirsutum* L.) (Malvaceae) crops, despite been controlled with insecticides for many years. This is in contrast to *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), which is resistant to many insecticides, but lacks a seasonal influx of moths. The rate of resistance evolution may therefore be affected by the seasonal influx of moths from regions where they overwinter (Sparks, 1979; Timilsena *et al.,* 2022). This might well be the case for *S. frugiperda* in SA with a seasonal influx by insecticide-susceptible individuals from other Sub-Saharan Africa (SSA) countries.

Secondly, migrating moths may assist in the introduction of resistance alleles into new areas leading, to resistance evolution in local populations (Arias *et al.,* 2019; Yainna *et al.,* 2021; Nguyen *et al.,* 2022). Many cases of indiscriminate insecticide usage leading to insecticide resistance of *S. frugiperda* has been reported in the Americas. For example, in Puerto Rico insecticides are sprayed thrice per week during the peak season and up to 25 sprays have been reported in one maize crop cycle to control *S. frugiperda,* resulting in its continuous exposure to insecticides (Belay *et al.,* 2012). *Spodoptera frugiperda* in Puerto Rico have shown an increased level of field-evolved resistance to a wide range of insecticides, including flubendiamide, chlorantraniliprole, methomyl, thiodicarb, permethrin, chlorpyriphos, zeta-cypermethrin, deltamethrin, triflumuron, spinetoram, spinosad, emamectin benzoate and abamectin (Gutiérrez-Moreno *et al.,* 2019). By 2014, about 3 000 tons of synthetic insecticides were used annually for control of *S. frugiperda* in Mexico (Blanco *et al.,* 2014). This caused an increased level of field-evolved resistance to chlorpyrifos and permethrin in Mexican *S. frugiperda* populations (Gutiérrez-Moreno *et al.,* 2019). Resistance, to a wide range of insecticides, has also been reported in *S. frugiperda* populations in Brazil, including emamectin benzoate (Muraro *et al.,* 2021), lufenuron (Do Nascimento *et al.,* 2015), chlorpyrifos, lambda-cyhalothrin (Carvalho *et al.*, 2013), spinosad (Okuma *et al.,* 2018), chlorantraniliprole (Bolzan *et al.,* 2019) and spinetoram (Lira *et al.,* 2020).

Control of *S. frugiperda,* particularly in SSA, also largely depends on chemical control using synthetic insecticides (Kumela *et al.,* 2019; Kansiime *et al.,* 2019; Tambo *et al.,* 2020; Sanou *et al.,* 2023). Evolution of resistance to synthetic insecticides in SSA has already been observed in *S. frugiperda* populations from Burkina Faso to deltamethrin and lambda-cyhalothrin (Ahissou *et al.,* 2021). The risk of resistance evolution, on a global scale, will therefore always remain a problem as long as insecticides are used in pest management programs (Tabashnik *et al.*, 2014).

It is, however, suggested that transcontinental movements of large numbers of *S. frugiperda* through natural migration may be limited (Nagoshi *et al.,* 2022). This may be ascribed to the different *S. frugiperda* strains which differ on the basis of geographic occurrences, as reported in west and east Africa (Nagoshi *et al.,* 2019). Studies on the genetic identity of the *S. frugiperda* introduced from the Americas into SSA indicated that the majority of the genetic group consists of heterogeneous hybrids from both the “corn” and “rice strain” (Nagoshi *et al.,* 2019). However, these introduced *S. frugiperda* mostly infests maize crops (Acharya *et al.,* 2021). Since continued introductions of *S. frugiperda* from its area of origin are likely, the possibility exists that alleles with resistance to certain insecticides may be introduced. This may rapidly alter the SSA population strain composition with regards to its host plant preference and susceptibility to insecticides (Nagoshi *et al.,* 2022).

Spodoptera frugiperda does, however, not pose a significant threat to commercial maize producers in SA. This is ascribed to the large-scale cultivation of transgenic *Bacillus thuringiensis* (Bt) Berliner (Caryophanales: Bacillaceae) maize. In SA, 1.62 million hectares of Bt maize (72% of the total maize area) were planted during the 2017-2018 cropping season (ISAAA 2017), which is also the only SSA country where Bt maize is currently cultivated (Clement, 2022; ISAAA, 2023). In SA, two Bt maize products provide resistance against *S. frugiperda* (Prasanna *et al.,* 2018). Firstly, MON810 maize expressing Cry1Ab toxin, which is intended for stem borer control (Prasanna *et al.,* 2018; Botha *et al.,* 2019) but confers partial resistance to *S. frugiperda* (Prasanna *et al.,* 2018). Secondly, MON89034 expressing Cry1A.105 + Cry2Ab2 toxins, which is intended for control of both stem borer and *S. frugiperda* (Prasanna *et al.,* 2018; Botha *et al.,* 2019). Cultivation of Bt maize reduces the use of synthetic insecticides and can therefore be used as an effective strategy for managing insecticide resistance (Burtet *et al.,* 2017). *Spodoptera frugiperda* is, however, well known to evolve resistance against Bt maize. Resistance of *S. frugiperda* to Bt maize has already been documented in many countries in its area of origin. For example, *S. frugiperda* was found to be resistant to Bt maize expressing Cry1F in Puerto Rico (Storer *et al.,* 2010), Brazil (Farias *et al.,* 2014) and in the southern states of US (Huang *et al.,* 2014), and also to Cry1Ab in Brazil (Farias *et al.,* 2014; Omoto *et al.,* 2016). The rapid evolution of resistance to Bt maize demonstrates that biotechnology cannot be the only mitigating strategy and that there is an urgent need to develop alternative control strategies for implementation in an integrated pest management (IPM) strategy.

2.7 Conclusion

This is the first report on the insecticide susceptibility status of a *S. frugiperda* population that invaded SA in 2017. This population had not been controlled with insecticides in SA prior to collection, and data on its susceptibility could serve as a baseline for future monitoring of possible resistance evolution. This study indicates that both the initially sampled population, and the one which was sampled 5 years later from an area where it was subjected to insecticide selection pressure, were still highly susceptible to chlorantraniliprole, indoxacarb, pyridalyl dichloropropene-derivitive and spinetoram. However, the responses in susceptibility of *S. frugiperda* larvae in 2022 (Mb-22), to the four insecticides differed significantly from the base line susceptibility data of the 2017 population (Mk-0117). Although this does not yet constitute field-evolved resistance, it emphasizes the importance of monitoring for changes in susceptibility to insecticides and highlights the importance of insecticide resistance management.

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CHAPTER 3: Susceptibility of *Spodoptera frugiperda* to entomopathogenic fungi in South Africa

Abstract

Chemical control using synthetic insecticides is the most widely used method for control of the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in Sub-Saharan Africa (SSA). This will, however, not provide long-term and sustainable solutions in the future. Biological control is an important pillar of integrated pest management, with entomopathogenic fungi (EPFs) as important biocontrol agents. However, there are currently no EPF bioinsecticides registered in South Africa (SA) against *S. frugiperda*. Very few studies have been done on the efficacy of commercial formulations of bioproducts against all *S. frugiperda* life stages. The objective of this study was therefore to assess the potential of two *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Clavicipitaceae) and two *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) based commercial biopesticides registered in SA against other pests, for their efficacy against *S. frugiperda*. The effect of the EPF formulations were evaluated on larval and prepupal mortality, moth emergence as well as fecundity and longevity of moths. For the bioassays, the working concentration of the biopesticide formulations was derived from the maximum recommended rates indicated for each biopesticide. Results indicated that *S. frugiperda* larvae were not susceptible to the biopesticides, and adult emergence as well as fecundity and longevity was not significantly affected. Prepupae were, however, susceptible to both *Metarhizium* formulations, with *Metarhizium anisopliae* ICIPE 78 having the highest mortality (56.67%). This biopesticide, which is commercialised against other pests in SA, could be a potential candidate for management of *S. frugiperda* when applied in soil for control of pupating larvae.

Keywords: *Beauveria bassiana,* biopesticides, EPF, fall armyworm, *Metarhizium anisopliae*

3.1 Introduction

The introduction of the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), into Sub-Saharan Africa (SSA), led to significant damage to maize (*Zea mays* L.) (Poaceae) crops (Stokstad, 2017; Huesing *et al.,* 2018). Chemical control remains the most frequently utilised method for its control (Kumela *et al.,* 2019; Kansiime *et al.,* 2019; Tambo *et al.,* 2020; Sanou *et al.,* 2023), however, it is not a long-term and sustainable solution (Young & McMillan, 1979; Yu *et al.,* 2003; Stokstad, 2017; Gutiérrez-Moreno *et al.,* 2019; Ahissou *et al.,* 2021; Chen *et al.,* 2023). *Spodoptera frugiperda* is expected to remain a significant pest, especially in the areas where resident populations occur. An integrated pest management (IPM) approach provides a valuable framework that can be used for its effective management (Huesing *et al.,* 2018).

Biological control using entomopathogenic fungi (EPF) is rapidly emerging as a prime substitute for synthetic insecticides for control of *S. frugiperda* (Cruz-Avalos *et al.,* 2018; Akutse *et al.,* 2019; Fakeer *et al.,* 2024; Jeon & Kim, 2024). The most commonly used biopesticides are *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Clavicipitaceae) and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) (Kidanu & Hagos, 2020), which are known to effectively produce and transmit a variety of toxin-causing infections in suitable hosts (Strasser *et al.,* 2010). Entomopathogenic fungi have various advantages when compared with synthetic insecticides. These include their high efficiency, cost-effectiveness, reduced residues in the environment and no effects on non-target organisms (Lacey *et al.,* 2001). Entomopathogenic fungi are also capable of infecting non-feeding life stages of insect pests. This is ascribed to their unique mode of infection since EPFs can infect hosts through cuticle adhesion and ingestion is not required (Aw & Hue, 2017). Nevertheless, EPFs also have its disadvantages, including environmental- and biological factors, that play a role in the successful infection of a host (Vega *et al.,* 2012). Temperature and humidity are the two most crucial environmental factors affecting the survival and efficacy of EPFs (Bugeme *et al.,* 2008; Vega *et al.,* 2012). Biological factors may include the host life stage, behaviour and immune responses (Vega *et al.,* 2012). During immature life stages, moulting may reduce the efficacy of EPFs owing to the shedding of conidia that are attached to the molted cuticle (Luz *et al.,* 2003). Additionally, some non-social insects have the ability to exhibit density-dependent prophylaxis, i.e., increased resistance to EPFs when reared under high-density conditions (Wilson & Reeson, 1998). However, once the EPF reaches the haemolymph, a range of antimicrobial responses can be initiated (Vega *et al.,* 2012).

An additional response could be that of behavioural fever, in which infected insects alter their behaviour (e.g., sun basking) to raise body temperature, with an adverse effect on the EPF that is present in the hemocoel (Elliot *et al.,* 2002). Another disadvantage of EPFs is that they infect and kill insect pests much slower than synthetic insecticides (Idrees *et al.,* 2022). This may be ascribed to the fact that different life stages of insect pests do not respond to EPF infection in the same way (Idrees *et al.,* 2022). It is therefore essential to do EPF bioassays on various life stages of an insect pest to obtain data on the susceptibility level of each life stage, which informs users of the most appropriate life stage of the insect pest to target for effective biological control programs (Idrees *et al.,* 2022).

The development and implementation of bioinsecticides in SSA is still in its early stages (Ndolo *et al.,* 2019). There are currently no EPF bioinsecticides registered in South Africa (SA) against *S. frugiperda* (Agri-Intel, 2024), but the successful use of EPFs such as *B. bassiana* and *M. anisopliae,* as seen in several other countries (Cruz-Avalos *et al.,* 2018; Akutse *et al.,* 2019; Fakeer *et al.,* 2024; Jeon & Kim, 2024), demonstrates the reliability of this biotechnology. Commercialization of EPFs on maize could provide an additional control option against *S. frugiperda* in SSA. There are very few studies that have been done on the efficacy of commercial formulations of bioinsecticides against all *S. frugiperda* life stages in SSA.

The aim of this study was therefore to assess the potential of two *B. bassiana* and two *M. anisopliae* based commercial biopesticides registered in SA against other crop pests, for their efficacy against *S. frugiperda*. The objectives of the study were to determine the efficacy of four EPF formulations for control of *S. frugiperda* larvae and prepupae, their effect on adult emergence as well as the fecundity and longevity of adult insects.

3.2 Materials and methods

3.2.1 Rearing of *Spodoptera frugiperda*

Spodoptera frugiperda larvae from a previously established reference colony (see 2.2.1), were used for testing the efficacy of EPFs for control of larvae and prepupae, and the resultant effect on surviving individuals in terms of adult emergence, their fecundity and longevity.

3.2.2 Entomopathogenic fungi used in this study

Four commercial products, which are not registered for control of *S. frugiperda* in SA, were used in the bioassays. The commercial EPF products with all complementary information are listed in table 3.1. The working concentrations of the respective biopesticides used in the bioassays were derived from their maximum recommended rates as per product label. This concentration was obtained for the wettable powder (WP) formulation, Eco-Bb, by quantifying conidia with a Neubauer Improved haemocytometer. For the emulsifiable suspension (ES) formulations, Broadband, Real *Metarhizium* and Real *Metarhizium* 78, the working concentrations were determined using the following formula: $C_1V_1 = C_2V_2$, where C_1 = concentration (initial), V_1 = volume (initial), C_2 = concentration (final) and V_2 = volume (final). A spray volume/rate of 300 l/hectare (ha) was used.

Table 3.1 Commercial entomopathogenic fungi and concentrations used in the respective bioassays.

Commercial name and manufacturer	Active ingredient	Experimental name	Application rate/ha	Batch Concentration	Working concentration (cfu/ml)*
Broadband®, BASF SA (Pty) Ltd©)	*Beauveria bassiana* PPRI 5339	Bband	1000 ml	4×10^9 cfu/ml	1.33×10^7
Eco-Bb®, Andermatt PHP (Pty) Ltd©	*Beauveria bassiana* R444	Ecobb	1000 g	2×10^9 spores/g	3.5×10^6
Real *Metarhizium*®, Real IPM SA (Pty) Ltd©	*Metarhizium anisopliae* ICIPE 69	Mt 69	400 ml	1×10^9 cfu/ml	1.33×10^6
Real *Metarhizium* 78®, Real IPM SA (Pty) Ltd©	*Metarhizium anisopliae* ICIPE 78	Mt 78	400 ml	1×10^9 cfu/ml	1.33×10^6

cfu = colony forming units

3.2.3 Viability assessment

Viability testing of the respective EPFs were conducted according to the methods of Akutse *et al.* (2019). Fungal suspensions were prepared by mixing 1 ml of the emulsifiable oil formulation with 9 ml sterile distilled water, containing 0.05% Triton X-100, in universal glass bottles containing five, 5 mm glass beads. Bottles were vortexed vigorously at 700 rpm to produce a homogeneous conidial suspension. The viability of conidia of an EPF was determined by spread plating 0.1 ml of conidial suspension on Potato Dextrose Agar (PDA) for *B. bassiana* formulations and Sabouraud Dextrose Agar (SDA) for *M. anisopliae* formulations. For the wettable powder, a 3 × 10^6 cfu/ml suspension was prepared. Three replicate plates for each formulation were used. The Petri dishes were then sealed with parafilm and incubated upside down at 25 ± 2 °C in darkness. Conidial germination was examined after 24 h and any further germination was prevented by adding lactophenol blue. For each Petri dish, percentage germination was calculated by counting the number of germinated conidia per hundred randomly selected conidia in a field, covered by three cover slips, under a microscope at 40× magnification (Olympus CX23). Conidia with visible germ tubes of about twice the diameter of the conidium were scored as viable. An EPF was considered satisfactorily viable if germination was above 70%.

3.2.4. Susceptibility of second- and sixth-instar *Spodoptera frugiperda* larvae to bioinsecticides

The susceptibility of second- (L2) and sixth-instar (L6) *S. frugiperda* larvae was evaluated in a bioassay, which consisted of the four biopesticides (Table 3.1) and a control treatment. The bioassays were conducted according to the methods of Idrees *et al.* (2021; 2022). Sterile distilled water containing 0.05% Triton X-100 was used to prepare 200 ml of the respective biopesticide formulations to the desired working concentrations (Table 3.1). Ninety pieces (50 mm × 50 mm) of maize leaf tissue Hybrid: DKC 80-10) was cut and used per biopesticide and control treatment in this assay. The leaf pieces were dipped in the biopesticide suspension, and placed with the adaxial surface upwards on a mesh net to airdry. Leaf pieces that served as the control were dipped in sterile distilled water containing 0.05% Triton X-100. One leaf piece was then transferred to each Petri dish (90 mm in diameter) and an individual larva was transferred onto the leaf piece, using either a fine paintbrush (L2) or a forceps (L6) (Figure 3.1a;b). The Petri dishes were then incubated at 25 ± 2 °C, 65% RH with a 16L:8D photoperiod (Figure 3.1c). The larvae were supplied with fresh maize leaf material every second day as food. Leaf pieces were treated again on the fourth day as indicated on the respective biopesticide labels. Larval mortality was recorded daily for 7 days. Thirty larvae of each instar were used per replicate and each biopesticide and the control treatment were replicated three times. A mycosis test, according to the methods of Akutse *et al.* (2019), was done to confirm mortality due to infection by the EPF. The cadavers were surface sterilized with 70% alcohol and then rinsed thrice in distilled water. Sterile Petri dishes were lined with sterile filter paper and moistened with sterile distilled water. The surface-sterilized cadavers were placed into these Petri dishes, which were sealed with parafilm. The Petri dishes were kept in an incubator at 25 ± 2 °C under darkness for 7-14 days to allow fungal growth. Mortality due to fungal infection was confirmed by the presence of hyphae and conidia on the surface of the cadaver. The L6 larvae that survived were provided with food until pupation.

Figure 3.1. A: **a -** second-instar and; **b -** sixth-instar *Spodoptera frugiperda* larva in a Petri dish containing a treated piece of maize leaf material. **c -** Petri dishes containing the treated maize leaf material and larvae were kept in an incubator.

3.2.4.1 Effect of bioinsecticides on *Spodoptera frugiperda* adult emergence

To evaluate the effect of L6 larval treatment with the respective biopesticides on *S. frugiperda* adult emergence, pupae from the susceptibility test (see 3.2.4) were removed on the day of pupation. Forty-five pupae (males and females) were collected per biopesticide and control treatment for observation of moth emergence. There were three replicates for each treatment. Fifteen pupae that were placed in a 500 ml plastic container (150 mm in height × 150 mm in diameter) covered with a mesh net (Figure 3.2), served as one replicate. The number of adults that eclosed per container was recorded every day for 11 days. The total number of adults that eclosed, those that failed to eclose as well as malformed adults were recorded.

Figure 3.2. Containers with pupae from sixth-instar larvae that survived the respective bioinsecticide and control treatments used to monitor for moth eclosion.

3.2.5 Susceptibility of *Spodoptera frugiperda* prepupae to bioinsecticides

Bioassays on prepupae were conducted according to the methods of Erasmus *et al.* (2021). Assays were conducted in 500 ml plastic containers (150 mm in height × 150 mm in diameter) aerated with plastic mesh-infused lids. Soil from a field (26° 67' 41'' S; 27° 10' 75'' E) was mixed with a commercially available seedling mix (Culterra, Johannesburg, SA) in a 1:1 ratio. The soil mixture was autoclaved at 121 °C for 15 minutes and was left to cool and aerate. This soil mixture (200 g) was added to transparent plastic containers to a depth of 8 cm. The soil in the respective containers was inoculated with 10 ml of a biopesticide formulation and mixed thoroughly by hand to obtain a homogenised inoculated substrate. Distilled water containing 0.05% Triton X-100 was applied as the control treatment. Twenty 2- to 3-day old prepupae were placed onto the substrate in each container and covered with a 2 cm layer of the substrate to simulate natural pupation conditions (Figure 3.3a). Containers were covered with a perforated lid and maintained at 28 ± 1 °C, 65% RH with a 14 L:10D photoperiod (Figure 3.3b). After four days the substrate was sprayed again with 10 ml of a biopesticide formulation. Mortality during the prepupal and pupal stages and adult emergence were recorded. The number of adults that eclosed per container was recorded daily for a period of 15 days. Moths that failed to eclose from pupae and cadavers of prepupae, pupae and adults were recorded on day 15. Mycosis tests were performed on these cadavers as described above (see 3.2.4).

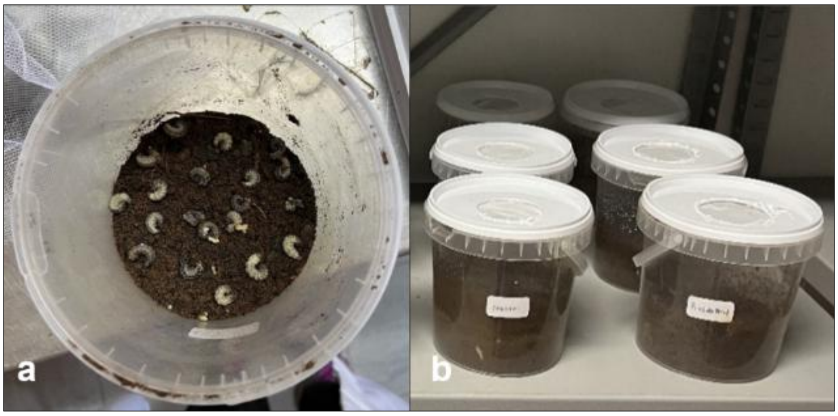

Figure 3.3 a - Twenty 2- to 3-day old *Spodoptera frugiperda* prepupae placed onto treated substrate and; **b -** containers inoculated with prepupae were kept in a rearing room.

3.2.6 Effect of bioinsecticides on fecundity and longevity of *Spodoptera frugiperda*

Based on the prepupal mortality results (see 3.2.3), one formulation for each fungal species, *viz.* Eco-Bb (WP) and Real *Metarhizium* 78 (ES) was selected for this bioassay. The experimental set up and soil inoculation was similar to that described in section 3.2.5. *Spodoptera frugiperda* pupae, 2- to 3-day old, were sexed by examining the position of the genital openings under a light microscope (Olympus CX23) according to Krutmuang *et al*. (2024) and Qianjin *et al.* (2019). Groups of male and female pupae were exposed separately to the treated soil. Twenty male and female pupae were used per replicate to ensure that enough adults eclosed for the bioassays. Pairs of females and males from the same treatment that emerged on the same day were transferred to new 500 ml transparent plastic containers (150 mm in height × 150 mm in diameter). Eight pairs were used per replicate and three replicates were used per treatment (n = 24 pairs). The containers were covered with a mesh net (Figure 3.4) and were maintained at 28 ± 1 °C, 65% RH with a 14 L:10D photoperiod. The moths were provided with a 10% sucrose solution on cotton balls, placed in 50 ml Falcon tubes as food. A piece of wax paper was placed in the containers as an oviposition substrate, and this was inspected for egg batches and replaced daily. One-day old eggs were counted under a light microscope (Olympus CX23). Eggs that were laid on the side and mesh net of the plastic containers were carefully removed with a fine paintbrush. The eggs were counted daily until the female died. The longevity of each individual insect was also recorded.

Figure 3.4. A single female and male *Spodoptera frugiperda* pair were kept in a container.

3.3 Data analysis

Laboratory experiments were arranged in a completely randomised design and statistical analyses were performed using R statistical package (R Core Team, 2024). When the control group data had only zeros (second- and sixth-instar mortality and mycosis data), the control group were excluded from the analysis. All binary data (conidial viability; insect/prepupa mortality; adult emergence and mycosis) were fitted to a Generalised Linear Model (GLM) with binomial distribution, followed by Tukey's HSD post-hoc test at $p < 0.05$. The mean fecundity per female was analysed using a GLM model assuming a negative binomial distribution. The Kaplan-Meier survivorship curves was used to test for differences in the longevity of adult insects exposed to the different treatments. Survival data was grouped to compare the longevity of (i) males, (ii) females, (iii) combined males and females of each treatment, and (iv) the individual groups from all the treatments. Pairwise log rank tests were conducted to compare survival of the different groups.

3.4 Results

3.4.1 Viability assessment

The results indicated that conidia germination of the respective EPF formulations used in the study exceeded 75% (Figure 3.5). The viability of the four formulations did, however, differ significantly ($\chi^2 = 258.1$; df = 3; $p < 0.001$). Viability of Bband was significantly higher compared to the other biopesticides (Figure 3.5).

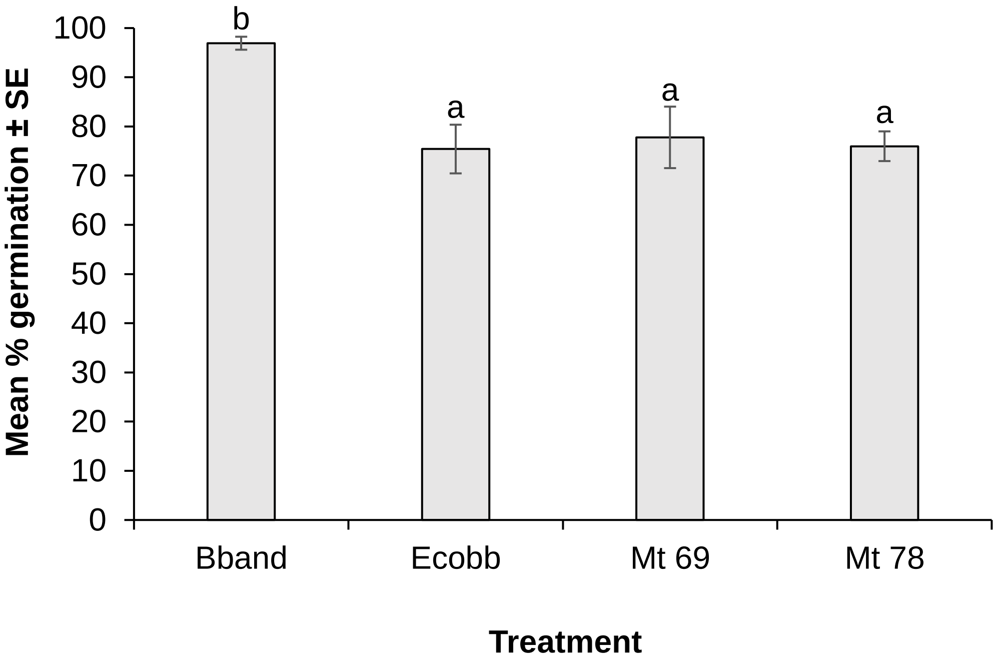

Figure 3.5. Percentage viability (±SE) of the respective entomopathogenic fungi (EPF) formulations used in this study. Bars capped with the same letter are not significantly different at $p < 0.05$ (Tukey's HSD post-hoc test).

3.4.2 Susceptibility of second- and sixth-instar larvae to bioinsecticides

The percentage larvae that died after exposure to the respective EPF's was very low (Table 3.2). L2 mortality ($\chi^2 = 0.66$; df = 3; $p > 0.05$) did not differ between the four treatments, with mortalities ranging from 10.0 - 13.3%. Mortality of L6 larvae did also not differ significantly ($\chi^2 = 1.65$; df = 3; $p > 0.05$) and ranged between 4.4 - 8.9% for the four treatments. Mortality in the control group for L2 and L6 was 0%. Mortality of larvae in the respective EPF treatments was, however, not all caused by the EPF infections as indicated by the varying mycosis results between the respective EPF treatments (Table 3.2). The *B. bassiana* formulations caused significantly higher mycosis levels (100.0 and 72.2%) compared to the *M. anisopliae* formulations (36.1 and 22.2%) in L2 ($\chi^2 = 8.64$; df = 3; $p < 0.001$). Mycosis levels of L6 larvae exposed to the EPF's did also differ significantly ($\chi^2 = 10.09$; df = 3; $p = 0.01$). Ecobb caused the highest mycosis of 77.8%, followed by Bband (33.3%) and Mt 78 (25.0%), while no mycosis occurred from cadavers of larvae treated with Mt 69. White fungal growth was visible from second- and sixth-instar mycosed larvae, indicating white muscardine disease (Figure 3.6a,b) and *M. anisopliae* mycosed larvae exhibited green fungal growth indicating green muscardine disease (Figure 3.7a,b).

Table 3.2. Percentage mortality and mycosis of second- and sixth-instar *Spodoptera frugiperda* larvae treated with the respective entomopathogenic fungi (EPF) formulations.

Treatment	**Mean mortality (%) ± SE**	**Mean mycosis (%) ± SE**	**Mean mortality (%) ± SE**	**Mean mycosis (%) ± SE**
	L2		**L6**	
Bband	11.11 ± 4.84a	100.0 ± 0.0d	4.44 ± 1.11a	33.33 ± 33.33a
Ecobb	10.0 ± 5.09a	72.22 ± 14.70c	8.89 ± 1.11a	77.78 ± 11.11ab
Mt 69	10.0 ± 1.92a	36.11 ± 7.35a	5.55 ± 2.22a	0.0 ± 0.0 a
Mt 78	13.33 ± 3.33a	22.22 ± 11.11ab	5.55 ± 4.01a	25.0 ± 25.0a
χ^2	0.66	8.64	1.65	10.09
p	> 0.05	< 0.001	> 0.05	= 0.01

*No L2 and L6 larvae died in the control groups. Values within a column followed by the same letter do not differ significantly at $p < 0.05$ (Tukey's HSD post-hoc test).

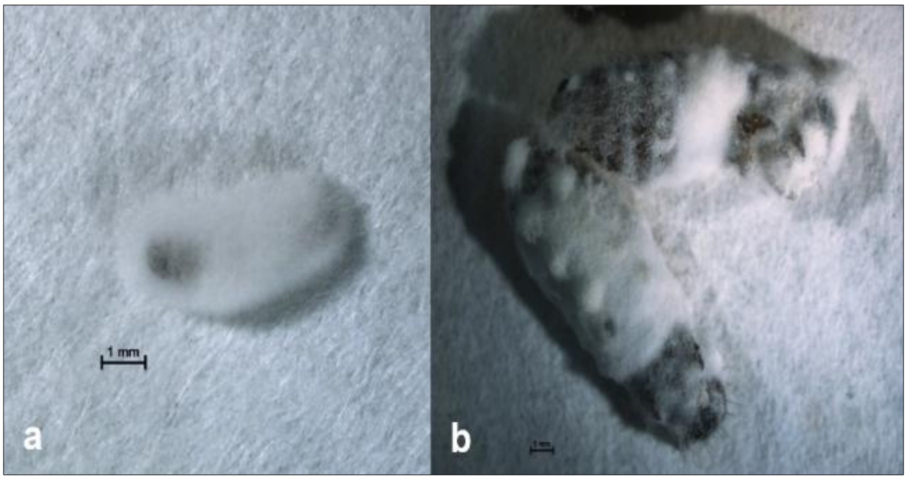

Figure 3.6. a - Second-instar and; **b -** sixth-instar *Spodoptera frugiperda* larvae mycosed with *Beauveria bassiana.*

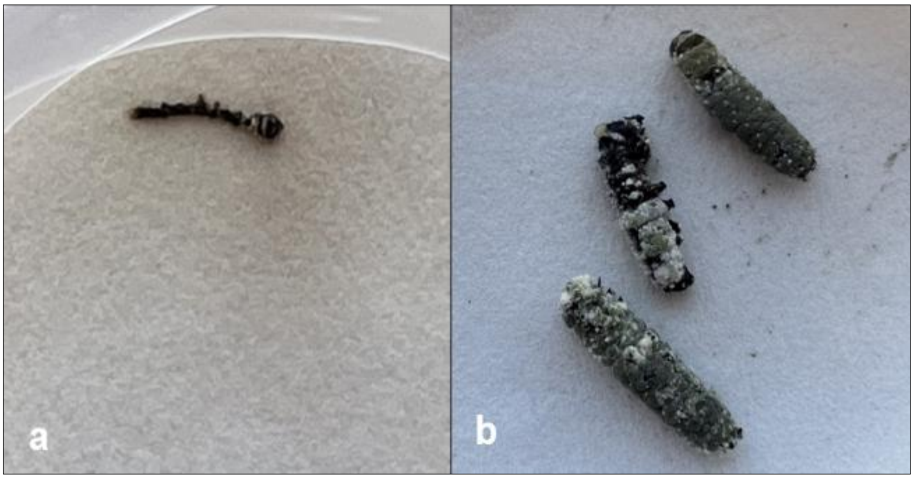

Figure 3.7. a - Second-instar and; **b -** sixth-instar *Spodoptera frugiperda* larvae mycosed with *Metarhizium anisopliae.*

3.4.2.1 Effect of bioinsecticides on *Spodoptera frugiperda* adult emergence

The proportion of moths that emerged from larvae that fed on fungus treated leaves compared to the proportion that emerged from the control treatment, did not differ significantly ($\chi^2 = 2.20$; df = 4; $p > 0.05$). However, fungal treatments of larvae caused a significantly higher number of malformed adults. A significantly higher proportion of malformed moths emerged from L6 larvae that fed on leaves treated with the *B. bassiana* formulations, Ecobb and Bband, compared to the *M. anisopliae* formulations Mt 69 and Mt 78 and the control ($\chi^2 = 9.83$; df = 4; p = 0.04) (Table 3.3). Figure 3.8

shows an adult that eclosed normally, and an adult that failed to eclose as well as a malformed adult can be seen below.

Table 3.3. Percentage emergence and malformed *Spodoptera frugiperda* moths from sixth-instar larvae treated with entomopathogenic fungi (EPF) formulations.

Treatment	**Mean emergence (%) ± SE**	**Mean malformation (%) ± SE**
Control	80.0 ± 3.85a	8.37 ± 0.40ab
Bband	66.67 ± 6.67a	21.30 ± 7.23c
Ecobb	75.56 ± 11.11a	20.19 ± 8.94c
Mt 69	75.56 ± 8.01a	2.78 ± 2.78a
Mt 78	75.56 ± 2.22a	3.03 ± 3.03a
χ^2	2.20	9.83
p	> 0.05	= 0.04

Values within a column followed by the same letter are not significantly different at $p < 0.05$ (Tukey's HSD post-hoc test).

Figure 3.8. A *Spodoptera frugiperda:* **a -** eclosed moth; **b -** moth that failed to eclose and; **c -** malformed moth.

3.4.3 Susceptibility of *Spodoptera frugiperda* prepupae to bioinsecticides

Spodoptera frugiperda prepupae that were exposed to EPF inoculated soil varied in susceptibility to the respective EPF's (χ^2 = 25.78; df = 4; $p < 0.001$) (Table 3.4). The highest mortality of prepupae, occurred in the Mt 69 and Mt 78 treatments. Mortality in the Mt 78 treatment was significantly higher than that in the two *Beauveria* and control treatments. Prepupal mortality in the two *Metarhizium* treatments (Mt 78 and Mt 69), was, however, not significantly different. Low, but similar mortalities occurred in the *Beauveria* treatments, *viz*. Bband (28.3%) and Ecobb (23.3%), which did not differ significantly from the percentage mortality of pre-pupae in the control treatment (18.3%). However, the level of mycosis did not differ significantly between the four formulations ($\chi^2 = 1.41$; df = 3; $p > 0.05$). The mycosed *B. bassiana* prepupae exhibited white fungal growth indicating white muscardine disease (Figure 3.9a), while mycosed *M. anisopliae* prepupae showed green fungal growth indicating green muscardine disease (Figure 3.9b).

Table 3.4. Percentage mortality and mycosis of *Spodoptera frugiperda* prepupae exposed to entomopathogenic fungi (EPF) formulations in soil.

Treatment	**Mean mortality ± SE (%)**	**Mean mycosis ± SE (%)**
Control	18.33 ± 6.01a	----
Bband	28.33 ± 4.41ab	33.33 ± 33.33a
Ecobb	23.33 ± 4.41ab	23.33 ± 14.53a
Mt 69	41.67 ± 6.01bc	27.78 ± 20.03a
Mt 78	56.67 ± 6.01c	38.25 ± 10.45a
χ^2	25.78	1.41
p	< 0.001	> 0.05

Values within a column followed by the same letter is not significantly different at $p < 0.05$ (Tukey's HSD post-hoc test).

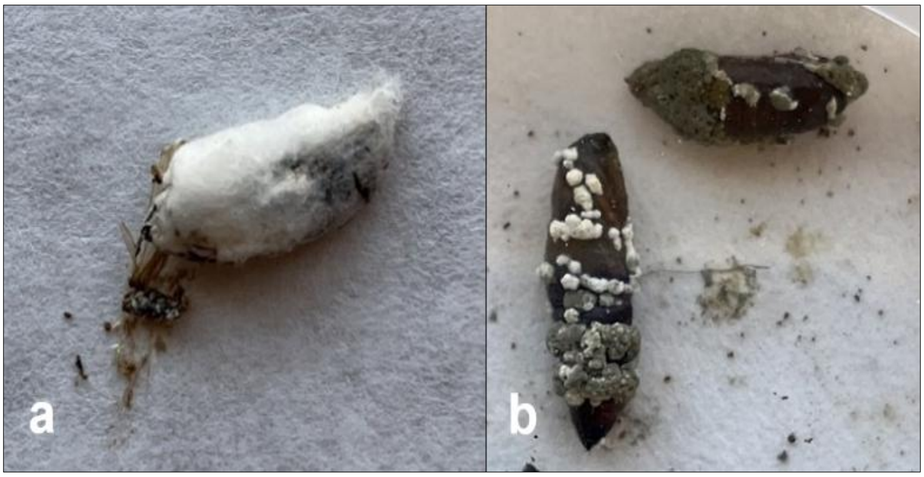

Figure 3.9 A *Spodoptera frugiperda* pupa exhibiting mycosis symptoms: **a -** *Beauveria bassiana* and; **b -** *Metarhizium anisopliae.*

3.4.4 Effect of bioinsecticides on fecundity and longevity of *Spodoptera frugiperda*

The number of eggs laid by female moths, of which both the male and female in the mating pair were exposed during their pupal stage to soil treated with the respective EPF's, was not significantly affected compared to those in the control treatment ($\chi^2 = 0.33$; df = 2; $p > 0.05$). The mean fecundity of Ecobb treated moths was 1088.0 ± 157.3 eggs/female, Mt 78 treated moths, 1153.5 ± 120.9 eggs/female and moths from the control, 1240.1 ± 136.4 eggs/female.

Exposure of pupae to soil inoculated with biopesticide formulations did also not affect the longevity of *S. frugiperda* moths that eclosed. This same trend was observed for female (log-rank: $\chi^2 = 0.07$; df = 2; $p > 0.05$) (Figure 3.10a) and male moth survival (log-rank: $\chi^2 = 0.17$; df = 2; $p > 0.05$) (Figure 3.10b), as well as for individual groups from all the treatments (log-rank: $\chi^2 = 0.26$; df = 5; $p > 0.05$) (Figure 3.10c). Similar results were obtained when longevity of the male and female moths was combined for each treatment ($\chi^2 = 1.39$; df = 2; $p = 0.05$) (Figure 3.10d).

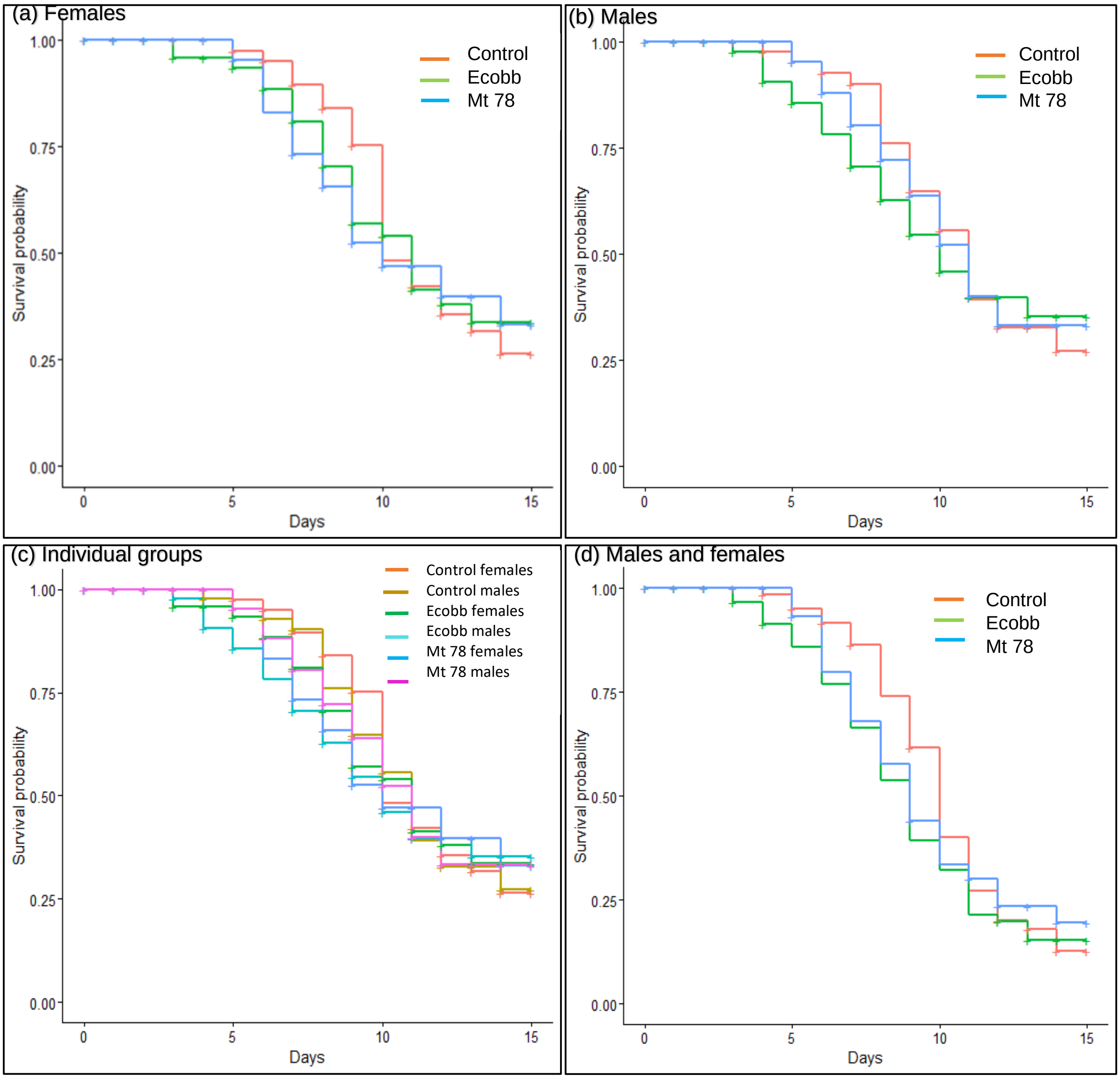

Figure 3.10. Plots of survival probability estimated for *S. frugiperda* moths after exposing the pupae to soil inoculated with *Beauveria bassiana* (Ecobb) and *Metarhizium anisopliae* (Mt 78) and for moths from the untreated control. (**a**) Females, (**b**) males, (**c**) individual groups from all the treatments and (**d**) combined male and female insects from each treatment.

3.5 Discussion

Second- and sixth-instar *S. frugiperda* larvae were not susceptible to the four commercial EPF’s (Bband, Ecobb, Mt 69 and Mt 78). It is important to note that the EPFs evaluated in this study are not registered against this pest in SA. Both the mortality and mycosis levels of second- and sixth-instar larvae were low. These results are comparable to that of Idrees *et al.* (2021), who recorded mortality of 2.2-10% and 2.2-5.6% in second- and sixth-instar *S. frugiperda* larvae, respectively, for fungal concentrations of 1 × $10^{6,\,7}$ cfu/ml of four different EPF species, *viz*. *Aspergillus* spp. P. Micheli (Eurotiales: Aspergillaceae), *Penicillium* Link (Eurotiales: Aspergillaceae), *Beauveria* and *Cladosporium* Link (Capnodiales: Davidiellaceae). These concentrations are within the ranges that were used in this study and allow for a reasonable comparison. The results are in contrast with those of Mekonnen *et al.* (2024) and Perumal *et al.* (2024) who reported that *S. frugiperda* larvae, pupae and adults were susceptible to EPFs. This could possibly be ascribed to differences in fungal concentrations, application methods and/or fungal species/strains.

There were some notable variations in the mycosis levels from the different treatments, with *B. bassiana* formulations showing higher mycosis levels than *M. anisopliae* formulations in the L2 and L6 cadavers. Entomopathogenic fungal infection is known to cause a significant reduction in feeding in some insects, resulting in insect death. This is attributed to the toxins produced by EPFs (Vinayaga Moorthi *et al.,* 2015). *Beauveria bassiana* and *M. anisopliae* produce substances that can deter an insect from further feeding leading to quick rejection of food. Alternatively, they produce toxins that prevent further feeding by disruption of the insect's structural integrity and physiological processes (Quesada-Moraga *et al.,* 2006). Reduced feeding has been demonstrated in larvae of the cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (Quesada-Moraga *et al.,* 2006) and *S. frugiperda* (Idrees *et al.,* 2021). Feeding performance assessments were, however, not done in this study. It is therefore suggested that it should be included in future studies, since it may explain the absence/low mycosis rates observed with some treatments. It will allow for death of non-mycosed individuals to be attributed to non-feeding where applicable. Nonetheless, mycosis is an important fitness characteristic for an EPF because sporulation allows for the self-propagation of the fungus in the environment (Rohrlich *et al., 2018)*. In this study, the *B. bassiana* formulations showed greater sporulation than *M. anisopliae* formulations from the feeding bioassays. However, when prepupae were exposed to inoculated soil, their mycosis levels were similar in all the four treatments. Entomopathogenic fungi may perform better in soil rather than foliar spray applications, since soil acts as a natural reservoir providing nutrition which allows for EPF proliferation (Hajek & St Leger, 1994). Soil is a known habitat for EPFs, including *Metarhizium* and *Beauveria* species (Pérez-González *et al.*, 2014).

When prepupae were exposed to inoculated soil, there was successful infection by both the *M. anisopliae* and *B. bassiana* formulations. This is ascribed to the contact mode of action of these EPFs. This study showed that soil drenching with EPFs resulted in mortality and mycosis of *S. frugiperda* prepupae, although not at a high frequency. Further investigation is therefore needed into the potential of soil drenching as an application method for EPFs to control the soil dwelling stages of this pest. In the field, it also seems the most practical method as soil provides protection of the fungus from ultraviolet radiation (Islam *et al.,* 2021). However, since sterile soil was used in this experiment, field evaluations are necessary to validate the results. Persistence of applied fungus depends on several other factors such as the soil physical properties, ambient temperature and moisture and the presence of other competing microorganisms (McGuire & Northfield, 2020).

Emergence, malformation, fecundity and adult longevity were evaluated to assess the sublethal effects of EPFs on *S. frugiperda* fitness. While there was no difference in adult emergence from the control treatment, EPF treatments caused significant malformation, similar to results by Montecalvo *et al.* (2021). It has been reported that EPF's can produce toxins that can result in improper cuticle formation, which may lead to malformation (Prasad & Veerwal, 2010). Malformation in adults, such as reduced body and wing size, may affect oviposition because adults are no longer able to fly, find a mating partner and reproduce (Vivekanandhan *et al*., 2024). *Metarhizium anisopliae* and *B. bassiana* produce toxins that cause the opening of Ca^{2+} channels in the membrane which disrupts chitin synthesis leading to deformities (Garrido-Jurado *et al.,* 2020). Since no effect on fecundity and moth longevity could be detected, it could be concluded that both the *Beauveria* and *Metarhizium* formulations used in this study, had no adverse effect on *S. frugiperda* which is concurrent with the findings of Idrees *et al.* (2023). Although the same EPF application method that was used and shown to be effective against prepupae was used for the pupae, the pupae may have become too sclerotised for fungus to enter and germinate to penetrate the pupal case (Goble *et al.*, 2011) to cause any significant sublethal effects to the adults that emerged.

Developing an effective formulation is crucial for the efficacy of EPFs in the field (Vega *et al.,* 2012). Entomopathogenic fungi can be formulated as liquid suspensions or dry solids, which may in turn affect their viability (degree of infectivity) (Vega *et al.,* 2012). Liquid formulations are typically water or oil based, including emulsion suspensions (ES), whereas solid formulations include wettable powders (WP) (Vega *et al.,* 2012). The results indicated that conidia germination of the respective biopesticide formulations used in this study exceeded 75%. However, Bband (ES) outperformed the other biopesticides by having the highest viability. Similarly, previous studies reported enhanced infectivity when conidia of *B. bassiana* (Prior *et al.,* 1988) or *M. anisopliae* (Bateman *et al.,* 1993) were formulated in oil rather than water. Enhanced infectivity has also been reported when conidia of *B. bassiana* were formulated in an ES rather than a WP formulation (Wraight *et al.,* 2016). This increased infectivity (viability) could be a result of broader distribution and greater adherence of the oil, as opposed to water, to the insect cuticle potentially enabling it to reach intersegmental membranes (Vega *et al.,* 2012).

3.6 Conclusion

This is the first report on the efficacy of commercial biopesticide formulations (EPFs) against *S. frugiperda* in SA. The larvae were not susceptible to the respective biopesticides, and adult emergence as well as fecundity and longevity was not significantly affected. Prepupae were, however, susceptible to the *Metarhizium* formulations, with *M. anisopliae* ICIPE 78 being the most promising. Further research on this biopesticide, which is already commercialised in SA, is needed to optimise its efficacy against prepupae in the soil. *Metarhizium anisopliae* ICIPE 78 could be a potential candidate for management of *S. frugiperda* when applied in the soil for control of pupating larvae.

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CHAPTER 4: Conclusion and recommendations

Maize (*Zea mays* L.) (Poaceae) is one of the world's leading staple crops in terms of production and is cultivated on around 197 million hectares globally (Erenstein *et al.,* 2022). The worldwide demand for maize is expected to increase in the future (Erenstein *et al.,* 2022). In Sub-Saharan Africa (SSA) maize is the main staple crop with over 300 million smallholder families relying on it as their primary food source (Huesing *et al.,* 2018). Despite the major significance of maize in SSA, yield levels remain low compared to the global mean (Nyaligwa *et al.,* 2017). Although insect pests pose a threat worldwide, they have a significantly bigger impact in smallholder farming systems in SSA, since many subsistence farmers do not have access to resources or knowledge about effective crop protection (Lenne, 2000). Contributing to the low maize yields in SSA are pests (Souto *et al.,* 2021) such as the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Huesing *et al*., 2018).

Synthetic insecticides often do not provide efficient control of *S. frugiperda*, and it can rapidly evolve resistance to insecticides (Young & McMillan, 1979; Yu *et al.,* 2003; Stokstad, 2017; Gutiérrez-Moreno *et al.,* 2019; Ahissou *et al.,* 2021; Chen *et al.,* 2023). Several cases of field-evolved resistance have been reported (Mota-Sanchez & Wise, 2024). This is mainly due to poor insecticide application methods (Carvalho *et al.,* 2013; Paredes-Sánchez *et al.,* 2021; Van den Berg & Du Plessis, 2022). However, *S. frugiperda* is a migratory species in which resistance alleles may also spread when new areas are invaded (Arias *et al.,* 2019; Yainna *et al.,* 2021; Nguyen *et al.,* 2022).

Insecticide susceptibility data can be used in insecticide resistance management (IRM) programs as a preventative measure, by providing an early warning to adapt control strategies (Forrester & Cahill, 1987; Miller *et al.,* 2010). Frequent monitoring of susceptibility levels in pest populations, by means of laboratory bioassays, allows for the early detection of resistance evolution enabling the implementation of preventative strategies (Ffrench-Constant & Roush, 2012). Monitoring the susceptibility status of *S. frugiperda* to insecticides over time is critical for its effective IRM in South Africa (SA) (Cook *et al.,* 2004). One of the objectives of this study therefore aimed to estimate the baseline susceptibility of *S. frugiperda* to four insecticides in SA.

Larvae from the first *S. frugiperda* population that invaded SA in 2017 were obtained and reared to serve as a reference population for future resistance screenings. Their susceptibility to chlorantraniliprole, indoxacarb, pyridalyl dichloropropene-derivitive and spinetoram was estimated in this study, to serve as baseline data for all future estimates of susceptibility levels of this pest. Through future monitoring, timely detection of changes in susceptibility levels can be obtained and used as an indication of resistance evolution. This study therefore provides a foundation for IRM of *S. frugiperda* in SA.

The latter is evident from the estimates for a *S. frugiperda* population, sampled from an overwintering area at Mbombela in 2022. Although the results indicated that no control failure is currently expected with applications of any of these insecticides, changes in susceptibility by *S. frugiperda* were detected. The resistance ratio (RR) between the reference - and test populations for the four insecticides tested, five years after its invasion into SA, differed significantly, and ranged from 1.8-12.5. The only concern at this stage in terms of resistance evolution for the Mb-22 population, is indoxacarb with a RR >10. The Mbombela population does, however, not currently have practical resistance to indoxacarb under field conditions, which is in line with the opinions of Mota-Sanchez *et al.* (2008) and Tabashnik *et al.* (2014) on resistance evolution and practical resistance. The use of synthetic insecticides in pest management programs always presents a risk of resistance evolution (Tabashnik *et al.,* 2014). It is recommended that regular monitoring of *S. frugiperda* should be done to detect changes in insecticide susceptibility to be used in effective IRM programs for this pest.

Spodoptera frugiperda is currently not a significant threat to commercial maize farmers in SA. This may be ascribed to the seasonal influx of moths from overwintering countries (Sparks, 1979; Timilsena *et al.,* 2022) which carry susceptible alleles into SA as well as the large-scale cultivation of transgenic *Bacillus thuringiensis* (Bt) Berliner (Caryophanales: Bacillaceae) maize (ISAAA 2017). Resistance evolution is not the only concern with the use of chemical control. It also poses potential health and environmental risks and affects non-target organisms. Alternative pest management tactics are therefore required to be included in integrated pest management (IPM) programs.

Control methods such as entomopathogenic fungi (EPF) has been identified as an alternative control tool to synthetic insecticides, for control of *S. frugiperda* (Cruz-Avalos *et al.,* 2018; Akutse *et al.,* 2019; Fakeer *et al.,* 2024; Jeon & Kim, 2024). Entomopathogenic fungi can therefore potentially be used across SSA to combat *S. frugiperda.* However, EPF bioinsecticides are not yet registered in SA against *S. frugiperda* (Agri-Intel, 2024), and very few studies have been done on the efficacy of the commercial formulations against the respective life stages of *S. frugiperda* in SSA. The results of this study contribute to knowledge on the susceptibility of the different life stages of *S. frugiperda* to four commercially available EPFs in SA. These were two *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Clavicipitaceae) and two *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) based commercial biopesticides registered against other pests in SA. *Spodoptera frugiperda* larvae were not susceptible to the biopesticides, and adult emergence as well as fecundity and longevity was not significantly affected. Prepupae were, however, susceptible to both *Metarhizium* formulations used, with, *Metarhizium anisopliae* ICIPE 78 having the highest mortality of 56.67%. This biopesticide, which is already commercialised in SA, could be presented as a potential candidate for management of *S. frugiperda* when applied in soil in an IPM program. It is recommended to further investigate the potential of soil drenching as an application method for EPFs to control the soil dwelling stages of this pest.

An IPM program aims to incorporate the simultaneous use of management strategies to control pests (Pretty & Bharucha, 2015), and not solely rely on synthetic insecticides since it is not a sustainable solution (Khun *et al.,* 2020). It may involve the use of synthetic insecticides, together with other compounds such as bioinsecticides. The latter been recognised as alternatives to synthetic insecticides (Khun *et al.,* 2020). Biopesticides account for only around 8% of all pesticides sold on a global scale (Glare *et al.,* 2012). This sector is rapidly growing at an annual growth rate of 16% compared to the 3% of synthetic insecticides (Khun *et al.,* 2020). The biological sector should increase at an even higher rate if biopesticides are to play a full role in reducing the overreliance on synthetic insecticides (Pretty & Bharucha, 2015). Synthetic insecticdes should therefore be incorporated with bioinsecticides in IPM programs to ensure a sustainable solution to control insect pests (Pretty & Bharucha, 2015).

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APPENDIX A: Declaration of language editing

Language editing statement

To whom this may concern,

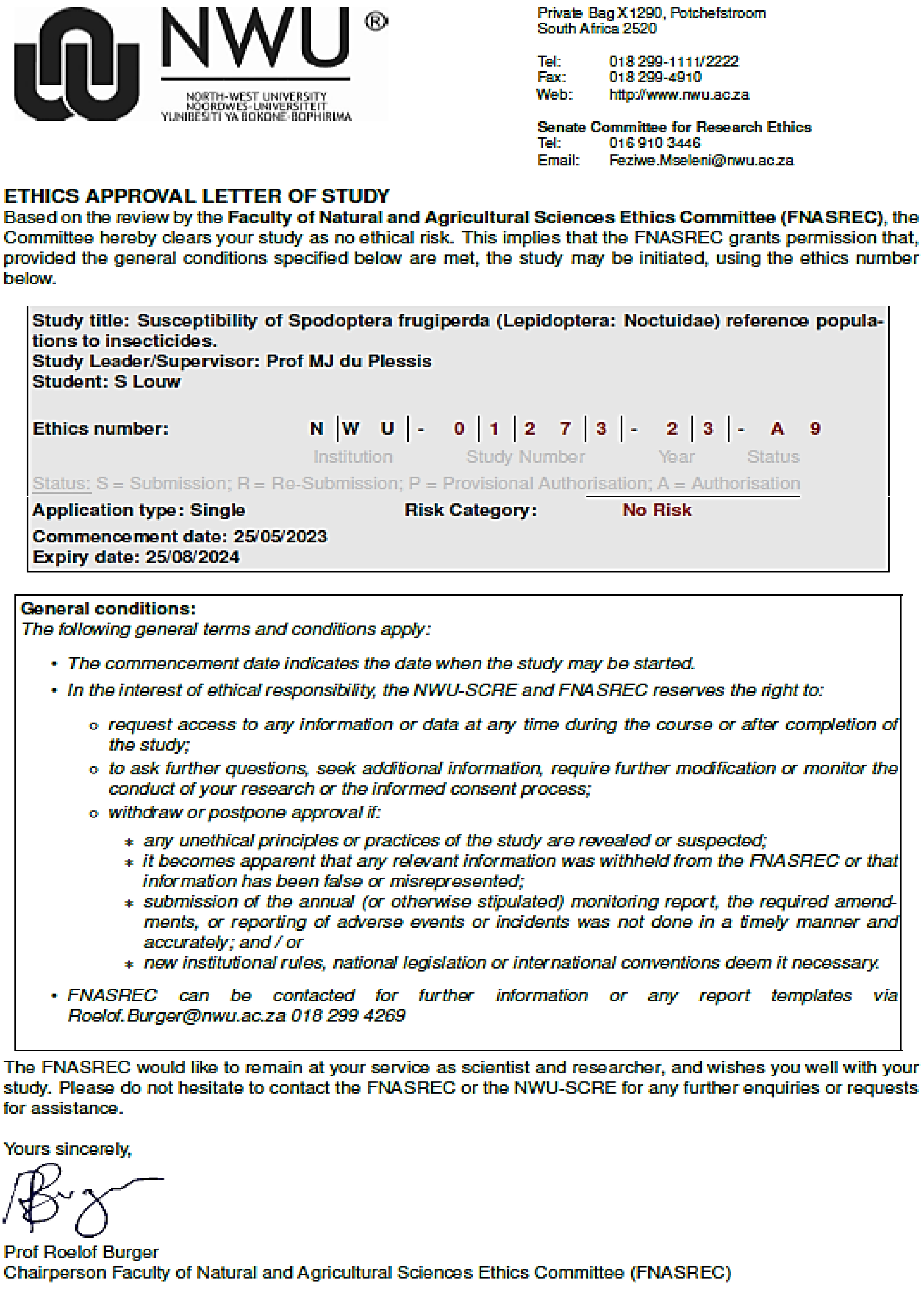
I, Prof. Johnnie Van den Berg, hereby declare that the thesis titled: “Susceptibility of a *Spodoptera frugiperda* (Lepidoptera: Noctuidae) reference population to insecticides and entomopathogenic fungi in South Africa” by Simoné Louw has been edited for language correctness and spelling. No changes were made to the academic content or structure of this work.

J van den Berg

Prof. Johnnie Van den Berg

Date: 27 November 2024

APPENDIX B: Ethical clearance



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Senate Committee for Research Ethics
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Email: Feziwe.Mseleni@nwu.ac.za

ETHICS APPROVAL LETTER OF STUDY

Based on the review by the **Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)**, the Committee hereby clears your study as no ethical risk. This implies that the FNASREC grants permission that, provided the general conditions specified below are met, the study may be initiated, using the ethics number below.

Study title: Susceptibility of Spodoptera frugiperda (Lepidoptera: Noctuidae) reference populations to insecticides.
Study Leader/Supervisor: Prof MJ du Plessis
Student: S Louw

Ethics number:	N W U -	0 1 2 7 3 -	2 3 -	A 9
	Institution	Study Number	Year	Status

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

Application type: Single **Risk Category:** **No Risk**
Commencement date: 25/05/2023
Expiry date: 25/08/2024

General conditions:
The following general terms and conditions apply:

- *The commencement date indicates the date when 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 monitor 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 necessary.*
- *FNASREC 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)