Environmental determinants of the abundance of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

by

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PLAGIARISM DECLARATION

UNIVERSITY OF PRETORIA
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LIST OF ABBREVIATIONS AND SYMBOLS

AcclimTemp	- Acclimation temperature
ARC-SCW	- Agricultural Research Council - Soil, Climate and Water
ATP	- Adenosine 5´- triphosphate
BBCH	- Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie
Bt	- *Bacillus thuringiensis*
CBD	- Convention on Biological Diversity
cm	- Centimetre
CO1	- Cytochrome oxidase 1
CTLs	- Critical thermal limits
CT_{max}	- Critical thermal maxima
CT_{min}	- Critical thermal minima
DAFF	- Department of Agriculture, Forestry and Fisheries
DALRRD	- Department of Land Reform and Rural Development
EPPO	- European and Mediterranean Plant Protection Organisation
FAO	- Food and Agricultural Organisation
FAW	- Fall armyworm
g	- Gram
GLZ	- Generalized linear model
h	- Hour
HSPs	- Heat shock proteins
IAS	- Invasive alien species
IPM	- Integrated pest management
KKS	- Klein Karoo Seed
km	- Kilometre
LD	- Light: Day
m	- metre
mg	- Milligram
min	- Minute
mm	- Millimetre
Nipagin M	- Methyl-4-hydroxybenzoane
T_B	- Thermal breadth
T_{opt}	- Thermal optimum

TPC - Thermal performance curve
ULTs - Upper lethal temperatures
USD - United States dollar
UV - Ultraviolet
% - Percent
°C - Degrees Celsius
µ - Micro

SUMMARY

Expanded global trade and improved modes of transportation have shortened the duration of movement between countries but have also increased and aided the spread of alien species. The latter has been aggravated by the changing climate, and is expected to worsen in the coming years. Invasive insect species are a particularly serious problem when they infest agricultural crops. One such alien invasive species is the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), a notorious pest of many grass crops, including maize (*Zea mays* L.). FAW is native to tropical and sub-tropical regions of North, Central and South America. It invaded South Africa in January 2017. This pest does not exhibit diapause and therefore temperature has a crucial influence on its fitness and geographic distribution. During harsh winter temperatures, FAW migrates to areas with milder temperatures and re-establishes when temperatures are conducive. Two host-preference based FAW strains are recognised. These are the corn (maize) and rice strain and both are present in South Africa. Analysis of *cytochrome oxidase 1* (*CO1*) and *triose phosphate isomerase* (*Tpi*) genes showed that the culture in this study contained a mixture of the rice and corn strains and an interstrain hybrid. Therefore, no distinction is made between strains when referring to FAW in this study.

In this study, lower and upper thermal tolerance of FAW were determined. The specific objectives were: (i) to determine the critical thermal minima (CT_{min}) and maxima (CT_{max}) of FAW and to investigate the effects of life stage (first instar, sixth instar and adults), thermal history and diet on critical thermal limits of FAW; and (ii) to determine the FAW infestation prevalence and relate this to the critical thermal limits of the pest to the recorded hourly temperatures in two maize fields in South Africa over 14 months.

FAW populations were reared on three different diets (maize, wheat, and chickpea-based artificial diet) and acclimated for 24 hours at one of three temperatures (20, 25 and 30°C). Critical thermal limits were determined by placing individual insects into a test tube immersed into a water bath and decreasing (for CT_{min}) or increasing (for CT_{max}) the temperatures at a constant rate ($0.1°C.min^{-1}$) until the insect lost muscle function. Both CT_{min} and CT_{max} differed significantly among life stages. CT_{min} of first instar larvae exhibited plasticity only in response to acclimation temperatures, whereas sixth instar larvae were sensitive to both acclimation and diet. Adult CT_{min} was not affected by either acclimation or diet. Acclimation and diet influenced the CT_{max} of first instar larvae, but only diet affected the CT_{max} of sixth instars. Only acclimation had an influence on adult CT_{max}. There were also complex interaction effects in both cold and heat tolerance.

To determine FAW infestation prevalence, maize fields at three farms, Loskop Prison farm at Brits, North West province, Baviaanspoort (Pretoria) and Zonderwater (Cullinan) Prison farms, Gauteng province, South Africa, were inspected. Hourly weather data near these three locations were obtained for a duration of 14 months and compared to the CT_{min} and CT_{max} of the life stages of FAW. There was a high infestation of FAW larvae on Loskop Prison farm (67 and 88 % infestation), but no FAW presence was observed at Zonderwater Prison or Bavaiaansport Prison farms. This was mainly attributed to the cultivars planted but may have also been due to the presence of natural enemies and state of the maize plants. The Brits farm cultivated non-Bt maize whereas the Zonderwater Prison farm grew Bt maize. The maize field at Baviaanspoort Prison farm was too dry for FAW infestation. Temperatures measured near these locations did not go above the CT_{max} of any FAW life stage, regardless of the season. However, winter temperatures did go below the CT_{min} of all life stages, although at differing frequencies and durations. These winter temperatures below CT_{min} may not be occurring at a frequency high enough and duration long enough to significantly reduce the FAW population in these regions. The difference in frequency and duration of environmental temperatures lower than CT_{min} between life stages indicates how temperature stress can vary for individual life stages of the same species, which may have implications for implementation of pest control strategies. However, countrywide studies that incorporate sampling of both larval stages and adults at regular intervals is needed to better understand FAW population dynamics in South Africa.

This study demonstrates the complexity of thermal tolerance and emphasises the importance of including different life stages in thermal biology studies. The biochemical changes responsible for the observed differences in thermal biology between life stages need further investigation.

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range (first to thirst quartile) and maximum (upper whiskers). Outliers are shown by black circles.

Figure 2.3 Average highest temperature (CT_{max}) at which there was an onset of muscle failure in (A) first instar larvae, (B) sixth instar larvae and (C) adults of *Spodoptera frugiperda* developing on different diets and acclimated at different temperatures [AcclimTemp (°C)]. The figures show minimum (lower whiskers), median (black line crossing inside the box), first quartile (lower shoulder of the box), third quartile (upper shoulder of the box), interquartile range (first to thirst quartile) and maximum (upper whiskers). Outliers are shown by black circles.

Figure 3.1 Prison farms (green circles [A, B and C]) visited and weather station sites (white circles [D and E]) where climate data were collected over 14 months (March 2017 to April 2018). Wildebeesthoek Weather station (E) is 33 km away from Loskop Prison farm (A). Roodeplaat Weather station (D) is 2.6 and 20.6 km from Baviaanspoort Prison farm (C) and Zonderwater Prison farm (B), respectively.

Figure 3.2 Hourly temperature range from (A) Loskop Prison farm over 14 months (April 2017-March 2018). Horizontal lines represent critical thermal limits of *Spodoptera frugiperda* life stages: CT_{min} of first instar larvae (purple); CT_{max} of first instar larvae (red); CT_{min} of sixth instar larvae (green); CT_{max} of sixth instar larvae (black); CT_{min} of adults (blue); CT_{max} of adults (yellow). (B) Frequency distribution and duration of temperatures recorded hourly from April 2017 to March 2018 near Loskop Prison farm for (B) first instar larvae, (C) sixth instar larvae and (D) adults. Shaded bars represent temperatures $<CT_{min}$. Mean CT_{min} was 4.1, 9.1 and 3.6 °C while mean CT_{max} was 47.1, 46.8 and 43.1 °C for first instars, sixth instars and adults, respectively. There were no instances where temperatures exceeded CT_{max} of any life stage and therefore there are no bars for CT_{max}. Errors bars signify 95% CLs.

Figure 3.3 Hourly temperature range from (A) Baviaanspoort prison farm and Zonderwater prison farm over 14 months (April 2017-March 2018). Horizontal lines represent critical thermal limits of *Spodoptera frugiperda* life stages: CT_{min} of first instar larvae (purple); CT_{max} of first instar larvae (red); CT_{min} of sixth instar larvae (green); CT_{max} of sixth instar larvae (black); CT_{min} of adults (blue); CT_{max} of adults (yellow). (B) Frequency distribution and duration of temperatures recorded hourly from April 2017 to March 2018 near Baviaanspoort and Zonderwater Prison farms for (B) first instar larvae, (C) sixth instar larvae, and (D) adults. Shaded bars represent temperatures $<CT_{min}$. Mean CT_{min} was 4.1, 9.1 and 3.6 °C while mean CT_{max} was 47.1, 46.8 and 43.1 °C for first instars, sixth instars and adults, respectively. There were no instances where temperatures exceeded CT_{max} of any life stage and therefore there are no bars for CT_{max}. Errors bars signify 95% CLs.

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CHAPTER 1:

General introduction and literature review

An increase in trade and ease of transportation between countries promotes the spread of invasive alien species (IAS) into novel areas. Invasive alien species are defined as non-indigenous species that negatively affect the economy, environment or animal and human health (CBD, 2009). The establishment of insects as IAS can affect crop production in invaded areas and trade between countries. However, the success of an invasive species in a new habitat is largely dependent on its ability to withstand abiotic barriers present at the arrival site (Hellmann *et al.*, 2008). Abiotic barriers include different environmental stresses and among them, thermal stress is the most important (Bale *et al.*, 2002; Deutsch *et al.*, 2008). Unfortunately, the increase in global temperatures caused by climate change can remove barriers for IAS (Bale *et al.*, 2002), with insects particularly predicted to benefit as they are ectotherms.

The tolerance of a species to thermal stress is of central importance to invasion biology as it plays a large role in determining the potential of a species to disperse, invade and successfully establish in a new habitat (Lee, 2002). Insect physiologists use different methods to determine the temperature range a species can tolerate, and ultimately its potential response to stressful temperatures. One such assay is the dynamic approach to determine critical thermal limits, CTLs (Terblanche *et al.*, 2011). This involves a gradual reduction or increase of temperature to measure the lower (CT_{min}) and upper (CT_{max}) temperatures at which an insect loses its basic motor function, respectively (Terblanche *et al.*, 2011; Garcia-Robledo *et al.*, 2018). The aim of this assay is not to determine temperatures that are lethal to the organism but to determine temperature points at which the organism cannot carry out basic muscle function and are therefore more prone to natural enemies and insecticidal chemicals (Nyamukondiwa & Terblanche, 2010).

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), has invaded and established populations outside its area of origin of tropical and sub-tropical regions of the United States of America to Argentina and the Caribbean for the first time in history. The pest was first detected in early 2016 in the west and central African countries of Togo, Nigeria, Benin, and the island nation of São Tomé and Príncipe (Goergen *et al.*, 2016).

By early 2017, FAW had already made its way into many sub-Saharan African countries (Abrahams *et al.*, 2017) including South Africa (Jacobs *et al.*, 2018). In the years 2018 to 2020, FAW spread even further to India (EPPO, 2018; Kalleshwaraswamy *et al.*, 2018a), Thailand (FAO, 2019e), Myanmar (FAO, 2019f), China (FAO, 2019d), Republic of Korea (FAO, 2019g), the Philippines (FAO, 2019h), Indonesia (FAO, 2019i), Pakistan (Khan *et al.*) and recently, Australia (FAO, 2020). This once geographically restricted organism is now cosmopolitan, and its caterpillars were intercepted at some European ports of entry prior to its invasion of Africa. In 2015, the pest status of FAW was reassessed and it was ranked as an A1 quarantine pest (pests absent in EPPO region) by the European and Mediterranean Plant Protection Organisation (EPPO) (EUROPHYT, 2018).

FAW is of high economic importance in agriculture as it is an herbivorous pest of many agricultural crops, with a preference for grasses like maize, wheat, sorghum, and sugar cane (Montezano *et al.*, 2018). In maize fields, FAW can cause yield losses of up to 34% (Lima *et al.*, 2010) and when the late instars feed on young seedlings, losses of 100% may occur (Fatoretto *et al.*, 2017). In Brazil, annual losses in maize yield due to this pest are estimated at US$400 million (Figueiredo *et al.*, 2005). The impact of FAW was expected to be higher in Africa due to inadequate knowledge of targeted pest management techniques, as such Abrahams *et al.* (2017) estimated the potential losses to maize crops in 12 invaded African countries (Benin, Cameroon, Democratic Republic of Congo, Ethiopia, Malawi, Mozambique, Nigeria, Uganda, Zimbabwe and Tanzania) to range between 8.3 and 20.6 million tonnes per annum, which translates to between US $2.5 and 6.2 billion per year. These estimated potential losses are conservative as they only take into consideration maize-associated losses and of only 12 African states infested by FAW.

FAW does not exhibit diapause during any life stage and therefore uses migration as a strategy to escape from harsh winter conditions to warmer conditions (Johnson, 1987). This suggests that the survival of the pest is more dependent on its migration potential which is mainly driven by temperature. In this regard, this review focuses on three topics related to FAW. First, I focus on the biology of FAW and the aspects that make this species a good invader. The second part focuses on its feeding patterns and field crop damage. Lastly, I highlight thermal biology and temperature effects on its distribution.

1.1 Biology of *Spodoptera frugiperda*

The following review of the biology of FAW is mainly based on work done in the South, Central and North America – its area of origin that has experienced several sporadic outbreaks over a century (Sparks, 1979; Montezano *et al.*, 2018).

Like most insects, FAW development is highly dependent on ambient temperature. A temperature dependent model constructed by Barfield *et al.* (1978) showed that FAW develop optimally (lowest mortality rate) at temperatures of 26.7 °C, and the highest mortality rates were below 18 °C and above 37 °C. Recently, Du Plessis *et al.* (2020) reported that the egg to adult optimal temperature for FAW development ranges between 26 and 30 °C, which further teases out the ideal temperature conditions for this pest. The same study reported a high mortality rate (71%) of FAW larvae when reared at a constant temperature of 18 °C. Barfield & Ashley (1987) reported that FAW developmental time was not only temperature-dependent, but it was also modified by the stage of maize they consumed. They found that FAW that fed on immature tissues of maize leaves had short developmental time compared to those that fed on tissues of matured maize leaves. In another study, Ali *et al.* (1990) noted that FAW developed poorly at temperatures below 25 °C and above 33 °C and completely ceased to develop at 38 °C. The environmental factors that affect FAW lifecycle and survival are summarised in **Figure 1.1**.

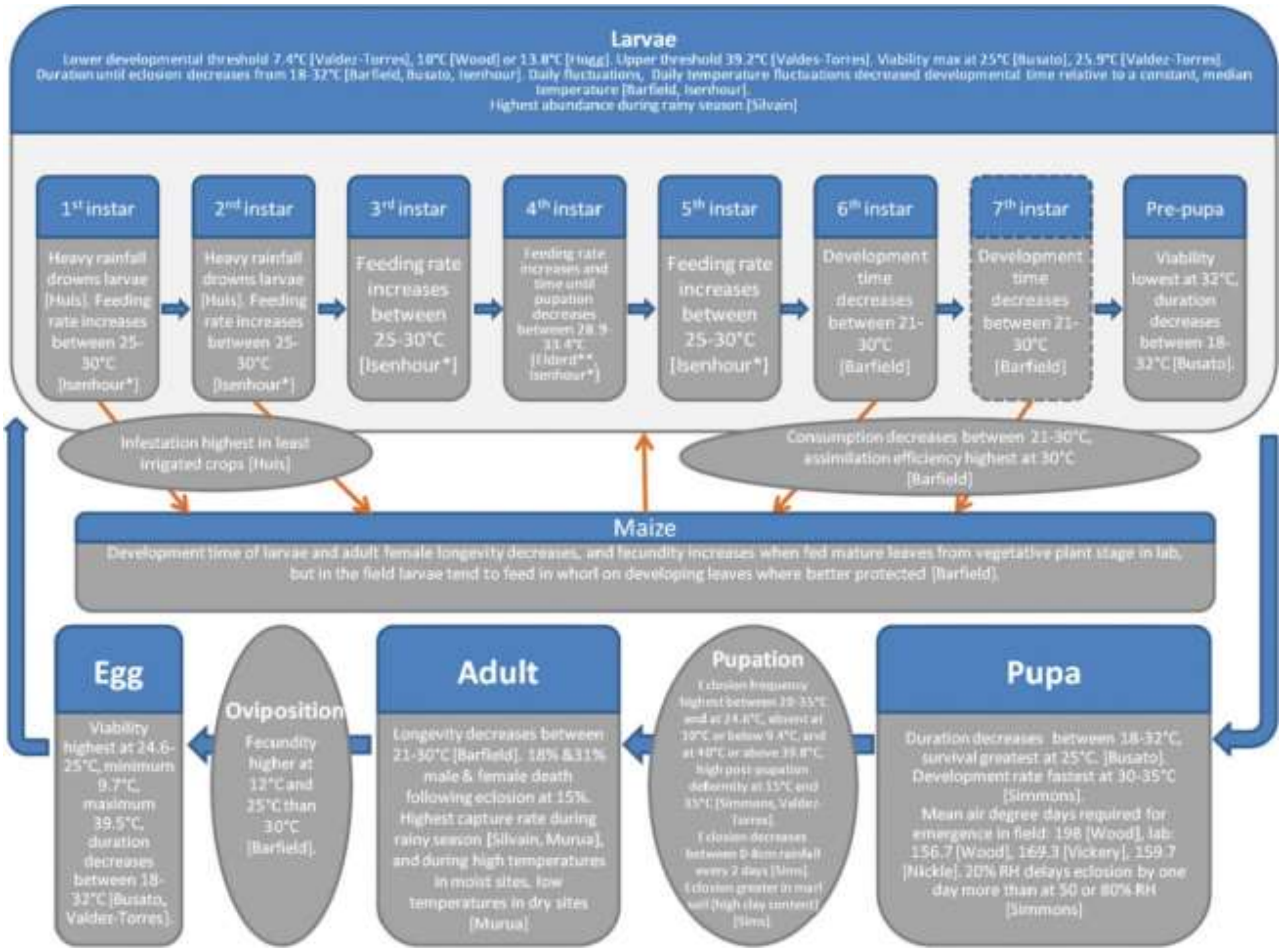

Figure 1.1: Environmental factors affecting the life cycle of *Spodoptera frugiperda*. This schematic diagram was originally presented by Early *et al.* (2018) and the literature cited can be found in the same paper.

The FAW has two distinct strains (Pashley, 1986). The two strains are morphologically indistinguishable, but they differ genetically, physiologically and ecologically (Pashley, 1988; Nagoshi & Meagher, 2004; Saldamando & Vélez-Arango, 2010). The maize strain usually feeds on maize and other broad grasses like sorghum, whereas the rice strain feeds mainly on narrow grasses like rice and bermudagrass (Nagoshi & Meagher, 2004; Saldamando & Vélez-Arango, 2010). The presence of both strains has been widely reported in the Americas (Pashley, 1988; Pashley, 1989; Prowell *et al.*, 2004; Saldamando & Vélez-Arango, 2010). These strains are sympatric but strain-specific variation in migration behaviour has been documented in the USA (Pashley, 1988; Nagoshi & Meagher, 2004). This could explain a frequently observed temporal difference in arrival and population density peak between the two strains. For example, in Louisiana and Florida, the maize strain is first detected in late spring and reaches its peak in early to mid-summer, coinciding with maize maturation; whereas the rice strain only becomes abundant in late summer after the maize strain is nearly absent (Pashley, 1993; Nagoshi & Meagher, 2004). Currently, studies conflict on whether both strains are present in sub-Saharan Africa. For example, a study done by Otim *et al.* (2018) reported that both strains are present in sub-Saharan Africa. Another study done by Jacobs *et al.* (2018) reported that both strains are present in South Africa. However, other studies indicated that the rice strain is scarce and that sub-Saharan Africa may have a novel interstrain hybrid instead (Nagoshi *et al.*, 2018; Nagoshi *et al.*, 2019). Accurate identification of the strain/s present in sub-Saharan Africa is important for effective pest management planning. It is, therefore, important that future studies draw particular attention to the FAW strains occurring in the region. This review does not cover the differences between the strains. Studies that have addressed either the differences between the strains or focused on one strain are listed on **Table 1.1**.

Table 1.1: List of literature that has focused on strain-specific studies of *S. frugiperda*

Study focus	**Reference**
Genetic differences between strains	Pashley (1986)
Strain genetic, insecticidal sensitivity and behavioural differences	Pashley (1988)
Genetic differences between strains	Pashley (1989)
Reproductive compatibility	Quisenberry (1991)
Genetic differences between strains	Lu & Adang (1996)
Genetic identification of strains	Meagher Jr & Gallo-Meagher (2003)
Field population behaviour of the two strains	Nagoshi & Meagher (2004)
Hybridization evidence and genetic mapping of the two strains	Prowell *et al.* (2004)
Strain identification and comparison	Nagoshi *et al.* (2007)
Mating behaviour and underlying genes of the two strains	Schöfl *et al.* (2009)
Strain genetic differentiation and host plant association	Saldamando & Vélez-Arango (2010)
Developmental differences	Meagher Jr & Nagoshi (2012)
Strain identification improvements	Nagoshi (2012)
Mating behaviour of maize strain	Cruz-Esteban *et al.* (2017)
Identification of FAW strains present in South Africa	Jacobs *et al.* (2018)
Strain distribution in sub-Saharan Africa	Nagoshi *et al.* (2018)
Genetic comparison of strains from sub-Saharan Africa	Nagoshi *et al.* (2019)

1.1.1 Adults and eggs

Reproduction and migration/dispersal are the main functions of FAW adults. The FAW moths have a wingspan that ranges between 3.2 to 4 cm (Hardke *et al.*, 2015; Capinera, 2017), and body length of approximately 1.6 cm (Du Plessis *et al.*, 2018). The male moth has some whitish and dark spots on the wings, giving it an ornamented appearance, whereas a female moth is normally dull greyish brown in colour (**Figure 1.2**). The availability of food greatly influences the adults' lifespan as Luginbill (1928) observed an average longevity of 4 days in unfed moths compared to 13 days on moths that were fed diluted honey solution.

Like most noctuids, FAW adults are active at night and hide during most of the day (Vickery, 1929). The moths are active in the evening to feed, mate and oviposit (Luginbill, 1928). The evening movements start during the early hours of darkness when the adults move with wind currents, followed shortly thereafter by upwind movements characterized by slow flight where the pest hovers and feeds, usually 1 to 10 m above the maize canopy (Sparks, 1979).

Fall armyworm moth. Left male, right female. D Visser ARC-VOP Roodeplaat

Figure 1.2: Adult male (left) and female (right) *Spodoptera frugiperda*.

Mating and sex pheromone

After feeding for about 2 hours after sunset, depending on the temperature and season, the female moth initiates mate calling by extending her ovipositor and emitting sex pheromones usually from the top of the crop canopy (Sparks, 1979). Two components of the FAW sex pheromone, (*Z*)-9-tetradecenyl acetate (Sekul & Sparks, 1967) and (*Z*)-9-dodecenyl acetate (Sekul & Sparks, 1976) were the first to be identified from FAW. Several other sex pheromone components have since been reported from FAW: (*Z*)-11-hexadecenyl acetate, (*Z*)-11-tetradecenyl acetate, (*Z*)-10-tetradecenyl acetate, (*Z*)-7-dodecenyl acetate, and (*E*)-7-dodecenyl acetate (Mitchell *et al.*, 1985; Tumlinson *et al.*, 1986; Descoins *et al.*, 1988; Pereira *et al.*, 2006). Several sex pheromones are used in pheromone lure traps to catch FAW (Mitchell *et al.*, 1985; Malo *et al.*, 2001; Meagher Jr *et al.*, 2019; Haenniger *et al.*, 2020).

The distance from which males respond to the female calling is highly dependent on wind velocity and temperature. Typically, males can respond to mating pheromones at a distance of 9 to 12 m when flying oblique to the wind and above the plant canopy. More than one male frequently responds to the mating pheromones of a female, which mates once per night and therefore only one male from a group of males will be able to mate with her. The rejected males revert to against-the-wind movement to trace another female's mating pheromone. Sparks (1979) reported an occasional sighting of males flying in groups of up to 50 individuals.

FAW exhibits 'sequential mating' behaviour (Sparks, 1979; Schöfl *et al.*, 2009). Generally the virgin females are the first to mate in the early hours of the night, followed by females that have mated once, and multiply-mated females mate last (Sparks, 1979; Simmons & Marti Jr, 1992). In a laboratory study Simmons & Marti Jr (1992) placed one FAW virgin female with two virgin males in a cage. They observed that fewer moths copulated on the second night compared to the first night and that the peak mating time was reached in the early hours of the dark on the first night whereas it occurred at later hours on the second night. The mechanism of this sequential mating is not yet understood and to the best of my knowledge the reasons for this phenomenon are yet to be investigated. However, it can be assumed that the mating pheromones released by the females play a role and I propose two possible hypotheses. Firstly, it is likely that the release of the mating pheromones tends to be delayed after every subsequent mating. This may be due to the energetic cost to females of synthesising and releasing pheromones while also laying eggs. In this case, the female may require time to recover after laying eggs before it can synthesise and release more mating pheromones for subsequent mating. Secondly, there may be changes in the concentration or even the ratio of the pheromone components released by FAW virgins and non-virgin female. Hirai & Mitchell (1982) showed that FAW sex pheromone components have different roles in sexual behaviour. They found that males exposed to a high dosage (1×10^1 µg) of either (*Z*)-9-tetradecenyl acetate or (*Z*)-9-dodecenyl acetate did not show any copulatory behaviour, however, the copulatory behaviour was exhibited when these two compounds were mixed. Some synergistic effects were observed in field studies when 2 and 10% quantities of (*Z*)-9-tetradecenyl acetate were mixed to 100 µg of (*Z*)-9-dodecenyl acetate and used in traps (Jones & Sparks, 1979). In this case, the non-virgin females lose the balanced concentration-ratio effective for stimulation of male sexual behaviour. This could explain why non-virgin females are the first to initiate mate calling but multiply-mated females are the last to copulate (Schöfl *et al.*, 2009). These uncertainties mean more effort needs to be put into understanding FAW sexual behaviour.

Oviposition

Generally, mated females lay eggs in batches on the underside of host plant leaves if FAW density is low. In cases where FAW densities are high, oviposition becomes indiscriminant and eggs are laid on any surface (Sparks, 1979). The numbers of eggs per batch depends on environmental conditions. Vickery (1929) reported a range of 9 to 349 eggs per batch whilst Kalleshwaraswamy *et al.* (2018b) reported 55 to 888 eggs per batch when FAW was reared at 26 °C on maize leaves. The common number of eggs per batch ranges between 100 to 200 (Malo & Hore, 2019). The female covers the egg batches with abdominal setae obtained from her lower abdomen **(Figure 1.3)** (Du Plessis *et al.*, 2018), perhaps to protect them from natural enemies and desiccation. Most of the eggs are laid during the first 4 hours of darkness, but oviposition can last until midnight (Johnson, 1987). On average, the female takes 3.5 days after emerging from the pupa before she can oviposit, and oviposition may continue for 4 to 17 days (Johnson, 1987; Kalleshwaraswamy *et al.*, 2018b). FAW eggs require between 2 to 11 days to develop depending on temperature and humidity (Luginbill, 1928; Johnson, 1987). A female can lay up to 1000 eggs during her lifespan, with 95 to 98% being viable (Kalleshwaraswamy *et al.*, 2018b).

Figure 1.3: Egg cluster of *Spodoptera frugiperda*. Notice the abdomen-derived white-cottony covering (or abdominal setae) on the eggs.

1.1.2 Larvae

FAW larvae go through six instars to complete larval development. The larvae have three different colour-based forms: dark form, green form, and brown form (Passoa, 1991). The latter form is regarded as the normal form as it is the most commonly encountered in the field (Passoa, 1991) and I will use it as a reference in the following description.

Description

The dorsal surface of eclosing larvae is normally off-white with small black spots (Hardke *et al.*, 2015) and they have a noticeably large black head (**Figure 1.4a**). The larvae change colour to greenish as they start to feed on the host plant. In the second instar, the head becomes proportional to the body and changes colour to orangish (Malo & Hore, 2019). In the late second to third instar, the larval dorsal surface colour changes to brownish with lateral white lines (Hardke *et al.*, 2015; Capinera, 2017; Malo & Hore, 2019). From fourth to sixth instars, the body bears white subdorsal and lateral lines with brown to black mottled head capsule (Capinera, 2017; Kalleshwaraswamy *et al.*, 2018b; Manjula *et al.*, 2019). The head capsule of the mature larvae is marked with a prominent inverted “Y” shape. On the eight abdominal segment, four distinct dark spots that are arranged in a square become prominent (**Figure 1.5b**). The larval body length from first to sixth instar ranges from an average of 1.7 mm to 34.2 mm, respectively (Luginbill, 1928; Hardke *et al.*, 2015; Capinera, 2017). The FAW larval stage tends to last about 14 days in the warm summer season and 30 days during the cool weather season (Capinera, 2017; Manjula *et al.*, 2019). When reared at 25 °C, Pitre & Hogg (1983) found average developmental time to be 3.3 and 3.7 days for first and sixth instars, respectively.

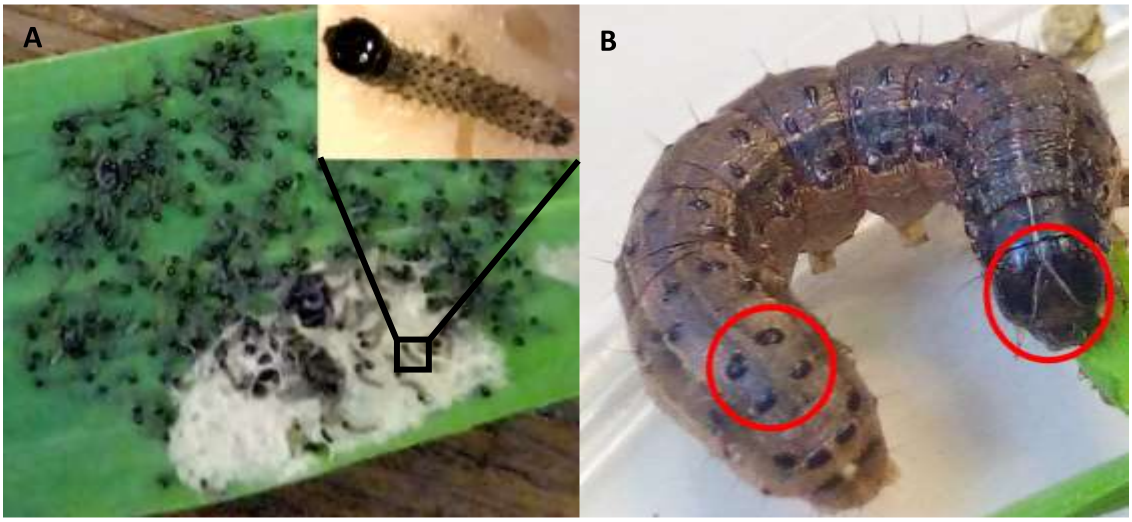

Figure 1.4: Larvae of *Spodoptera frugiperda*. (A) Mass of neonates a few hours after hatching. Notice the large black head and dark spots with tiny spikes on the zoomed picture. (B) Late instar larva with noticeable inverted Y marking on the head capsid and four dots arranged in a square on the last segment (circled in red).

General and feeding behaviour

FAW larvae commence feeding on the eggshells and on the host plant soon after hatching. However, they also avoid feeding in close proximity to each other (**Figure 1.4b**) by dispersing using silk threads and dropping to the ground or other nearby host plants. While parachuting on the silk threads, the larvae may be blown by wind to some distance away from the natal oviposition site (Luginbill, 1928; Malo & Hore, 2019) in order to minimize chances of being cannibalised as they develop (to be discussed in the next section) (Abrahams *et al.*, 2017). The dropping behaviour of the larvae may also be a means to escape natural enemies considering that larvae release silk threads and spin downward immediately when disturbed. However, older larvae do not escape in this way but curl up, cease to move, and if disrupted while in this state, engage in combat and/or move to conceal themselves under foliage (Luginbill, 1928).

The amount of feeding by FAW larvae increases as they grow and develop. FAW larvae mostly feed actively from late evening through to early morning, but also feed during the day when hungry (Luginbill, 1928). During the day, especially on a sunny day, the larvae conceal themselves under foliage.

Although the two FAW strains are distinguished based on the preferred host plant for the larvae, the larval development is largely dependent on temperature and diet and not so much on larval strain-diet combination. For example, when Meagher Jr *et al.* (2004) reared both strains on maize, they found that rice strain larvae developed significantly faster than the maize strain based on larval weight and larval duration comparisons. However, no significant differences in larval weight and larval duration between the two strains were found by Meagher Jr & Nagoshi (2012) when they were reared on maize plants.

Cannibalism in FAW larvae

The FAW larvae are cannibalistic, feeding on each other, especially from the fourth instar on the smaller larvae (Abrahams *et al.*, 2017). Increased interference between larvae and food scarcity are the most likely triggers for cannibalistic behaviour (Vickery, 1929; Chapman *et al.*, 2000; Murúa *et al.*, 2006; Pannuti *et al.*, 2015). However, on maize plants, the larvae, particularly the first three instars, can hide between the young maize leaves without much interference with each other; however, in the later instars the amount of foliage may not be sufficient (Malo & Hore, 2019) and cannibalism due to crowding occurs. Chapman *et al.* (2000) observed that cannibalism accounted for about 40% mortality of second to fourth instar FAW larvae feeding on one maize plant.

The cannibalistic behaviour of the FAW larvae makes migration and seeking other plants necessary to avoid competition caused by crowding. This explains the above-mentioned wide dispersal of neonates just after hatching from eggs. No cannibalistic behaviour has been reported in African armyworm, *Spodoptera exempta* Walker (Lepidoptera: Noctuidae), and as a result the larvae of this species move *en masse*. Therefore, the biology and ecology of the African armyworm is different from FAW (Abrahams *et al.*, 2017). At this stage, there has been no report on whether the FAW larvae attack other maize pests and *Spodoptera* species found in Africa and the implications of this potentially predatory behaviour on the pests' community ecology.

1.1.3 Pupa

In the field, fully developed larvae stop feeding, drop down to the ground and burrow 2 to 10 cm into the soil where pupation takes place. Pupation can also occur in covered plant parts like maize cobs and stalk tunnels formed by the larva when burrowing. In the soil, the larva makes a loose, 20 to 30 mm in length, oval-shaped cocoon by tying together soil particles with silk. In cases where the soil is too hard, leaf debris and other material can be used by the larvae (Capinera, 2017; Malo & Hore, 2019). The duration of the pupal stage is affected by temperature. For example, the pupal stage may last 6 days and 32 days at average soil temperatures of 29 °C and 16 °C, respectively (Vickery, 1929). The pupal stage of FAW reared on maize leaves at 26°C took 9 to 12 days (Kalleshwaraswamy *et al.*, 2018b). Eclosion occurs at temperatures between 10 °C and 35 °C, although deformities increase at 35 °C (Simmons, 1993). Pitre & Hogg (1983) studied winter effects on pupal survival in Florida and recorded 11.6% survival in northern Florida, 27.5% survival in central Florida and 51% survival in warm winters of southern Florida.

A freshly formed pupa is greenish in colour and turns reddish-brown as it ages. FAW pupae measure 14 to 22 mm in length and about 5 mm in width (Capinera, 2017; Malo & Hore, 2019). Sex identification can be done based on the genital slits of the pupae (**Figure 1.5**) (Kalleshwaraswamy *et al.*, 2018b). The space between anal and genital notches is longer for the female pupae than for male pupae. Moreover, the male genital notch is more pronounced compared to that of the female notch (**Figure 1.5**).

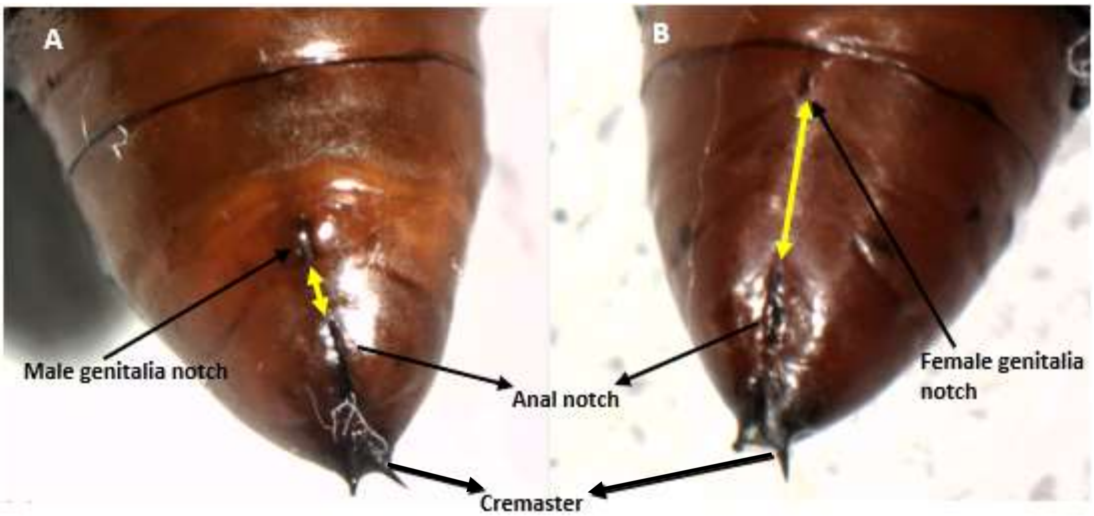

Figure 1.5: *Spodoptera frugiperda* pupae. (A) Male pupa (notice short distance between anal and genital notches indicated by yellow arrow), and (B) female pupa (notice longer distance between anal and genital notches indicated by yellow arrow). *Refer to the text for further description.*

1.2 Host plant damage

The larva is the only FAW developmental stage that causes plant injury and ultimately, yield loss. FAW outbreaks are unpredictable and when they occur, the larvae severely damage any above-ground plant material. These outbreaks, compounded by the voracious feeding of the larvae, lead to devastating crop losses (Hardke *et al.*, 2015).

Plant injury caused by FAW does not always translate into yield loss (Abrahams *et al.*, 2017; Malo & Hore, 2019). For some crops, like maize, larval feeding on leaves alone does not always cause much damage since it is not the reproductive structure that is harvested. This is in part caused by the ability of the plant to compensate for some lost leaf area. However, when present in large numbers, the plant may not be able to recover, with FAW caterpillars completely devouring and defoliating the plant (Abrahams *et al.*, 2017; Malo & Hore, 2019). Another deciding factor that relates level of attack with yield loss is the timing of plant injury caused by FAW feeding. Earlier attack on young plants can allow the plant to compensate for loss of leaves relative to later attack, as the damage may also occur on developing maize cobs (Abrahams *et al.*, 2017). FAW is a polyphagous pest, the damage it causes is more serious on grasses like maize, rice, and wheat.

Early instar larvae cause damage by initiating foliage feeding on one side of the leaf and the opposite epidermal layer remains intact, producing a “window effect”. Another distinctive damage symptom of early instar feeding is pin or shot holes (Maruthadurai & Ramesh, 2020), which typically happen when early larvae feed on fresh immature maize leaves. From the second to third instar, feeding style changes as the larvae start to eat the leaves inward from the edge (Capinera, 2017). Feeding of FAW in corn whorl results in characteristic damage with the appearance of row perforations on the leaf on older maize plants. Later instar larvae feeding on young maize plants can cause complete damage to the whorl (Maruthadurai & Ramesh, 2020). As mentioned before, there is usually one or two larvae per plant during late instars. These older larvae normally stay inside the maize whorls. The infested whorls are usually filled with frass (faecal matter), which makes it difficult for traditional spray insecticides and some natural enemies to reach the larvae (Abrahams *et al.*, 2017; Malo & Hore, 2019; Maruthadurai & Ramesh, 2020). The older larvae are the ones that cause extensive damage on the cobs by boring into the developing cobs to reduce both yield quality and quantity (Pannuti *et al.*, 2015). Feeding on plant growth points like bud and whorl destroy the growth point of the plant and may completely kill the plant (Capinera, 2017). Feeding on the cob may reduce the crop value and ultimately yield and it is one of the costliest types of damage as the pest attacks the plant after investment of fertilizer, irrigation, and time.

Results of socio-economic surveys focusing on farmer perceptions of FAW impacts in Africa have been reported by several authors. Yield loss due to FAW damage was estimated between 22% and 67% in Ghana and between 25% and 50% in Zambia (Abrahams *et al.*, 2017). In Kenya, an estimated yield loss of 37% and 33% was reported in the years 2017 and 2018, respectively (De Groote *et al.*, 2020). These studies might not report accurate estimates of FAW impact as they depend on farmer perception. McGrath *et al.* (2018) proposed a better method for FAW damage estimation, which involves identifying sampling points and using an appropriate sampling pattern like a “W” scouting pattern to pick up individual plants that show FAW damage. For example, Baudron *et al.* (2019) studied FAW damage in Zimbabwe using this method and obtained FAW yield damage of 11.5%. This loss is much lower than those estimated by the above studies from other African countries. However, Maruthadurai & Ramesh (2020) studied field damage of FAW in India and observed an incidence ranging from 16 to 52% on fodder maize. The large discrepancy on the estimated FAW damage impacts emphasise the uniqueness of each FAW infestation case. This is not a surprise as this pest is known to have sporadic but devastating outbreaks in the Americas (Luginbill, 1928; Sparks, 1979).

1.3 Pest distribution and environmental limitations

Characteristics of the potential recipient area, and pest biological traits including physiological tolerance to adverse climatic conditions found in the recipient area are key determining factors for potential invasion (Arim *et al.*, 2006; Van Kleunen *et al.*, 2010). As the climate changes and alien species impinge on new regions, it is important to accurately predict the likely response of pests to extreme environmental conditions predicted to be brought by climate change and recipient regions. FAW has biological traits and physiological strategies to tolerate adverse environmental conditions, and this gives it an advantage when invading new areas. These biological traits and physiological strategies include relatively short generation time, high reproduction rate, ability to feed on multiple hosts, tolerance of adverse climatic conditions, and good dispersal and migration abilities. The effectiveness of these traits for enabling FAW invasiveness is evidenced by its fast spread from Africa to the Indian subcontinent, Oceania, Asia and the Middle East in under five years (**Figure 1.6**).

Intercontinental dispersal is thought to be aided by air transport, with FAW being stowaways as aircraft transit from one destination to the next (Cock *et al.*, 2017; Early *et al.*, 2018). Intra-continental spread of FAW is possibly aided by both intra-continental transportation links and wind-aided flight of adults (Westbrook *et al.*, 2016; Early *et al.*, 2018). FAW adults have been reported by Early *et al.* (2018) to ascend into the sky with the aid of the prevailing winds and fly for hundreds of kilometres in a single night.

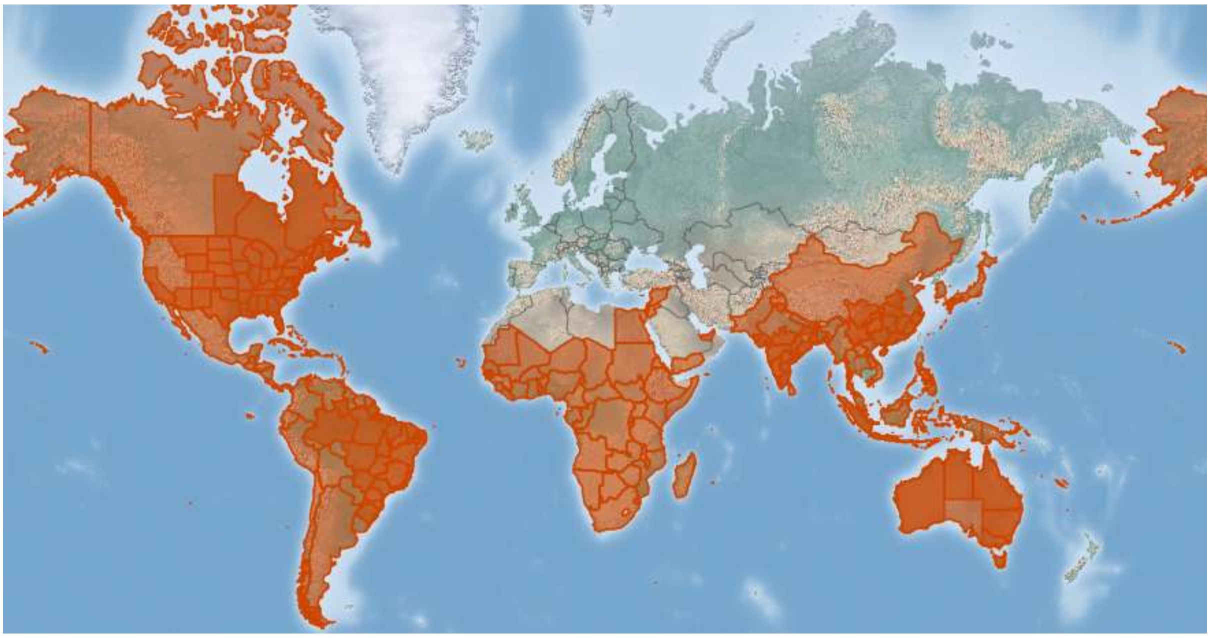

Figure 1.6: Current global distribution of *Spodoptera frugiperda* as of 2021. Source: CABI (2021)

1.3.1 Effects of temperature on insect distribution

It has long been noted that temperature plays a key role in insect development, fitness, activity and geographical distribution (Early *et al.*, 2018). Limits on insect geographical distribution may be set by temperature by acting on differential survival rates (Chen *et al.*, 2011). Plants at higher latitudes where temperatures are lower may grow too slowly to support insect development while plant development may be too quick in mid-low latitudes (Bale *et al.*, 2002). So, insects tend to have physiological adaptations to the latitude they inhabit. These varying physiological adaptations of species with latitude coincide with regions experiencing the greatest climate heating (see Deutsch *et al.*, 2008). However, species in the tropics and in mid-low latitudes are the most sensitive to temperature change and are predicted to be affected most by the warming climate. The species in tropical and mid-low latitude regions are already living close to their optimal thermal requirements, therefore any increases in temperature may be deleterious (see review by Chown & Terblanche, 2006; Deutsch *et al.*, 2008). In contrast, temperate species (associated with higher latitudes) are eurythermal (tolerance to wide range of temperatures) and currently live in cooler climates than their optimal thermal requirements, so it is expected that their fitness will be enhanced by a warming climate (Pörtner *et al.*, 2000; Chown & Terblanche, 2006; Deutsch *et al.*, 2008). Moreover, warming temperatures are predicted to favour insects with high acclimation ability, usually from the temperate regions and less so for those from less variable tropical (stenothermal species) and polar environments (Ghalambor *et al.*, 2006). It is also likely that the tropical and sub-tropical species will migrate to regions that were previously too cool for them but are now experiencing higher temperatures due to global warming. These adaptations to climate change by means of dispersal are expected to be more pronounced in polyphagous insects (Bale *et al.*, 2002) like FAW. This tracking of climate change rather than adapting to it is the main driving force for the currently observed and predicted likely changes in geographic distribution of insects (Coope, 1978; Bale *et al.*, 2002).

Dispersal and at a larger scale, migration of insects has spiked in the past century in response to climate warming (Deutsch *et al.*, 2008). For migratory Lepidoptera, the increase in temperatures heightens their potential for migration, as supported by the high number of butterflies and moths reported in new territories where they did not exist before (Sparks *et al.*, 2007; Diarra *et al.*, 2014; Goergen *et al.*, 2016). For example, Sparks *et al.* (2007) investigated the influence of temperature on, and the incidence of, migration of Lepidoptera into the UK over the period from 1982 to 2005 and recorded that there were 14 more species of migratory butterflies or moths for every 1 °C increase in temperature. *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and FAW are two examples of destructive moths that have recently expanded their geographic distribution to the African continent (Diarra *et al.*, 2014; Goergen *et al.*, 2016). These moths are both native in the Americas and are well-known pests of tomato plants and cereal crops, respectively.

1.3.2 Thermal tolerances of insects and their ecological implications

Performance and therefore fitness of insects is largely affected by environmental temperatures. Performance of ectotherms, including insects, tends to follow a general thermal performance curve (TPC) (**Figure 1.7**) (Huey & Stevenson, 1979; Angilletta Jr *et al.*, 2002). Some useful biological parameters are illustrated by a TPC. First is the thermal optimum (T_{opt}), a temperature range where performance or fitness is at its maximum. Secondly, are critical thermal minima (CT_{min}) and maxima (CT_{max}), which are the lowest and highest temperatures where performance can occur. The area under the curve (from CT_{min} to CT_{max}) is the thermal niche, which is the range of temperatures that support basic physiological function. Any temperatures below or above the thermal niche are detrimental to the insect. Finally, the performance breadth is a range of temperatures that allows good performance. Performance breath is usually expressed as a percentage of the maximal performance (Angilletta Jr *et al.*, 2002). This percentage is usually chosen arbitrarily. For example, 60% performance breath ($T_{B/60}$) is a temperature range where performance is equal to or greater than 60% of the maximum performance.

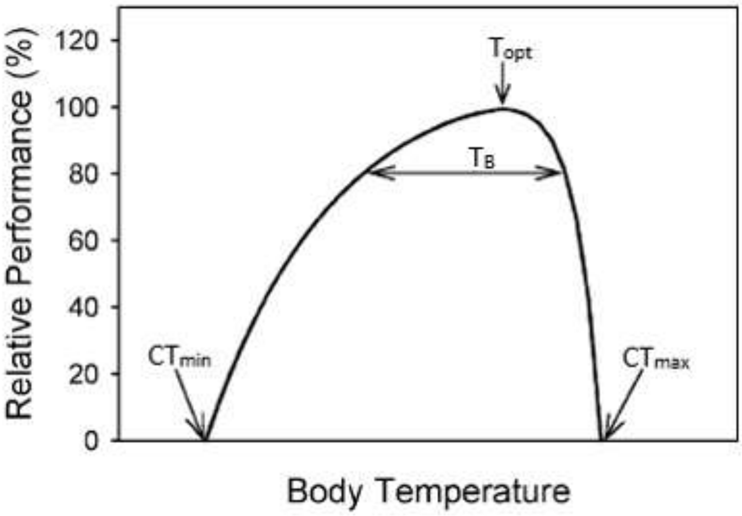

Figure 1.7: Thermal performance curve of ectothermic organisms showing the general relationship between performance of an organism and temperature (visit text for description). Source: Angilletta Jr *et al.* (2002).

Any changes in environmental temperature that negatively affect insect behavioural and physiological traits is called thermal stress. Some of the behavioural and physiological traits that are usually affected by thermal stress include foraging ability, feeding rate and growth (Barfield *et al.*, 1978; Ali *et al.*, 1990; Du Plessis *et al.*, 2020), locomotion (Deere & Chown, 2006), immune function, and sensory perception. The effects of thermal stress on these behavioural and physiological traits greatly reduce insect performance and fitness. Rapid laboratory assays, such as the measurement of critical thermal limits (‘CTLs’), are usually performed to assess the thermal tolerance of ectotherms by estimating the thermal niche of species (Huey *et al.*, 1992; Chown *et al.*, 2009).

Factors such as quality of diet, thermal history and life stage of the organism may influence thermal tolerance. These changes are examples of phenotypic plasticity where a single genotype can produce different phenotypes when exposed to different conditions (Pigliucci *et al.*, 2006). For example, Villanueva *et al.* (2011) showed that shredder larvae, *Sericostoma vittatum* Rambur (Trichoptera: Sericostomatidae), feeding on a nutrient-poor plant were more sensitive to increased temperature stress. In addition, the study demonstrated that during high temperature stress, the insect exhibited poor nutrient assimilation, suggesting that the high temperature stress affected the metabolism of the organism and that it is host plant-dependent. Kleynhans *et al.* (2014a) reported that there was host-plant-dependent variation in CTLs of the stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae), feeding on two different host plants, *Saccharum* sp. or *Cyperus papyrus* L. Even for a polyphagous organism like FAW, there are differences in its biology when feeding on various hosts. For example, FAW developed better on millet (short generation time, high reproductive success and survival) than on either cotton or soybean (Barros *et al.*, 2010). Meagher Jr *et al.* (2004) reported comparable development of FAW larvae feeding on maize and sorghum but poor development when compared to those feeding on cowpeas. Differences in nutritional composition are responsible for diet-dependent variation in thermal tolerance (Andersen *et al.*, 2010; Colinet *et al.*, 2013). There is still relatively little known about the effects of diet (and the mechanism thereof) on insect physiology. However, it is possible that the diets interfere with insect stress protective mechanisms. One such mechanism is expression of heat shock proteins (HSP), which are produced by the organism in response to stress (Feder & Hofmann, 1999; Sørensen *et al.*, 2003; King & MacRae, 2015). Andersen *et al.* (2010) compared two diets, carbohydrate-enriched and protein-enriched, on fruit flies, *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) and found diet-dependent effects on thermal stress. They found that fruit flies fed on a protein-rich diet had increased tolerance to thermal stress and that HSP 70 was upregulated in these flies compared to those on a carbohydrate-enriched diet. However, the same study showed that flies fed on a carbohydrate-enriched diet had superior tolerance to cold stress compared to those on a protein-enriched diet. This high tolerance to cold stress could be due to the increased lipid content offered by the carbohydrate (Hoffmann *et al.*, 2001; Mayntz *et al.*, 2005), which influences cellular water uptake and loss (Koštál *et al.*, 2004). Similar results were reported in *D. ananassae* Doleschall (Diptera: Drosophilidae) (Sisodia & Singh, 2012). These studies did not include a control (non-enriched) diet, which makes it impossible to deduce whether the carbohydrate-enriched diet improved heat-stress tolerance when compared to a control. One study found that carbohydrate-rich diet, compared to the non-enriched diet, improved heat stress tolerance of canopy ants, *Azteca chartifex* Forel (Hymenoptera: Formicidae) (Bujan & Kaspari, 2017). In this case it was proposed that carbohydrates increase synthesis of sorbitol, which is used in protein stabilisation during thermal stress (Salvucci, 2000). Further examination of biological interactions between host plant and insect herbivores is needed to better understand the mechanisms by which host plants affect thermal biology of pests.

Thermal history, usually measured as acclimation temperatures in the laboratory setting, is a crucial form of phenotypic plasticity that provides information on how easily an organism can manipulate its physiology to tolerate changing temperatures (Terblanche *et al.*, 2011). Acclimation-dependent responses to temperature stress have been reported in many species. For example, the fruit flies (Diptera: Tephritidae) *Ceratitis capitata* (Wiedemann) and *C. rosa* (Karsh) had improved heat stress tolerance when acclimated to high temperatures (Nyamukondiwa & Terblanche, 2010). In the same study, they were also better at tolerating cold stress when acclimated at cold temperatures. Kleynhans *et al.* (2014b) examined CT_{min} of different geographic populations of stalk borer, *E. saccharina*, and found that the CT_{min} differed significantly among geographic populations (i.e., the geographic lines with higher average local temperature had poor cold tolerance) and that there was a positive correlation between the CT_{min} and the local climate. The same study also demonstrated that there was a trade-off with living in the cooler regions as the longer development time of cold-tolerant geographic lines had higher survival. Acclimation to low temperatures involves genetic, cellular, tissue, organ and even whole animal changes that result in improved performance during future cold stress (and Chown & Terblanche, 2006; see Bowler & Terblanche, 2008).

The plasticity seen in response to cold is less pronounced or absent when measuring CT_{max}. Generally, CT_{max} occurs at high temperatures close to those that cause injury and mortality (see review by Chown & Terblanche, 2006). The taxonomically widespread and conserved mechanisms, like HSPs, that prevent cellular damage operate close to denaturation temperatures (Feder & Hofmann, 1999; Chown & Terblanche, 2006; King & MacRae, 2015). This suggests that more taxa are likely to be affected negatively by a warming climate. For example, variation of CT_{max} is less than that of CT_{min} among different species of South African dung beetles when studied across latitude (Gaston & Chown, 1999). Similarly, in the tsetse fly *Glossina pallidipes* Austen (Diptera: Hippobosoidea), Terblanche *et al.* (2006) found variation in the order of 5 °C on CT_{max} as compared to changes of as high as 10 °C on CT_{min}. These studies sound a warning regarding species occupying environments that have temperatures close to their upper thermal limits, because there is little capacity for acclimation to improve their performance when higher temperatures are experienced. In addition, warming can lead to mismatches between insect developmental schedules and food availability, which may affect insect survival and reproduction (Buckley *et al.*, 2017).

Several studies have shown that thermal tolerance is influenced by insect life stage, and it has been reported to be diminished in advanced life stages. For example, in a study of critical thermal limits of field collected kelp flies, *Paractora dreuxi* Seguy (Diptera: Helcomyzidae), average CT_{max} of larvae was 35.5 °C as opposed to 30.2 °C for the adults (Klok & Chown, 2001). Similarly, CT_{min} of larvae was -5.1 °C and that of adults was -2.7 °C. In widow spiders (Araneae: Theridiidae) *Letrodectus geometricus* Koch and *L. mactans* Fabricius, CT_{max} of early developmental stages was higher than that of the late developmental stages (Barnes *et al.*, 2019). In contrast, a study on FAW found that tolerance to cold stress improves with development (Keosentse *et al.*, 2021). Similarly, a study by Chen *et al.* (2019) on upper lethal temperatures (ULTs) of the corn leaf aphid, *Rhopalosiphum maidis* Fitch (Hemiptera: Aphididae), demonstrated that ULTs increased with the developmental stages. It is intuitive to attribute these differences to variation in the mobility of the life stages as most insects are more mobile in the adult stage. However, these results cannot be generalized to all taxa. For example, in *D. melanogaster*, eggs were the most tolerant development stage to cold stress followed by adults, pupae and larvae (Jensen *et al.*, 2007). This variation is not linear or non-linear but seems to be complex and might be influenced by several interacting factors (Bowler & Terblanche, 2008). Further understanding of the factors involved may require that future studies use transcriptomic and metabolomic approaches.

1.4 Rationale

FAW causes devastating yield losses to maize and other cultivated crops in the grass family. The effects of this pest are even more devastating for smallholder and small-scale farmers who do not have adequate skills and resources to mitigate crop damage. Temperature plays an important role in FAW development and the distribution of this species and therefore deserves special consideration when predicting its likelihood of occurrence (Du Plessis *et al.*, 2020). There is still a gap on how thermal tolerance of this species reacts to thermal history and different diets, i.e., how well FAW populations survive for generations on alternative hosts in the absence of maize. Incorporation of these two confounding factors on studies that investigate thermal stress tolerance is important for the accurate development of FAW distribution and migratory models.

1.5 Aim and objectives

The primary aim of the study was to determine the critical thermal limits of different stages of FAW, and how they are affected by diet and thermal history, and to relate these thermal limits to the abundance of FAW in certain parts of Gauteng and North-West Provinces.

The study objectives were to:

- establish thermal limits of FAW by determining lower (CT_{min}) and upper (CT_{max}) critical thermal limits for activity (Chapter 2),
- determine how CT_{min} and CT_{max} compare between life stages and how it is affected by diet and acclimation temperatures (Chapter 2);
- relate FAW CT_{min} and CT_{max} to the recorded environmental temperatures during the summer crop season (Chapter 3); and
- investigate how thermal limits could affect population dynamics of the FAW (Chapter 3).

CHAPTER 2:

Effects of developmental diet, life stage and thermal acclimation on thermal tolerances of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Abstract

Insect thermal tolerance is a key trait for the survival and distribution of species. As fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) only recently invaded South Africa, it is imperative to understand the factors that lead to its persistence as an economic pest of different crops. This study determined the effects of different larval diets (chickpea-based artificial diet, maize plants or wheat plants), life stages and acclimation temperature on thermal tolerance traits of the FAW. First instars, sixth instars and moths were reared either on chickpea-based artificial diet, maize, or wheat at 25 °C, and were then acclimated for 24 h at either 20, 25 or 30 °C. Larvae and adults were then subjected to a controlled, ramped increase or decrease in temperature (0.1 $°C.min^{-1}$) to determine the critical thermal maximum (CT_{max}) and minimum (CT_{min}), respectively. Both CT_{min} and CT_{max} differed significantly among life stages. CT_{min} of first instars varied only with acclimation temperature, whereas sixth instars were sensitive to both acclimation and diet. Adult CT_{min} was not affected by either acclimation or diet. CT_{max} of first instars was affected by both acclimation and diet, whereas only diet influenced CT_{max} of sixth instars, and adults showed plasticity in response to acclimation temperature. This study demonstrates the complexity of thermal tolerance and emphasises the importance of considering differing thermal stress responses among life stages when predicting the ecological and evolutionary significance of climate change.

2.1 Introduction

Temperature is one of the most important environmental factors affecting insect population dynamics (Chown & Nicolson, 2004; Angilletta Jr & Angilletta, 2009). Temperature influences insect population dynamics by affecting the rate of insect biochemical and physiological processes (reviewed on Bowler & Terblanche, 2008; Villanueva *et al.*, 2011). The ability of an insect to withstand thermal stress is therefore an important trait for its fitness and survival (Loeschcke & Hoffmann, 2007). Due to this, thermal tolerance is an important factor limiting the geographic range of insects.

It is widely accepted that thermal tolerance is a trait not purely determined by genetic architecture, but also influenced by other factors that affect insect fitness (Huey *et al.*, 1992; Nyamukondiwa & Terblanche, 2009; Terblanche *et al.*, 2011). Such confounding factors include thermal history, host nutritional quality, and life stage. For example, acclimation temperature, a laboratory mimic of thermal acclimatisation in the field, is positively correlated with thermal tolerance in both *Ceratitis rosa* (Karsh) (Diptera: Tephritidae) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Nyamukondiwa & Terblanche, 2010). Verhoef *et al.* (2014) also reported a similar result in two *Culicoides* species; lower and upper acclimation temperatures improved lower and upper thermal stress tolerances, respectively. The effects of host plant quality (Villanueva *et al.*, 2011; Kleynhans *et al.*, 2014a; Abarca *et al.*, 2018) and that of life stage (Marais *et al.*, 2009) on thermal tolerance have also been reported but are both understudied and poorly understood. In *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), supplementing the diet with high sugar concentrations led to metabolic imbalances and reduced tolerance to cold stress (Colinet *et al.*, 2013). However, dietary carbohydrates improved high temperature tolerance in canopy ants, *Azteca chartifex* Forel (Hymenoptera: Formicidae) (Bujan & Kaspari, 2017). It is possible that carbohydrates increase body glycogen content which acts both as energy (ATP) and a water source, and that water is used in evaporative cooling (Gibbs *et al.*, 1997). Differences in thermal sensitivity and other important traits needed to respond to variation in environmental conditions may differ between the life stages of holometabolous insects as they usually inhabit different microhabitats (see Kingsolver *et al.*, 2011). The life stages also exhibit differences in their ability to move and avoid potentially stressful conditions, leading to life stage-specific mechanisms to survive.

The fall armyworm ('FAW'), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a polyphagous and migratory pest that is native to the Americas (Johnson, 1987). Until recently, this devastating pest of many agricultural crops has been restricted to its native range in North and Central America, although with occasional interceptions in Europe that never led to population establishment (EUROPHYT, 2018). However, both the rice and maize strains (Pashley, 1986; Pashley, 1988) of FAW are now established outside America (Cock *et al.*, 2017). Populations were reported from North and West African countries in 2016 (Goergen *et al.*, 2016), South Africa in 2017 (Jan Hendrik, 2017), from India in 2018 (Kalleshwaraswamy *et al.*, 2018a), and by January 2019, the pest had been reported from Yemen (FAO, 2019a), Thailand (FAO, 2019b), China (FAO, 2019c) and Australia (FAO, 2020). The relatively short generation time (Castro & Pitre, 1988), high migration potential (Johnson, 1987; Nagoshi & Meagher, 2008; Nagoshi *et al.*, 2018) and polyphagous nature (Barros *et al.*, 2010) of FAW make it a successful invasive species. Thermal limits to the development of FAW were studied by Du Plessis *et al.* (2020), who found its development to be optimal between 26 and 30 °C, and estimated the lower developmental threshold at 13.01 °C. An earlier study by Ali *et al.* (1990) reported a larval thermal development range of 21 to 33 °C. Keosentse *et al.* (2021) reported that the lower thermal tolerance of FAW varied with developmental stage and tended to improve in later developmental stages. The same study also reported a lack of plastic response to cold stress in FAW larvae. Although the thermal limits for development of FAW have been studied, thermal stress tolerance of this species has not been given enough attention and when studied, upper thermal limits and other factors that affect thermal tolerance, such as developmental diet and acclimation, have been overlooked. However, this information on thermal tolerance and factors affecting thermal stress on FAW is important for predicting whether phenotypic plasticity in these traits exist to support its rapid expansion into new regions.

In this chapter, I determine how the thermal tolerance of various life stages of this species differs (a) when exposed to different thermal history (acclimation temperatures of 20, 25, and 30 °C), and (b) when feeding on different diets (cowpea-based artificial diet, maize, and wheat). Analysis of mitochondrial *cytochrome oxidase 1* (*CO1*) and *triose phosphate isomerase* (*Tpi*) genes (unpublished data) showed that our FAW culture contained both rice- and corn-strains as well as an interstrain hybrid. My predictions are that FAW thermal tolerance will be affected by acclimation temperatures and that effects will vary with life stage and diet. I expect adult FAW thermal limits to be less plastic to acclimation temperatures than the immature stages as it can avoid thermal stress behaviourally by means of flight. Most FAW reports in sub-Saharan Africa are on maize and sorghum (Njuguna *et al.*, 2021) and our culture was also developed from insects taken from maize. Based on this, I further predict that individuals developing on maize will perform better than those on wheat. This study has important implications, particularly in instances where the potential distribution of the pest is to be predicted but there are narrow grass (wheat) rather than broad grass (maize) hosts available for the pest.

2.2 Materials and methods

2.2.1 Source and rearing of insects

The FAW culture used in this experiment was started from moths sourced from the ARC-Plant Health and Protection's Roodeplaat campus, Pretoria, South Africa in June 2018. The original specimens for the culture were collected from an infested maize field in the Onderstepoort area (Pretoria, South Africa) during January 2017. Molecular analysis done on a mitochondrial genetic marker, *CO1*, showed that the FAW culture used for this study contained both rice- and corn-strains and the *Tpi* gene marker confirmed that both strains are present as well as an interstrain hybrid (unpublished data).

Rearing of the FAW was done in the insect laboratories of the Department of Zoology and Entomology at the University of Pretoria, Hatfield, South Africa. The culture was maintained in square glass cages (30 cm × 30 cm × 30 cm) kept in incubators under a 14L: 10D photoperiod at a temperature of 25 ± 2 °C and 75 ± 10% relative humidity. The rearing cages had muslin cloth covering half the surface of two opposing sides in order to facilitate ventilation. Adult mated females were provided with wheat plants on which to lay eggs in glass cages containing pelleted vermiculite (approximately 1.5 cm depth) for moisture regulation. These oviposition cages were monitored daily to check if the eggs had hatched. The culture was maintained by transferring two-day-old larvae from wheat plants to glass cages (30 cm × 30 cm × 30 cm) with of a chickpea-based artificial diet (2 cm × 2 cm × 2 cm cubes) in Petri dishes and reared through to the adult stage. Larvae pupated in the vermiculite at the bottom of the cage. Emerging adults were supplied with honey solution as food and a wheat plant for oviposition and the process was repeated. For the experiments, neonates on hatching were provided with different diets (maize plants, wheat plants or a chickpea-based artificial diet) in different glass cages with 1.5 cm depth pelleted vermiculite on the floor and moistened cotton wool in a Petri dish to maintain a high humidity in cages with artificial diet. The humidity in cages with plants was sufficiently high and did not need any moistened cotton wool. The maize diet consisted of yellow grain maize plants, *Zea mays* L. (hybrid KKS 4520), that were not treated with any pesticides nor genetically modified. The wheat diet consisted of wheat plants, *Triticum aestivum* L. var. Duzi. For both, plants were grown from seeds in potting soil mixture consisting of composted bark, compost and topsoil (3:1:1) in plant pots (700 mL) at 24 °C. Plants were watered three times a week. Maize seedlings at BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) growth stage 13 and wheat seedlings at BBCH growth stage 12 (Meier, 2018) were used for oviposition by the moths or as larval diet . To ensure that larvae had enough food and to prevent cannibalism, the maize and wheat plants were checked daily to determine when plants needed to be replaced. Maize was selected as a preferred diet as the insect culture was developed from insects taken from maize fields. Wheat was selected as a non-preferred host that farmers plant in South Africa and could therefore be available and be exposed to FAW damage.

The artificial diet was prepared following a modified diet created for *Helicoverpa armigera* (Lepidoptera: Noctuidae) by Kfir (1994). The diet was prepared by dissolving bacteriological agar (2 g) in 73 ml warm distilled water with 52 g of chickpea flour, yeast (7 g), ascorbic acid (700 mg), sorbic acid (300 mg), phosphoric acid (480 mg), and nipagin M (methyl-4-hydroxybenzoane) (480 mg). The artificial diet was prepared in a sterile laminar flow cabinet with autoclaved and UV-treated equipment. The diet was cut into cubes (2 cm × 2 cm × 2 cm) and placed on a UV-sterilized Petri dish bottom which was then placed inside the cages. The diet was stored at 5 ± 2 °C and was used within three days of preparation.

2.2.2 Acclimation procedure

One day old 1st instar larvae and 6th instar larvae used for experiments were first acclimated at either 20, 25 or 30 °C for 24 h on their developmental diets. The moths obtained from each of the three diets were also acclimated at either 20, 25 or 30 °C for 24 h prior to experiments. The insects acclimated at 25 °C were considered a control group for ageing and acclimation effects (Nyamukondiwa & Terblanche, 2010). These life stages were selected because they permit measurement of the effect of diet (all have varying feeding capabilities), life stages (all different life stages and physiologies) and all occupy relatively difference micro-environment which can influence their thermal tolerance (Chown & Terblanche, 2006). The acclimation of insects was done at a similar time (12h00 ± 20 min) so that they had completed the 24 h mark at a similar time on days of the experiment. An acclimation duration of 24 h is often sufficient to acclimate insects to novel constant temperatures (Weldon *et al.*, 2011). Individuals from each acclimated cohort were individually loaded into 10 ml glass test tubes for the experiments below. A total of ten insects per life stage, acclimation temperature and diet were acclimated.

2.2.3 Critical thermal limits (CTLs)

These tests were used to determine the temperature at which an individual insect lost its coordinated muscle function, thereby incapable of performing basic physiological activities and behaviours. This behavioural endpoint was marked by the failure of the insect to respond to a gentle stimulation by a fine paintbrush. An individual insect from each acclimation temperature was placed in a labelled glass test tube (10 ml) and sealed at the mouth with a cotton wool ball. The test tubes were then placed into a test tube rack and submerged into a programmable water bath (CC-K25, Huber Kältemaschinenbau, Offenburg, Germany). As noted above, this was done until a total of ten insects per diet, life stage and acclimation temperature had been tested to determine CT_{min} and CT_{max}. One test tube contained a type T (copper/constantan) thermocouple connected to a temperature data logger (0.05 °C accuracy; TC-08, Pico Technology, St. Neots, UK) to measure the temperature in the test tubes. The temperature data logger was connected to a personal computer to record the temperature change during tests. Both CT_{min} and CT_{max} tests were started at a setpoint temperature of 25 °C and the temperatures were ramped down (CT_{min}) or ramped up (CT_{max}) at a rate of 0.1 °C/min. This rate of temperature change is similar to those measured in the field, and aligns with Chown *et al.* (2009) who reported that measures of critical thermal limits vary with methodological context. The temperature at which each insect lost its coordinated muscle function (**Figure 2.1**) was recorded as its CT_{min}/CT_{max}. Each individual insect was used only once for each test.

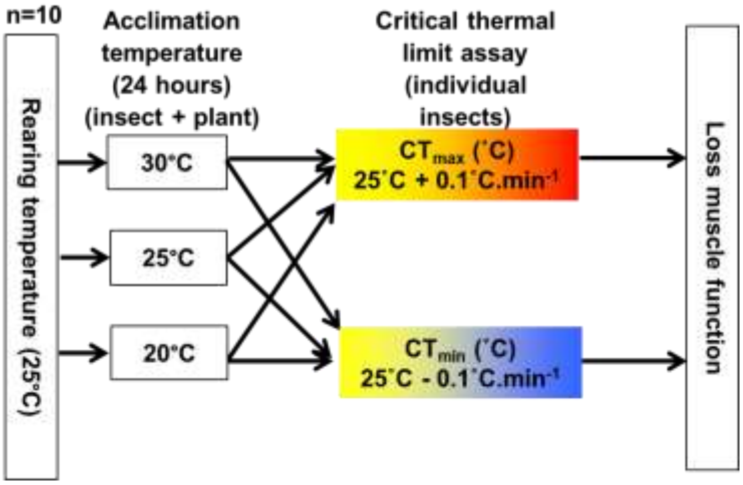

Figure 2.1: Diagram representing experimental design for determining CT_{max} and CT_{min} of *Spodoptera frugiperda*. (see Materials and Methods for full description). Adapted from Nyamukondiwa & Terblanche (2010).

2.3 Data analyses

All statistical analyses were carried out using R software (version 3.5.3). The data were first subjected to Shapiro-Wilk tests to check for normality. The data were not normally distributed so all data analyses were performed using generalized linear models (GLZ) with gaussian family and identity link to assess the effects of life stage, diet and acclimation temperature on CT_{min} and CT_{max}. After running a full model separate models to investigate higher order interaction terms from the full model were carried out. Where there was a significant effect, *post hoc* Tukey's multiple comparisons test was used to establish which treatments differed from each other.

2.4 Results

Critical thermal minima (CT_{min}) differed significantly between the life stages, with sixth instar larvae having the highest CT_{min} overall (i.e., least tolerant to cold stress) regardless of diet and acclimation (**Table 2.1**, **Figure 2.2**). Sixth instar larvae were significantly more susceptible to cold stress than first instars (mean difference of 5.81 ± 0.72 °C) and moths (mean difference of 5.24 ± 0.72 °C). The average CT_{min} for first instars, sixth instars and moths were 5.1, 9.7 and 3.7 °C, respectively. No difference was observed between CT_{min} of first instars and moths (mean difference of 0.57 ± 0.72). Variation in values of CT_{min} was greater in first instars than observed in either sixth instar larvae or moths (**Figure 2.2**). The effects of acclimation and diet on CT_{min} differed among life stages, with a significant second-order interaction between life stage, diet and acclimation in the full model (**Table 2.1**). The results of separate GLZs for each life stage reveal that CT_{min} is sensitive to both diet and acclimation but the sensitivity is life stage related (**Table 2.1**).

Table 2.1: Effects of life stage, diet, acclimation and their interactions on CT_{min} of first instar larvae, sixth instar larvae and moths of *Spodoptera frugiperda*.

Factors	***d.f***	X^2	***p***
CT_{min}			
Life Stage	2	78.0720	**<0.0001*** **
Diet	2	0.2100	0.9001
Acclimation	2	1.0620	0.5880
Life stage *x* Diet	4	3.6180	0.4601
Life stage *x* Acclimation	4	30.3780	**<0.0001*** **
Diet *x* Acclimation	4	2.2190	0.6955
Life stage *x* Diet x Acclimation	8	19.3380	**0.0131* **
CT_{min} First instar larvae			
Diet	2	1.5368	0.4638
Acclimation	2	18.0364	**0.0001*** **
Diet *x* Acclimation	4	7.8833	0.0960
CT_{min} Sixth instar larvae			
Diet	2	8.3509	**0.0154* **
Acclimation	2	17.1801	**0.0002*** **
Diet *x* Acclimation	4	1.5562	0.8166
CT_{min} Moths			
Diet	2	0.7700	0.6805
Acclimation	2	3.8866	0.1432
Diet *x* Acclimation	4	8.1212	0.0872

Statistically significant values are indicated in bold and asterisk/s.

For example, in first instar larvae only acclimation influenced CT_{min} (**Figure 2.1A**), whereas in sixth instar larvae, CT_{min} was sensitive to both diet and acclimation (**Figure 2.1B**), and neither of those factors influenced moths (**Figure 2.1C**). There was no significant difference in CT_{min} of first instar larvae acclimated at 20 and 25 °C, but these larvae had significantly lower average CT_{min} than first instar larvae acclimated at 30 °C. The sixth instar larvae feeding on maize plants were significantly more tolerant to cold stress compared to those on wheat, however there was no significant difference between individuals on either maize and artificial diet, or wheat and artificial diet (**Figure 2.1B**). Sixth instar larvae acclimated at the coolest temperature (20 °C) had lower CT_{min} than those acclimated to the hottest temperature (30 °C), but neither of these acclimation temperatures differed from the control (25 °C). Interaction terms did not have any significant on CT_{min} within each life stage.

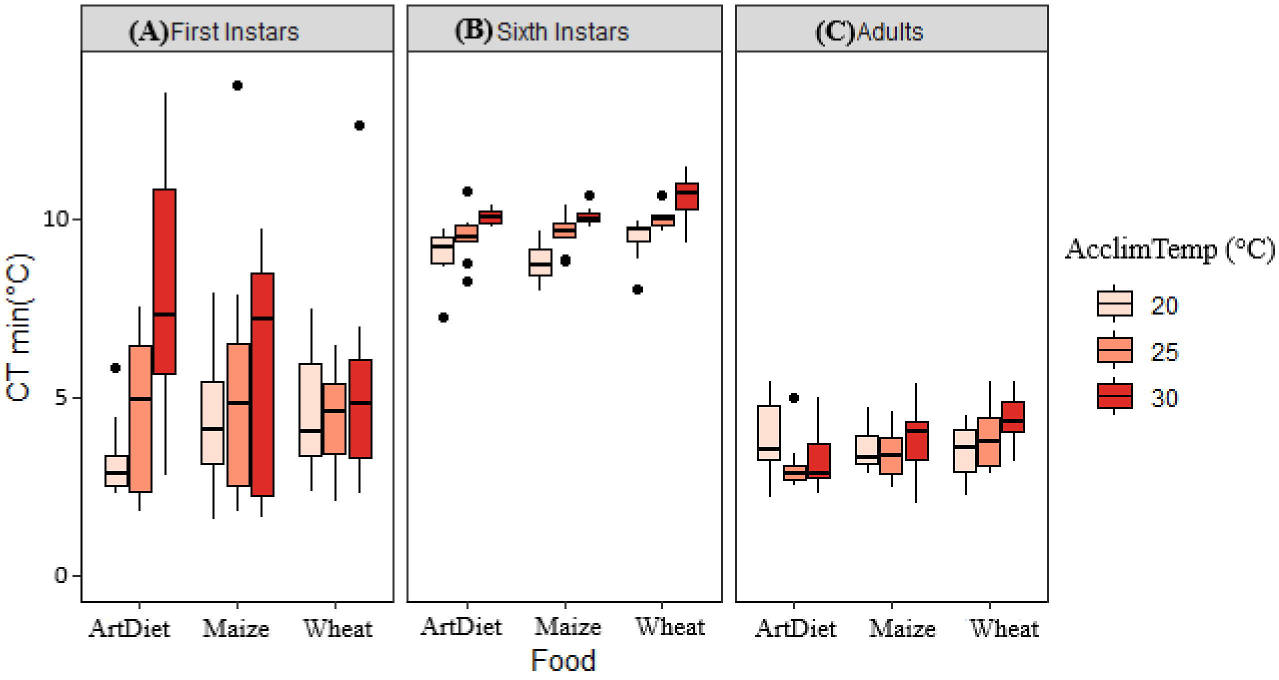

Figure 2.2: Average lowest temperature (CT_{min}) at which there was an onset of muscle failure in (A) first instar larvae, (B) sixth instar larvae and (C) moths of *Spodoptera frugiperda* developing on different diets and acclimated at different temperatures [AcclimTemp (°C)]. The figures show minimum (lower whiskers), median (black line crossing inside the box), first quartile (lower shoulder of the box), third quartile (upper shoulder of the box), interquartile range (first to thirst quartile) and maximum (upper whiskers). Outliers are shown by black circles.

All main effects (life stage, diet and acclimation) affected tolerance to heat stress (CT_{max}) of FAW (**Table 2.2**). Life stage had a particularly pronounced effect with moths (44.2 °C) being significantly less tolerant to heat stress than both first (48.0 °C) and sixth instars (46.5 °C). The mean difference in CT_{max} between first instars and moths was 3.90 ± 0.36 °C, and between sixth instars and moths was 3.35 ± 0.36 °C. No significant difference on heat stress between sixth- and first-instars (mean difference of 0.55 ± 0.36 °C). There was a complex, significant relationship between all three effects in the full model (**Table 2.2** & **Figure 2.3**). Separate GLZs for each life stage revealed that the CT_{max} of first instar larvae was significantly affected by diet and marginally affected by acclimation, sixth instar larvae were only affected significantly by diet, and the moths were only affected significantly by acclimation (**Table 2.2**).

Table 2.2: Effects of life stage, diet, acclimation and their interactions on CT_{max} of first instar larvae, sixth instar larvae and moths of *Spodoptera frugiperda*.

Factors	***d.f***	***X^2***	***P***
CT_{max}			
Life stage	2	76.5950	**<0.0001*** **
Diet	2	7.1660	**0.0278***
Acclimation	2	16.3130	**0.0003*** **
Life stage *x* Diet	4	14.5990	**0.0056*** **
Life stage *x* Acclimation	4	12.7640	**0.0125***
Diet *x* Acclimation	4	5.7970	0.2148
Life stage *x* Diet x Acclimation	8	8.4100	0.3945
CT_{max} First instar larvae			
Diet	2	16.5706	**0.0003*** **
Acclimation	2	6.7261	**0.0346***
Diet *x* Acclimation	4	6.2920	0.1784
CT_{max} Sixth instar larvae			
Diet	2	19.5451	**<0.0001*** **
Acclimation	2	1.3222	0.5163
Diet *x* Acclimation	4	9.2340	0.0555
CT_{max} Adults			
Diet	2	4.0246	0.1337
Acclimation	2	8.5686	**0.0138***
Diet *x* Acclimation	4	3.1338	0.5357

Statistically significant values are indicated in bold and asterisk/s.

There were no significant interaction effects revealed within life stages in the separate GLZs. First instar larvae developing on artificial diet were better at tolerating heat stress than those developing on wheat. However, larvae that developed on artificial diet were not significantly different from those on maize, and there was no significant difference between those that developed on maize and those on wheat (**Figure 2.3A**). First instar larvae acclimated at 20 °C had lower CT_{max} compared to those acclimated at 30 °C, but there was no difference between first instars acclimated at 20 and 25 °C, and those acclimated at 25 and 30 °C (**Figure 2.3A**). For sixth instar larvae, those fed maize had the poorest tolerance to heat stress compared to both artificial diet and wheat, but there was no difference between artificial diet and wheat (**Figure 2.3B**). The moths exhibited larger variation in CT_{max} than other life stages, particularly those that developed on wheat and acclimated at 20 and 25 °C (**Figure 2.3C**). Moths acclimated at 20 °C had lower heat tolerance than those acclimated at 30 °C across diets but did not differ significantly with those acclimated at 25 °C. No significant difference was observed between adults acclimated at 25 °C and 30 °C.

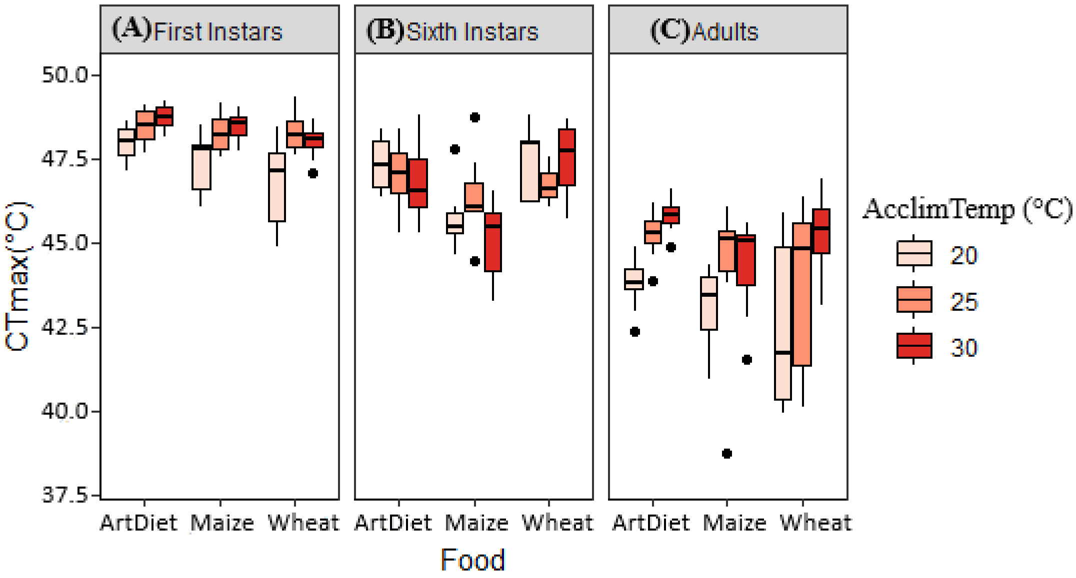

Figure 2.3: Average highest temperature (CT$_{max}$) at which there was an onset of muscle failure in (A) first instar larvae, (B) sixth instar larvae and (C) moths of *Spodoptera frugiperda* developing on different diets and acclimated at different temperatures [AcclimTemp (°C)]. The figures show minimum (lower whiskers), median (black line crossing inside the box), first quartile (lower shoulder of the box), third quartile (upper shoulder of the box), interquartile range (first to thirst quartile) and maximum (upper whiskers). Outliers are shown by black circles.

2.5 Discussion

This study demonstrated that the thermal tolerance of *S. frugiperda* is largely influenced by the life stage, but also by acclimation temperature and diet in a rather complex manner. Life stage as a factor had a large effect on both CT$_{min}$ and CT$_{max}$. Sixth instar larvae were the most sensitive life stage to cold stress followed by first instar larvae with the moths being the most tolerant. The opposite is true for CT$_{max}$, adults were the most sensitive life stage followed by the sixth instar larvae and the first instars were the most tolerant of high temperate stress.

Life-stage related effects on thermal tolerance have been reported for FAW by Keosentse *et al.* (2021). These authors investigated cold stress of four larval instars (3rd, 4th, 5th and 6th) and moths. They reported that adults had higher tolerance to cold stress compared to all the tested larval instars. These results that are in keeping with the current study. However, Keosentse *et al.* (2021) also reported an increase in cold tolerance in later instars. The results contrast with the current study. Another study on a moth in the genus *Colias* showed that larvae had much lower temperatures for maximal rates of feeding and growth compared to the adults (Kingsolver *et al.*, 2011). Other studies on species from different insect orders have also shown that cold tolerance decreases with life stage. For example, Klok & Chown (2001) found that adult *Paractora dreuxi* Seguy (Diptera: Helcomyzidae) had significantly higher CT_{min} (lower tolerance to cold stress) than the larval stage. They also showed that adults had lower CT_{max} (lower tolerance to heat stress) than the larval stages. In mealworms, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), the beetles were more tolerant to high temperature stress than either larvae or pupae (Vorhees & Bradley, 2012). Variation in thermal tolerance between life stages can be attributed to multiple factors including the cost of the mechanism to avoid extreme temperature, morphological and physiological distinctness of each life stage, and the microhabitat each life stage inhabits (reviewed by Bowler & Terblanche, 2008; Kingsolver *et al.*, 2011). In our study, high tolerance to cold stress exhibited by adult *S. frugiperda* could be because the moths have evolved mechanism/s to tolerate cold stress during migration from areas experiencing cold winter conditions to milder climates to start a new population (see Johnson, 1987; Nagoshi *et al.*, 2012). It may also be that the adults are mainly nocturnal and so have evolved to tolerate cooler conditions experienced at night. Smith (1957) proposed that two types of acclimation exists in adult insects. First, is physiological acclimation, attained during the adult stage and attributed to the temperature regimes experienced during the adult stage. Physiological acclimation is not innate and lasts only for as long as the new thermal conditions persist (Chown & Terblanche, 2006). It is unlikely that adult *S. frugiperda* use this mechanism for CT_{min} as the adults showed no plasticity in response to acclimation temperatures. Secondly, there is a more fixed form of acclimation, which is influenced by the temperatures experienced during the pre-adult stages and is termed developmental acclimation. This form of acclimation was beyond the scope of this study, but it needs to be investigated in the future, especially for migratory insects like the FAW as the adults of the two strains may inhabit geographically different habitats in the pre-adult stages.

Several studies attributed the well-observed trend of decline of heat tolerance in later stages to increased mobility across ontogeny (Bowler & Terblanche, 2008; Marais *et al.*, 2009; Vorhees & Bradley, 2012). This allows the winged adults to behaviourally compensate for stressful temperatures by escaping to avoid heat stress with ease. For CT_{max} in this study, the moths were the most sensitive to heat stress perhaps because they avoid heat stress by flying to less stressful environments. However, adult FAW showed plasticity in response to acclimation as the CT_{max} of adults acclimated at 30 °C was 2.1 °C higher than those acclimated at 20 °C. This indicates that adults use different stress responses to heat and cold stress (e.g., behavioural for CT_{min} and both physiological and behavioural for CT_{max}). The high tolerance of first instar larvae to heat stress could be attributed to behavioural characteristics of this life stage. The first instar larvae feed on the surface of the leaves where temperatures are high (see Kingsolver *et al.*, 2011). First instars may therefore have evolved physiological mechanisms to mitigate heat damage better than sixth instars, which burrow into plant parts, and adults. It is also important for first instars to acclimatise to variation in temperatures as the leaf may experience larger fluctuations in temperature within and among days than other plant parts. This could explain the observed plasticity in thermal tolerance of first instars; acclimation at 30 °C led to an average CT_{min} of 6.4 °C compared to an average CT_{min} of 4.1 °C for 20 °C acclimation and 4.8 °C for 25 °C acclimation. Similar plasticity in thermal tolerance was observed in CT_{max} of first instars; acclimation at 20, 25 and 30 °C led to an average CT_{max} of 47.1, 48.3 and 48.4 °C, respectively. Sixth instars normally burrow into stalks or cobs which have a high moisture content. Because water has high heat capacity, the temperatures in these plant structures seldom reach temperatures equal to that of the leaf surfaces and therefore the late instars are usually protected from high temperatures (discussed by Kingsolver *et al.*, 2011). This could also explain the lack of significant thermal acclimation in heat tolerance in sixth instars observed in this study. Moreover, the sixth instars are close to metamorphosis, a process that is energy demanding and energy may be invested in metamorphosis rather than on acclimating to varying temperatures. However, this was not the case with cold stress. Although sixth instar larvae feed in a somewhat protected part of the plant, they showed a general trend of beneficial acclimation (Deere & Chown, 2006) to cold stress because larvae acclimated at low temperatures were recorded to have lower CT_{min} values. It may be that sixth instar larvae use different stress responses for heat and cold stress, i.e., physiological for CT_{min} and behavioural for CT_{max} (e.g., hiding in plant pockets and feeding tunnels for cold stress).

Thermal stress affects insect fitness by negatively impacting cellular metabolism, which subsequently leads to increased accumulation of harmful reactive oxygen species (Farahani *et al.*, 2020). Consequently, insects have evolved stress response mechanisms such as heat shock proteins (HSPs), to counteract the damage caused by thermal stress (Farahani *et al.*, 2020). HSPs are stress-induced proteins that function as chaperones, promoting correct folding of proteins and keep them stable to remain functional (Yi *et al.*, 2018). Diet influences these mechanisms by functioning as a source of nutrients needed in biosynthesis of the molecules needed for these stress response mechanisms. Carbohydrates and proteins, for examples, have been reported to affect thermal stress tolerance in some species. In *D. melanogaster*, adults feeding on a carbohydrate-enriched diet were more tolerant to cold stress compared to those on a protein-enriched diet (Andersen *et al.*, 2010). In the same study, *D. melanogaster* fed with the protein-enriched diet had improved heat stress tolerance compared to those on the carbohydrate-enriched diet. However, carbohydrates improved CT_{max} in canopy ants, *A. chartifex* (Bujan & Kaspari, 2017). It is therefore possible that the observed heat stress tolerance (high CT_{max}) in the first instar FAW larvae feeding on artificial diet in the current study is due to high protein (up to 35%) and carbohydrate (up to 65%) contents of the cowpea flour (Prinyawiwatkul *et al.*, 1996), which was the main ingredient of the diet. However, this assumption does not apply to the sixth instar larvae. In the sixth instar larvae, those developing on maize were the poorest in tolerating heat stress, even though maize has higher protein (~12%) and carbohydrate (~21%) (Cetinkaya *et al.*, 2020) content than wheat protein (~6%) and carbohydrate (~5.4%) (Lorenzo *et al.*, 2015). It may be that high heat stress tolerance in first instars is further aided by the emerging neonates consuming the protein-rich egg shells (Sparks, 1979; Capinera, 2017), thereby adding protein content to the diet of the early instars. Thus, the protein-rich eggshell improves the fitness in first instars and that benefit is lost as the larvae grow through to later instars. The large variation in CT_{min} of first instar larvae (range of 12.2 °C, compared to 4.2 °C for sixth instars, and 3.5 °C for adults) may be because neonates consume different quantities of eggshells (Luginbill, 1928); those that consumed more would be more fit than those that consumed less. In addition, this variation in thermal tolerance in first instars could also be due to them having spent less time on the diet. This could explain why diet did not influence the CT_{min} of first instars but did influence the CT_{min} of sixth instars. The effect of diet on thermal tolerance of the immature life stages and not in adults further highlights the importance of inclusion of life stage and diet in thermal physiology studies. The lack of diet effects in adults can be attributed to the fact that only the larval stages feed on foliage whereas adults feed on nectar (sugar solution in this study). It can also be deduced from this study that the diet used during the larval stages does not influence the thermal tolerance of the adult stage.

2.6 Conclusion

The current study highlights that the evaluation of thermal tolerance should be done across life stages and researchers should be critical of using thermal biology data from one life stage and assume its relevance for all individuals and life stages. Even though acclimation did not affect CTLs of all life stages, there was a general pattern of beneficial acclimation in most cohorts. The mechanism for this thermal plasticity is not fully known but points to differences among life stages and, to some extent, capacity to respond to cold and hot conditions. The study further highlights that the diet is an important factor that can confound thermal biology studies if not considered, even for a polyphagous pest like FAW. Such information is useful for understanding ecological and evolutionary responses of FAW to varying and extreme climatic conditions, and how the pest may perform in different regions or times of the year when the host plants cultivated differ. The current study demonstrates that phenotypic plasticity of thermal tolerance in FAW varies among life stages with regard to acclimation temperature and developmental diet, and that the variation is sometimes complex and challenging to interpret.

CHAPTER 3:

Evaluating fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) prevalence using thermal limits of three life stages in South African maize fields

Abstract

Maize is a vital crop in South Africa, serving both as feed and a staple food. It is the most produced crop in the country and grown in diverse environments. The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a pest of grasses, especially maize. It has recently invaded Africa, Asia and Oceania. The life cycle of FAW is dependent on environmental temperatures as it lacks the ability to enter diapause. In this study, I determined the infestation of FAW in maize fields near Pretoria, South Africa, and related temperatures recorded near the fields to the critical thermal minima (CT_{min}) and maxima (CT_{max}) of first instars, sixth instars and adults of FAW. One maize field at Loskop Prison farm had high levels of infestation of 88% and 67% during two sampling occasions. No FAW infestation was observed in the maize fields at Baviaanspoort and Zonderwater Prison farms. There was no instance where field temperatures were higher than the CT_{max} of FAW, regardless of the life stage and season. During winter months, however, temperatures did go below the CT_{min} of the life stages. At Loskop Prison farm, there were up to 21 occasions with a duration of up to 5.5 hours where temperatures were below the CT_{min} of sixth instar larvae (9.1 °C). In contrast, the temperature fell below the CT_{min} of first instars (4.1 °C) and adults (3.6 °C) only once for 1 hour. Near the Baviaanspoort and Zonderwater Prison farms, temperatures were lower and went below the CT_{min} of each stage more frequently and for longer periods. However, lack of FAW infestation at these sites were more likely due to the poor condition of plants and the Bt maize cultivar that had been planted, respectively. These results may be incorporated into models for population dynamics and be used in pest monitoring, forecasting, early warming and planning for integrated pest management of FAW.

3.1 Introduction

Maize (*Zea mays* L.) is the most produced grain crop and a staple food for a large proportion of the South African population. The crop is produced in all provinces of the country in diverse environments (Du Plessis, 2003). Maize is primarily grown for human consumption, animal feed, and some is processed (Burger & Rheeder, 2017). This contributes to food security, but also contributes to employment and the economy via foreign income from exports. In the 2019/2020 season, the maize industry produced an estimated total of 15.2 million tons, of which 11 million tons were consumed locally, 1.6 million tons exported, and the rest was used as livestock feed and in processing (DALRRD, 2020). However, maize yield in South Africa is not optimal due to the presence of pests. This is in part due to maize generally being planted in warm areas with average temperatures of above 23 °C (Du Plessis, 2003). These high temperatures favour pest development, so maize suffers significant pest damage.

The fall armyworm ('FAW'), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a polyphagous species native to tropical and sub-tropical regions of the Americas. It is regarded as a major pest of maize and other grasses. Larval stages are voracious feeders and are responsible for host damage. The young larvae feed on the leaves and cause a window effect whereas the later instar larvae can tunnel into the maize ear and stem. Adult FAW feed on nectar. FAW lacks the ability to diapause and does not survive harsh winter temperatures, rather using migration as a cold-temperature survival strategy (Nagoshi & Meagher, 2008). In North America, for example, FAW overwinters in south Florida and Texas and only migrates back to reinvade most of the central United States and Canada when temperatures are conducive to survival (Luginbill, 1928; Sparks, 1979; Nagoshi & Meagher, 2008). The inability to diapause means FAW survival is highly dependent on prevailing temperatures. Therefore, it is expected that FAW abundance will be greatly reduced when environmental temperatures pass critical thermal thresholds for activity.

The association of environmental temperature and population fluctuations plays a considerable role in potential insect pest distribution and management (Nyamukondiwa *et al.*, 2013). Several studies have successfully linked laboratory-derived thermal limits to field temperatures (Nyamukondiwa *et al.*, 2010; Nyamukondiwa & Terblanche, 2010; Nyamukondiwa *et al.*, 2013; Verhoef *et al.*, 2014). One of the main reasons is that insects are ectothermic organisms, so their fitness and survival are reliant on environmental temperatures, making a relationship between thermal limits and environmental temperatures a good predictor of potential establishment of an insect population in an area (Kelley, 2014). This then means environmental temperatures that lie outside the thermal limits of an insect can be a barrier for insect establishment and ultimately, invasion (Nyamukondiwa *et al.*, 2010; Verhoef *et al.*, 2014; Timilsena *et al.*, 2021). However, not enough studies have looked at the thermal limits of different life stages of FAW and linked this with location-specific weather data. This is important as there are data showing that the thermal biology of ectotherms differs among life stages (Bowler & Terblanche, 2008; Marais *et al.*, 2009; Vorhees & Bradley, 2012; Mutamiswa *et al.*, 2019).

This chapter investigates if FAW critical thermal limits to activity are within the temperatures experienced in maize fields near Pretoria and Brits, South Africa. I did this by performing FAW surveys of maize fields at three farms for which temperature records were also obtained and related these results to the critical thermal limits of FAW reported in Chapter 2. I predicted that FAW critical thermal limits to activity will be within the temperatures experienced in the maize fields when close to harvest. However, during the cooler months of the year, after which maize has been planted, I predicted that field temperatures will fall below the lower critical thermal limits. These predictions are mainly guided by results from the CLIMAX model that predicted that FAW will not survive winter season of the interior part of South Africa (Timilsena *et al.*, 2021). They also derive from the observation that infestation has mainly been reported on maize fields (Njuguna *et al.*, 2021), and that maize is mainly planted during the warm (late spring/early summer) season (DAFF, 2018) in South Africa.

3.2 Materials and methods

Field sites

Three maize farms, Loskop Prison farm (25° 35' 36.78"S, 27° 41' 55.1934"E) (1128 m above sea level) located in North West province, and Baviaanspoort (25°40'12.1"S, 28°20'58.6"E) and Zonderwater Prison farms (25° 41' 4.257"S, 28° 33' 6.5874"E), both located at 1276 m above sea level in Gauteng province, were visited for sampling of *Spodoptera* species **(Figure 3.1)**. All three farms were in the Highveld region of South Africa, which is characterised by an average annual rainfall of 400 to 900 mm (Low & Rebelo, 1996; Dyson, 2009). Mean maximum and minimum temperatures range from 21 to 24 °C and 5 to 6 °C, respectively (Low & Rebelo, 1996).

Loskop Prison farm was surrounded by natural vegetation, and several cultivated tomato and vegetable fields were located a few kilometres from the sampled field. The farm was infested with weeds, including within the maize field. The maize crops in the field looked stunted and damage from various insect pests was noted, as well as symptoms of loose smut and maize streak diseases. Zonderwater Prison farm was surrounded by small plots of vegetables and no other farm was visible within a radius of several kilometres. The maize looked healthy with no visible disease symptoms or insect damage. The planted maize cultivar was genetically modified (Bt maize). At the Baviaanspoort Prison farm, the field was surrounded by a mountainous landscape with natural vegetation and serious weed infestation was visible. Although the maize was dry, it looked stunted and had no visible healthy cobs, presumably from lack of proper care (weeding, watering and fertilising the soil).

Although three sites were visited, sampling could only be done in the Loskop Prison farm for the reasons mentioned above. The maize plants at Baviaanspoort Prison farm were too dry, and there was no evidence of insect damage in maize plants at this and the Zonderwater Prison farm. The farms were visited in Autumn (March-April) and maize was at harvest stage.

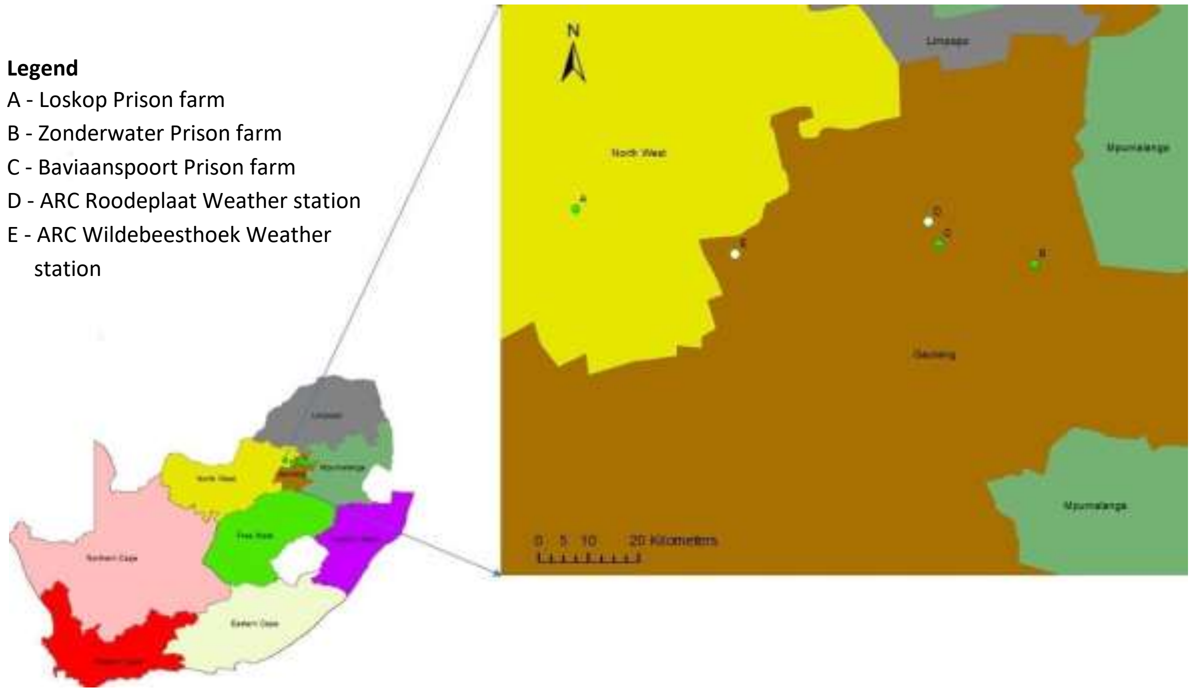

Figure 3.1: Prison farms (green circles [A, B and C]) visited and weather station sites (white circles [D and E]) where climate data were collected over 14 months (March 2017 to April 2018). Wildebeesthoek

Weather station (E) is 33 km away from Loskop Prison farm (A). Roodeplaat Weather station (D) is 2.6 and 20.6 km from Baviaanspoort Prison farm (C) and Zonderwaters Prison farm (B), respectively.

Collection of samples

Sample 1 (33 maize stalks) and Sample 2 (42 maize stalks) were collected on 31 March 2018 and 3 April 2018, respectively from the Loskop Prison farm. All collected maize stalks had cobs on them as it was close to harvesting. Maize stalks were collected by breaking them off close to the ground. All the above ground parts of the stalk were left intact and stalks were transferred into large sampling sleeves before being transported by road to the aphid sorting laboratory of the Department of Zoology and Entomology, University of Pretoria, South Africa. Plant sampling was done so that stalks were collected evenly from the field. This was achieved by allowing three people to spread out in the field until all sampling sleeves were full. Each maize stalk was individually investigated for larvae of *S. frugiperda*. The number of larvae per stalk were counted and recorded.

Relation of critical thermal limits to field conditions

To assess the role of stressful temperatures on the presence of *S. frugiperda* at the field sites, the frequency and duration of temperatures more stressful than measured critical thermal limits (Chapter 2) were determined. Weather data from the weather stations located closest to the three prison farms were supplied by the South African Agricultural Research Council - Soil, Climate and Water (ARC-SCW), Pretoria, South Africa. The weather station closest to Loskop Prison farm (Wildebeesthoek Weather station) was 33km away. Another weather station (Roodeplaat Weather station) supplied weather data for both Baviaanspoort and Zonderwater Prison farms and was 2.6 and 20.6 km away, respectively. The weather data were for the period 01 March 2017 to 30 April 2018.

The frequency and duration of temperatures lower than the critical thermal minimum (CT_{min}) or higher than the critical thermal maximum (CT_{max}) were calculated using a macro developed for Microsoft Excel (Sinclair, 2001). The mean critical thermal minimum (CT_{min}) and maximum (CT_{max}) for first instar, sixth instar and adult *S. frugiperda* were obtained as described in Chapter 2. Only data from individuals that developed on maize in that study were used here to align with the host from which samples were collected. Insects used for CT_{min} and CT_{max} were reared at 25 °C, but their critical thermal limits when acclimated to 20 °C were selected because it was closest to the average temperature of 18.72 °C and 19.87 °C recorded near the Loskop Prison farm and Baviaanspoort Prison/Zonderwaters Prison farms, respectively.

3.3 Results

Infestation incidence

Sample 1 from Loskop Prison farm had a total of 33 maize stalks; 29 were infested with FAW larvae and no larvae were recovered from the other 4 stalks although they had insect damage (87.9% infestation incidence). The infested stalks had 100 larvae in total; 98 were alive and 2 dead. Most of the recovered larvae were in the late instar stage (81 late instar larvae compared to 19 early instar larvae). The stalk with the highest infestation had six larvae.

A total of 42 maize stalks were obtained for Sample 2 from Loskop Prison farm. The infestation incidence of the collected stalks was 66.7% (28 infested and 14 uninfested). A total of 52 larvae were collected from all stalks of which 49 were alive and 3 were dead. All recovered larvae from Sample 2 were at late instar stages. The stalk with the highest infestation rate had 5 larvae.

Relation of critical thermal limits to field conditions

The frequency and duration of field temperatures that were stressful for *S. frugiperda* depended on the life stage that may have been affected. First instars, sixth instars and adults reared on maize had a CT_{min} of 4.1, 9.1, and 3.6 °C, respectively (Chapter 2). For Loskop Prison farm, there were months where field temperatures were below the average CT_{min} for *S. frugiperda*. The frequency of these events were was more pronounced for sixth instars, where a frequency of up to 21 occasions and duration of up to 5.5 hours were recorded over a period of nine months. For first instars, temperatures lower than CT_{min} occurred twice, one hour each and in different months. Adults experienced temperatures lower than their CT_{min} only once for a duration of one hour (**Figure 3.2**). Temperatures lower than the CT_{min} mainly occurred between early May to late August for sixth instars, mid-July and mid-August for first instars and only in late August for the adults. Field temperatures did not go above CT_{max}, regardless of the life stage.

A similar pattern was observed for Baviaanspoort and Zonderwaters Prison farms; the frequency of field temperatures lower than CT_{min} was more pronounced for sixth instars compared to the other life stages. Field temperatures lower than the CT_{min} of sixth instars occurred at a frequency of up to 36 occasions and duration of up to 9.3 hours. For first instars, field temperatures lower than CT_{min} occurred at a frequency of up to 19 occasions and duration of up to 4 hours. Adults experienced field temperatures lower than their CT_{min} for a duration of 4.3 hours at a frequency of up to 12 occasions (**Figure 3.3**). Field temperatures frequently passed below the CT_{min} of sixth instar larvae between mid-April to late August whereas the CT_{min} of first instars and adults was sporadically passed between late May to July, but also on a few occasions in late August. There were no instances where field temperatures went above CT_{max} of any life stage.

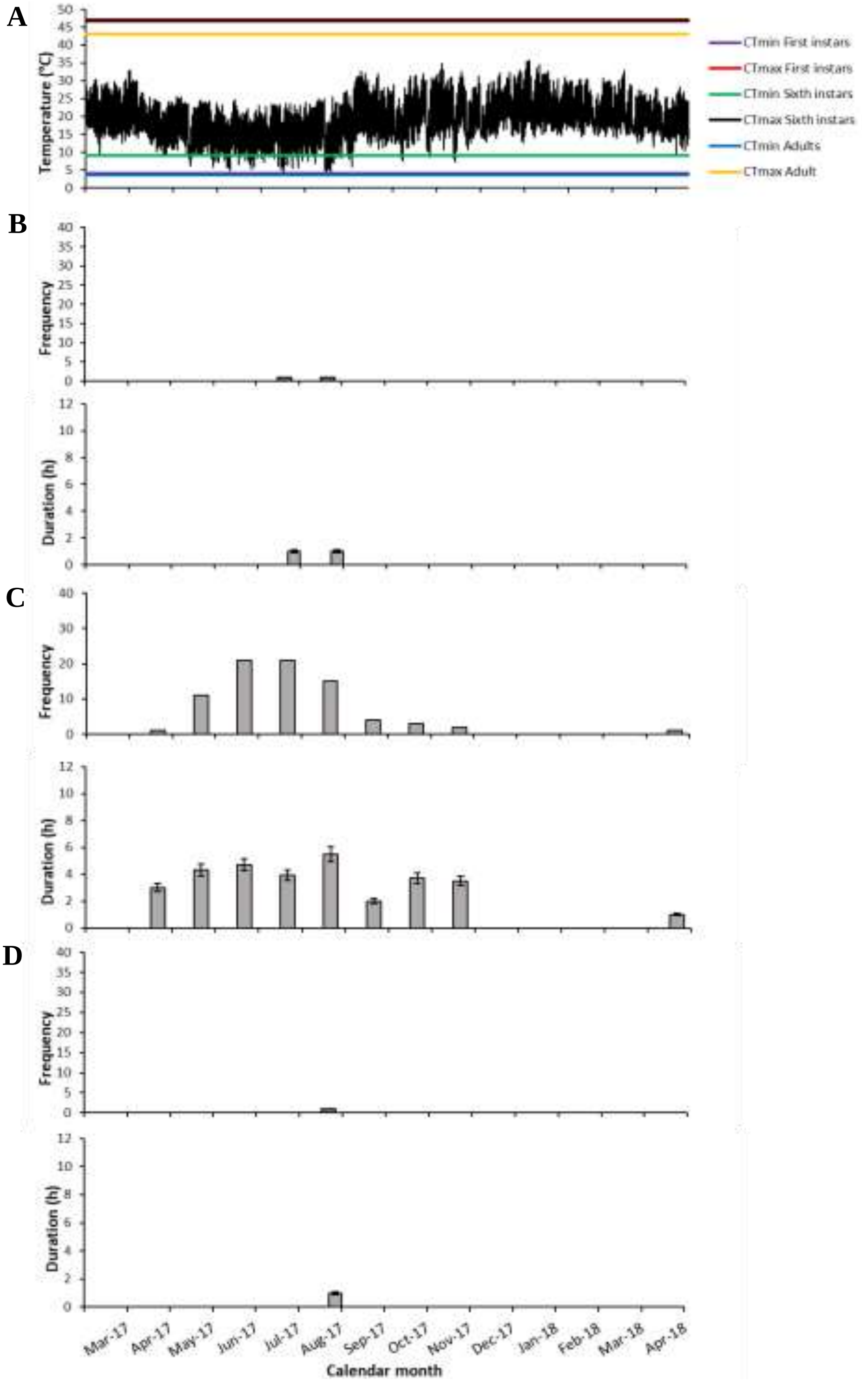

Figure 3.2: Hourly temperature range from (A) Loskop Prison farm over 14 months (April 2017-March 2018). Horizontal lines represent critical thermal limits of *Spodoptera frugiperda* life stages: CT$_{min}$ of first instar larvae (purple); CT$_{max}$ of first instar larvae (red); CT$_{min}$ of sixth instar larvae (green); CT$_{max}$ of sixth instar larvae (black); CT$_{min}$ of adults (blue); CT$_{max}$ of adults (yellow). (B) Frequency distribution and duration of temperatures recorded hourly from April 2017 to March 2018 near Loskop Prison farm for (B) first instar larvae, (C) sixth instar larvae and (D) adults. Shaded bars represent temperatures <CT$_{min}$. Mean CT$_{min}$ was 4.1, 9.1 and 3.6 °C while mean CT$_{max}$ was 47.1, 46.8 and 43.1°C for first instars, sixth instars and adults, respectively. There were no instances where temperatures exceeded CT$_{max}$ of any life stage and therefore there are no bars for CT$_{max}$. Errors bars signify 95% CLs.

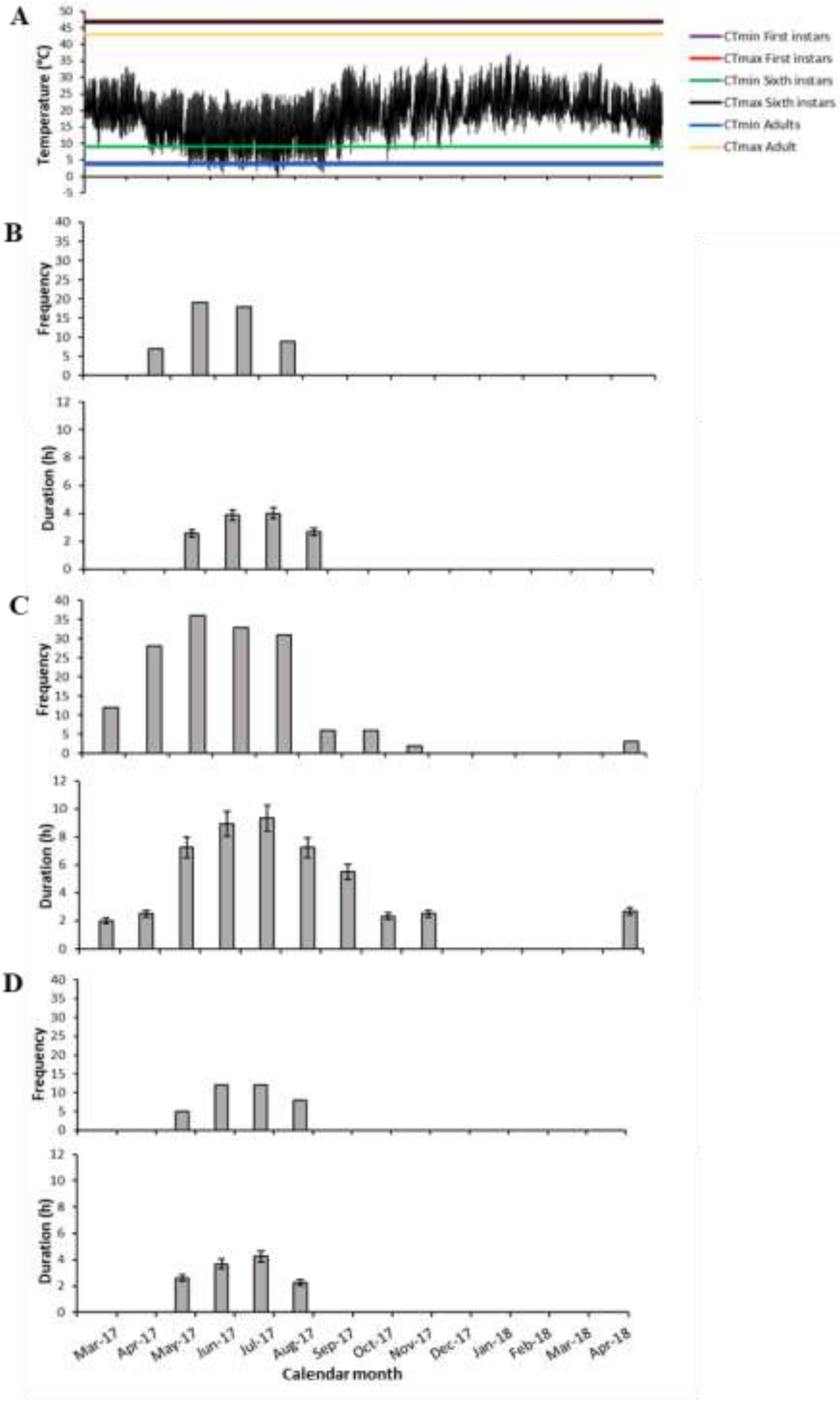

Figure 3.3: Hourly temperature range (A) from near Baviaanspoort prison and Zonderwater prison farms over 14 months (April 2017-March 2018). Horizontal lines represent critical thermal limits of *Spodoptera frugiperda* life stages: CT_{min} of first instar larvae (purple); CT_{max} of first instar larvae (red); CT_{min} of sixth instar larvae (green); CT_{max} of sixth instar larvae (black); CT_{min} of adults (blue); CT_{max} of adults (yellow). (B) Frequency distribution and duration of temperatures recorded hourly from April 2017 to March 2018 near Baviaanspoort and Zonderwater Prison farms for (B) first instar larvae, (C) sixth instar larvae, and (D) adults. Shaded bars represent temperatures $<CT_{min}$. Mean CT_{min} was 4.1, 9.1 and 3.6 °C while mean CT_{max} was 47.1, 46.8 and 43.1 °C for first instars, sixth instars and adults, respectively. There were no instances where temperatures exceeded CT_{max} of any life stage and therefore there are no bars for CT_{max}. Errors bars signify 95% CLs.

3.4 Discussion

Evolutionary history, phenotypic plasticity, and ability to tolerate varying temperatures facilitates insect population persistence and individual activity, both spatially and temporary (Mutamiswa *et al.*, 2018). In the current study, FAW was present in high numbers in the sampled maize field at Loskop Prison farm, indicating that this environment was suitable for this insect to thrive. It is important to note that sampling was only done twice (31 March 2018 and 03 April 2018), and that the field temperatures on both occasions were not below or above the thermal limits of this insect. The high CT_{max} could be attributed to the sub-tropical to tropical native range of this insect which may predispose FAW to be more tolerant to high temperature stress. The recorded field temperatures were always below average CT_{max} of this insect. The highest field temperatures recorded were 35.72 °C near Loskop Prison farm and 37.22 °C near Baviaanspoort and Zonderwaters Prison farms. These temperatures are well below 47.1, 46.8 and 43.1 °C, which are the CT_{max} of *S. frugiperda* first instars, sixth instars and adults, respectively, when reared on maize and acclimated to 20 °C. This implies that these regions experience temperatures that are conducive for the survival of this pest, which means that FAW is likely to thrive here during spring and summer. As a pest native to the tropical and subtropical regions of the Americas, the optimal temperature range for development of FAW of 26 and 30 °C (Sparks, 1979; Capinera, 2017; Du Plessis *et al.*, 2020) aligns with the recorded temperature ranges of the three prison farms.

In winter months, field temperatures tended to go below the CT_{min} of FAW at all three sites. These results are in line with the CLIMAX model that predicted that FAW will not survive colder winter temperatures of this part of South Africa (Timilsena *et al.*, 2021). In this study, the environmental temperatures lower than CT_{min} were occurred most often and for longer periods of time for the sixth instars. Environmental temperatures infrequently dropped below the CT_{min} of other life stages (first instars and adults). It is therefore likely that only first instars and adults of FAW survive at these sites during winter and the most damaging sixth instars die of causes related to their inactivity. At field temperatures below CT_{min}, sixth instar larvae are more prone to attack by natural enemies as they will be motionless and therefore cannot escape predation or parasitism. Heightened vulnerability to natural enemies during cold weather has been reported on females of the oak pest *Parthenolecanium quercifex* Fitch (Hemiptera: Coccidae) (Meineke *et al.*, 2014). Planning of integrated pest management (IPM) programs can take advantage of this to, for example, use it as a predictive model for the right time to release natural enemies or to spray insecticides. The differences in thermal tolerance between life stages has also been reported by Vorhees & Bradley (2012) in mealworms, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae). The differences in the observed thermal limits between life stages may be due to the substantial differences in environmental temperatures they experience. This also derives from the mobility of each life stage and the microenvironment it tends to inhabit (Bowler & Terblanche, 2008; Marais *et al.*, 2009). The sixth instar fall armyworm tends to make a tunnel on the maize whorl, ear, or cob. Because these areas are mostly moist and water has high heat capacity, their temperatures are unlikely to vary much. Thus, the sixth instar larvae respond to thermal stress behaviourally rather than physiologically.

The absence of FAW or any insect damage on Bt maize at Baviaanspoort Prison farm presents a salient example of the management tactics for the pest. It is possible that the Bt maize cultivar planted provided complete protection against the FAW population present in South Africa. This, however, should not be celebrated early as there are several reports of FAW populations that are resistant to Bt maize. For example, Huang *et al.* (2014) reported that *S. frugiperda* in the southern region of the USA was resistant to Cry1F Bt maize. This population of FAW was thought to represent migration of insects from Puerto Rico, which put emphasis on the need to achieve long distance pest management when dealing with a migratory pest like FAW. In Brazil, FAW has been reported to be resistant to several Bt maize cultivars and only one Bt maize (Vip3Aa20) remains effective (Fatoretto *et al.*, 2017). The quick development of resistance of FAW to Bt maize is thought to result from genetic characteristics, biology and ecology of the pest, and lack of proper adoption of insecticide resistance management strategies (reviewed by Fatoretto *et al.*, 2017). It is also possible that absence of FAW was a consequence of the pest not having reached Baviaanspoort Prison farm at the time of sampling. This might be the case as the site is isolated and has no farms nearby. This might suggest a role for environmental management tactics like removal of alternative hosts (Speight, 1983; Halbert, 2008) as an important component for control of FAW. However, FAW disperses rapidly by flight and is also a generalist, using a wide range of grasses for development. Thus, control of FAW should not be dependent solely on isolation but should involve area-wide IPM which uses multiple control tactics across an entire landscape and includes coordinated actions by all role players in a region (see review by Knipling, 1980; Njuguna *et al.*, 2021).

3.5 Conclusion

The results of this chapter show that FAW will likely survive the temperatures experienced in the region around Pretoria and Brits. There are infrequent events in winter months where field temperatures are well below the CT_{min} of *S. frugiperda*, particularly for sixth instars, but there are no instances where temperatures rose above the CT_{max} for this pest. The absence of FAW at Zonderwater Prison farm, despite temperatures rarely reaching stressful levels, suggests that South African populations of the pest are still susceptible to at least some Bt maize varieties in the country. However, adoption of area-wide integrated pest management is vital as this pest is able to develop resistance to Bt maize cultivars, is able to use a wide range of plants as hosts, and is also able to migrate long distances. FAW has already established in South Africa, and therefore there is a need to develop sustainable and practical means for dealing with this pest. Results from the current study can be used as a steppingstone towards a model for prediction of areas where FAW can establish.

CHAPTER 4:

General discussion and conclusion

This project defined some measures of upper and lower thermal tolerance for the fall armyworm (‘FAW’), *Spodoptera frugiperda*, and demonstrated the effects of life stage, thermal history, and diet on thermal tolerance. Invasive species across taxa have physiological phenotypes that are important for invasion success. Some of these traits may include broad thermal tolerance breadth and a capacity for phenotypic plasticity (Kelley, 2014). It is, therefore, not a surprise that thermal limits to activity are usually used by thermal biologists to assess the abundance and potential geographic distribution of ectotherms (Vorhees & Bradley, 2012). However, there are several factors that influence thermal limits of ectotherms. Some of these factors include diet, life stage and thermal history (Bowler & Terblanche, 2008; Terblanche *et al.*, 2011). The aim of this project was to provide insight into the thermal limits of activity and their plasticity for an alien herbivorous pest of maize that has recently invaded Africa. I went on to investigates if FAW critical thermal limits to activity are within the temperatures experienced in maize fields near Pretoria and Brits, South Africa.

In the present study, both CT_{min} and CT_{max} of FAW differed significantly between life stages (Chapter 2). First instar larvae showed acclimation only in their ability to tolerate cold. First instar larvae acclimated at lower temperatures (20 °C) had improved cold stress tolerance compared to those acclimated at 25 and 30 °C. Sixth instar larvae exhibited acclimation to temperature and diet-induced plasticity in thermal tolerance. Maize offered more cold stress tolerance when compared with other diets. Lower temperature acclimation (20 °C) improved cold stress tolerance of sixth instar larvae compared to those acclimated at 25 and 30 °C. Although not always statistically significant, a decrease in acclimation temperature of sixth instars led to better tolerance of cold, meaning that acclimation was generally beneficial. In adults, CT_{min} was not affected by either thermal history or diet. These results correspond with those of several other sources that have reported that thermal tolerance tends to vary between life stages and phenotypic plasticity may not be uniform between life stages (Terblanche *et al.*, 2007; Bowler & Terblanche, 2008; review by Terblanche *et al.*, 2011; Vorhees & Bradley, 2012; Keosentse *et al.*, 2021). The observed variation in phenotypic plasticity between immature life stages and adults supports the notion that flexibility in thermal tolerance is largely influenced by mobility of a life stage. Unlike the less mobile immature life stages, adult FAW may not have needed to evolve the capacity for thermal acclimation as they can use behaviour to escape harsh temperatures by flight. The effect of diet on sixth instars and not on first instars and adults may be attributed to the fact that the sixth instars had time to develop on that particular diet (in this study: maize, wheat or artificial diet), with the nutritional content of the diet affecting resources available for the insect to tolerate stress.

Both acclimation and diet had an effect on CT_{max} of first instars and only diet influenced sixth instar CT_{max}. Adults showed thermal plasticity to high temperature stress tolerance (CT_{max}). A complex interaction between acclimation temperatures, diets and life stages was observed. The fact that adult FAW show phenotypic plasticity in high temperature stress tolerance may make this pest a good invasive species as adults are responsible for initiating new populations in distant locations. High phenotypic plasticity to warmer temperatures is a particularly important trait in a warming climate (Leonard & Lancaster, 2020; Rodrigues & Beldade, 2020). Being a sub-tropical to tropical insect may have played a role in FAW tolerance to high temperature stress. The differences in effects of acclimation temperatures on tolerance of cold and heat shows that CT_{min} and CT_{max} may be controlled by different mechanisms. Moreover, the presence of the complex interaction effects further point to the same hypothesis.

Species with broad geographic ranges tend to have higher tolerance of high temperature stress (Kelley, 2014). The thermal tolerance range must still be above the normal and occasional highest environmental temperatures reached on the invaded site. FAW infested non-Bt maize fields and caused high damage on one of the visited farms near Pretoria, South Africa (Chapter 3). In contrast, there was no sight of this pest on another maize field, and this was attributed to the Bt maize cultivar planted there. Temperatures in the field recorded over 14 months on both fields indicated that *S. frugiperda* may be able to survive in these regions for most of the year. There were no instances where the temperatures in the field at these sites went beyond average CT_{max} of any life stage of *S. frugiperda*. Du Plessis *et al.* (2020) reported that the minimum temperature threshold for FAW development is 12.57 °C which was higher than the CT_{min} of 4.1, 9.1 and 3.6 °C for first instars, sixth instars and adults, respectively, recorded in the current study. However, field temperatures, were mostly above the temperature threshold for the development (determined by Du Plessis *et al.*, 2020) of FAW. An exception was during winter months where temperatures would occasionally go below CT_{min} of *S. frugiperda*, especially for the sixth instars. However, even during these winter months, the frequency and duration of these sub-CT_{min} temperatures were probably not sufficient to completely rid the field of *S. frugiperda*. Similar results were reported by Keosentse *et al.* (2021) on a FAW population in

Botswana. There are other places in the country that have milder winter temperatures than the site of focus for this study. Given its ability to move long distances, it is possible that the places with milder winters can serve as overwintering sites of this pest. Thus, regardless of occurrence of cold temperatures as reported on this study, areas with warmer winters can be the source of moths.

4.1 Future directions

The presented results from this project highlight some gaps in knowledge and give rise to new ideas and questions. FAW exhibits plasticity in thermal tolerance that is life stage dependent. Future studies need to focus on mechanisms for this plasticity to shed light on differences in the cellular mechanisms involved between life stages. Furthermore, whilst FAW showed acclimation plasticity, current results do not tell whether it is physiological acclimation that is obtained during the current life stage or it is a fixed-acclimation that is attained from the temperatures experienced by earlier life stages. At the time of this research, to the best of my knowledge, there was no study that had attempted to compare thermal tolerance of FAW to that of native Lepidopteran pests and investigated if there are any differences in the mechanisms involved in phenotypic plasticity observed between these species. Such knowledge can further shed light on how thermal tolerance differs between notorious invasive and native species and how their population dynamics will compare. Due to resource constraints, this research focused on a relatively small area for field surveys. Country-wide studies performed over multiple seasons are necessary for realistic and accurate models for predictions of population dynamics of FAW in the country.

4.2 Concluding remarks

FAW show phenotypic plasticity in thermal tolerance traits in response to diet and thermal history, and this plasticity varies with life stage. This means these factors can confound results in thermal biology studies if they are not considered or incorporated into the study design. Critical thermal limits of FAW suggest that this pest can survive in many regions of South Africa. However, it is unlikely to persist in some areas, particularly in those regions that experience field temperatures below its CT_{min}. It is clear that for effective control of FAW, area-wide IPM that incorporates several control measures and involves many role players is needed. The thermal tolerance of *S. frugiperda* determined in this work can contribute to building models that predict potential areas of establishment that can be assimilated into IPM programme design.

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