

The effect of temperature on the development and reproduction of *Busseola fusca* (Lepidoptera: Noctuidae)

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Dissertation submitted in fulfilment of the requirements for the degree *Magister Scientiae* in *Environmental Sciences* at the Potchefstroom Campus of the North-West University

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May 2015

ACKNOWLEDGEMENTS

I am very grateful to my study leader, Prof. Hannalene du Plessis, for her enthusiasm, encouragement and support. Furthermore, I would like to thank her for helping me with the statistical analyses. Her assistance and guidance helped a lot towards the success of this dissertation.

I want to thank my co-supervisor, Prof Johnnie van den Berg, for his advice and support as well as his assistance in finishing this dissertation.

I want to thank my mom and dad, Marianna and Uli, for their love, encouragement, patience and financial support to make a success of this dissertation. And also my sister, Yolandi, for her love and support.

I also want to thank my friends and colleges who have been with me while working in the laboratory and field as well as to finish my dissertation.

ABSTRACT

Busseola fusca is an indigenous lepidopteran pest species in tropical Africa, attacking several grain crops. Crop loss caused by this pest can be as high as 100 % depending on conditions. Despite it being a major pest in Africa, occurring in contrasting climatic zones, only a few studies have been published on its developmental biology. The effect of temperature on the development of *B. fusca* was studied at five different temperature regimes namely 15, 18, 20, 26 and 30 \pm 1 $^{\circ}$ C and 70 \pm 30 % relative humidity (RH) with 14L: 10D photoperiod. The number of instars for *B. fusca* was also determined. The most favourable temperature as well as the upper threshold temperature for larval development was found to be between 26 and 30 $^{\circ}$ C. Total development period was 152.6 to 52.6 days, respectively, at 15 $^{\circ}$ C, and 26 - 30 $^{\circ}$ C. The thermal constants for *B. fusca* was 99.50, 536.48, 246.25 and 893.66 $^{\circ}$ D and lower temperature threshold was 10.36, 8.14, 8.99 and 8.84 $^{\circ}$ C, for completion of the egg, larval, pupal, and egg-to-adult stages, respectively. The number of larval instars was determined by using head capsule widths that ranged from 0.31 - 2.68 mm. Clear distinctions of head capsule widths could be made from instar 1 to 3, yet overlapping occurred from instar 4 to 6. No distinction could be made between instars 7 and 8 in terms of head capsule width. All successive instars, except for instar eight, increased in size according to Dyar's ratio. The effect of temperature on reproduction of *B. fusca* was studied at 15, 20, 26 and 30 \pm 1 $^{\circ}$ C, 70 \pm 30 % RH with 14L: 10D photoperiod. Oviposition occurred at all the temperatures evaluated, but no fertility was recorded at 30 $^{\circ}$ C. The total number of eggs laid by *B. fusca* females was 300 - 400 eggs and the optimum temperature for oviposition and fertility was determined to be between 20 and 26 $^{\circ}$ C. Results from this study on the thermal constants and lower and upper threshold temperatures of *B. fusca* can be used to predict the impact of climate change on the distribution and population growth of this pest.

Key words: *Busseola fusca*, degree-days, development, fertility, instars, reproduction, temperature

OPSOMMING

Titel: Die effek van temperatuur op die ontwikkeling en voortplanting van *Busseola fusca* (Lepidoptera: Noctuidae)

Busseola fusca is 'n inheemse plaag op verskeie graangewasse in tropiese Afrika. Hierdie plaag is van ekonomiese belang en kan tot 100 % gewasverlies veroorsaak, afhangende van die toestande. Alhoewel *B. fusca* 'n ernstige plaag in Afrika is en in teenstellende klimaatsones voorkom, is slegs 'n paar studies oor die insek se ontwikkelingsbiologie gepubliseer. Die effek van temperatuur op die ontwikkeling van *B. fusca* was by vyf verskillende temperature bestudeer, naamlik 15, 18, 20, 26 en 30 ± 1 °C en 70 ± 10 % relatiewe humiditeit (RH) met 'n 14L: 10D fotoperiode. Die aantal instars was ook bepaal. Die mees geskikte - en hoogste drempeltemperatuur was tussen 26 en 30 °C. Volledige ontwikkeling het van 152.6 dae by 15 °C tot 52.6 dae by 26 - 30 °C verminder. Die termiese konstante vir ontwikkeling van *B. fusca* eiers, larwes, papies en die eier-tot-volwasse stadium was 99.50, 536.48, 246.25 en 893.66 °D en laagste drempeltemperatuur was 10.36, 8.14, 8.99 en 8.84 °C, onderskeidelik. Die wydte van kopkapsules was gemeet om die instars te bepaal en het gewissel van 0.31 - 2.68 mm. Daar was duidelike verskille tussen die eerste drie instars se kopkapsule-wydtes, maar oorvleueling het van die vierde instar af voorgekom. Daar was geen onderskeiding tussen die sewende en agtste instar se kopkapsule-wydtes nie. Al die opeenvolgende instars, behalwe instar agt, se kopkapsules het vergroot volgens Dyar se verhouding. Die effek van temperatuur op voortplanting van *B. fusca* was by 15, 20, 26 en 30 ± 1 °C en 70 ± 30 % RH met 'n 14L: 10D fotoperiode bestudeer. Eierlegging het by al die temperature plaasgevind, maar al die eiers wat by 30 °C gelê was, was onvrugbaar. Die totale aantal eiers wat deur *B. fusca* wyfies gelê was, het tussen 300 en 400 eiers gewissel. Die mees geskikte temperatuur vir eierlegging en vrugbaarheid van *B. fusca* wyfies was tussen 20 en 26 °C. Resultate van die studie met betrekking tot die termiese konstantes asook hoogste en laagste drempeltemperatuur van *B. fusca* kan gebruik word om die impak van klimaatsverandering op die verspreiding en populasietoename van hierdie plaag te bepaal.

Sleutelwoorde: *Busseola fusca*, graad-dae, instars, ontwikkeling, temperatuur, voortplanting, vrugbaarheid

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	i
ABSTRACT.....	ii
OPSOMMING	iii
TABLE OF CONTENTS	iv
LIST OF FIGURES	vii
LIST OF TABLES.....	viii
 CHAPTER 1	 1
Introduction	1
1.1 General introduction	1
1.2 Lepidopteran pests, crop losses and management strategies	2
1.2.1 Lepidopteran pests of grain crops in Africa	2
1.2.2 Stem borer pests of maize and sorghum in southern Africa.....	4
1.3 <i>Busseola fusca</i> as a pest of maize in Africa.....	4
1.3.1 Distribution	4
1.3.2 Pest status	5
1.3.3 Effect of climate on <i>Busseola fusca</i>	5
1.3.4 Control	6
1.4 Life cycle of <i>Busseola fusca</i>	8
1.5 Diapause.....	9
1.6 Oviposition	11
1.7 Temperature dependent development and reproduction	11
1.8 Objectives of this study.....	13
1.8.1 General objective	13
1.8.2 Specific objectives.....	13
1.9 References.....	14

CHAPTER 2.....	29
The effect of temperature on the development of <i>Busseola fusca</i> (Lepidoptera: Noctuidae).....	29
2.1 Abstract.....	29
2.2 Introduction	30
2.2.1 The effect of temperature on insect development	30
2.2.2 Larval development of <i>Busseola fusca</i> (life cycle)	30
2.2.3 Geographical distribution of <i>Busseola fusca</i>	31
2.2.4 Climate Change.....	32
2.2.5 Head capsules.....	32
2.2.6 Objectives	32
2.3 Materials and Methods	33
2.3.1 <i>Busseola fusca</i> stock colony.....	33
2.3.2 Temperature-dependent egg development.....	33
2.3.3 Temperature-dependent larval and pupal development.....	34
2.3.4 Number of instars	35
2.3.5 Data analysis.....	36
2.4 Results	37
2.5 Discussion.....	39
2.6 References.....	46
 CHAPTER 3.....	 72
The effect of temperature on reproduction of <i>Busseola fusca</i> (Lepidoptera: Noctuidae).....	72
3.1 Abstract.....	72
3.2 Introduction	73
3.2.1 The effect of temperature on reproduction.....	73
3.2.2 <i>Busseola fusca</i> moths	74
3.2.3 Monitoring of <i>Busseola fusca</i> moths	75

3.2.4	Objectives	75
3.3	Materials and Methods	76
3.3.1	<i>Busseola fusca</i> collection sites	76
3.3.2	Fecundity and longevity of female moths	76
3.3.3	Statistical analysis	76
3.4	Results	77
3.5	Discussion	78
3.6	References	81
CHAPTER 4	89
Conclusion	89
4.1	References	91

LIST OF FIGURES

Figure 1.1:	Life cycle of <i>Busseola fusca</i>	27
Figure 1.2:	The male (top) and female (bottom) pupae of <i>Busseola fusca</i> distinguished by the genital scars on sternum 9 of males and sternum 8 of females.....	28
Figure 2.1:	Containers used for rearing of <i>Busseola fusca</i> : a) oviposition chambers with one cut maize stem per moth pair; b) desiccator with small plastic containers for egg development; c) test tubes used for larval development; d) plastic container with water and test tubes containing pupae.	59
Figure 2.2:	Temperature and RH recorded inside a maize stem with an iButton®.....	59
Figure 2.3:	<i>Busseola fusca</i> larvae where fed with compact unfolded leaves from the second to the fifth instar (above) and a piece of maize stem from the six instar onwards (bottom).	60
Figure 2.4:	Damage by <i>Busseola fusca</i> larvae through tunnelling into maize stems.....	61
Figure 2.5:	Piece of maize plant cut longitudinally and kept together with an elastic band for daily observation of <i>B. fusca</i> larvae.....	62
Figure 2.6:	The relationship between <i>Busseola fusca</i> development rates and rearing temperature for larval instar one to six.	63
Figure 2.7:	The relationship between development rates and rearing temperature for eggs, larvae, pupae and egg-to-adult stages of <i>Busseola fusca</i> . (Development rates for larvae and egg-to-adult stage include instars 7 and 8).	64
Figure 2.8:	Frequency distribution of head capsule widths of <i>Busseola fusca</i> larvae. Each coloured line indicates the range of a specific instar.....	65
Figure 2.9:	Relationship between head capsule width and instar of <i>Busseola fusca</i> larvae. The linear regression shows a straight line which fitted Dyar's rule.	66
Figure 2.10:	Head capsules of <i>Busseola fusca</i> larvae indicating instars one to seven.....	67

LIST OF TABLES

Table 1.1:	Important lepidopteran pests of grain crops in Africa.	3
Table 2.1:	Mean development time (days \pm S.E.) of different life stages and larval survival of <i>Busseola fusca</i> at constant temperatures. The range of days to develop is shown in brackets.	68
Table 2.2:	Linear regression equations describing the relationship between development rate (1/days) and temperature (15 - 26 °C) and the thermal requirements of different developmental stages of <i>Busseola fusca</i>	69
Table 2.3:	Mean development time in days and degree-days (°D) for <i>Busseola fusca</i> at constant temperatures from 15 - 26 °C. Degree-days were calculated using the lower threshold temperature for development determined for each developmental stage (eggs = 10.36 °C, larvae = 8.99 °C, pupae = 8.14 °C and egg-to-adult = 8.84 °C).	70
Table 2.4:	Mean head capsule widths and ranges for each <i>Busseola fusca</i> larval instar stage and Dyar's ratio.	71
Table 3.1:	Mean fecundity and longevity (\pm S.E.) of <i>Busseola fusca</i> moths at constant temperatures.	87
Table 3.2:	Fertility (\pm S.E.) of <i>Busseola fusca</i> eggs at constant temperatures.	88

CHAPTER 1

Introduction

1.1 General introduction

The most important cereal crops in Africa are maize, millet, rice and sorghum. Since the 16th century, maize (*Zea mays* L.) has been used in Africa and by the 17th century it was cultivated widely in Africa (Polaszek & Khan, 1998).

Climate change is a worldwide phenomenon that affects agricultural productivity. This may result in a decrease in crop production and an increase in food costs and food insecurity. However, climate change impacts vary among regions and may have either positive or negative effects, depending on how the impact affects agricultural productivity (Calzadilla *et al.*, 2014). Climate change affects crop production through five main factors: temperature, precipitation, elevated carbon dioxide levels, varying environments and water availability (IPCC, 2007; World Bank, 2007). Crop production is mostly dependent on temperature and soil moisture availability. Temperature largely determines the length of the growing season and development rate of crops. In certain areas, higher temperatures will lead to a decrease in the number of frost-days thereby creating climates more conducive for crop production. However, in arid and semi-arid regions, higher temperatures will result in shorter cropping cycles and reduction in crop production due to reduced water availability (IPCC, 2007).

South Africa is largely a semi-arid country, with agro-ecological zones that ranges from desert and semi-desert areas in the north-western region, to sub-humid and wet areas in the eastern coastal region (Calzadilla *et al.*, 2014). The average rainfall in South Africa is 464 mm per year, compared to a world average of 857 mm per year (United Nations, 2009). Prediction models show that South Africa will have a much drier climate in future with a small increase in temperature, leading to a decrease in the total production of crops (Calzadilla *et al.*, 2014). Various adaptive methods have been integrated to alleviate climate change in South Africa such as development of irrigation systems and improvements in agricultural productivity (Hussain & Hanjra, 2004; Molden *et al.*, 2007; FAO, 2008; Calzadilla *et al.*, 2013). The methods mentioned above have been discussed by Calzadilla *et al.* (2013). Irrigation is practiced throughout South Africa and accounts for 62 % of the water used in the country (DWAF, 2004). It can therefore be said that agriculture will be highly affected by

climate change. Farmers practicing rainfed agriculture have already been affected by climate change (FAO, 2011).

Future crop production patterns will depend on the severity of climate change which, for example, may cause longer or shorter growing seasons (Adams *et al.*, 1990; Matthews *et al.*, 1997; Aggarwal & Mall, 2002; Xiong *et al.*, 2008). Methods such as changing planting and harvest dates (Winters *et al.*, 1998; Susanna *et al.*, 2007; Lobell *et al.*, 2008) and introducing new crop cultivars with longer growing seasons (Jørgen & Marco, 2002; Ogden & Innes, 2008) have been used in order to adapt to global changes (Li *et al.*, 2014). Due to these adaptive methods, crop phenology may be influenced which may lead to longer growing seasons (Zhang *et al.*, 2013).

Crop phenology changes as temperature varies over time and space (Chmielewski *et al.*, 2004; Hu *et al.*, 2005; Tao *et al.*, 2006; Sacks & Kucharik, 2011; Siebert & Ewert, 2012). The growth and development of maize is mainly affected by temperature, radiation, photoperiod and water. These factors may vary in time and space. Different planting dates therefore result in crops experiencing different environmental conditions (Tsimba *et al.*, 2013), which may also influence stem borer - host plant interactions.

1.2 Lepidopteran pests, crop losses and management strategies

1.2.1 Lepidopteran pests of grain crops in Africa

Maes (1997) reported 20 economically important stem borer species in Africa. These species vary in distribution, relative abundance and pest status (Megenasa, 1982; Songa *et al.*, 1998; Ndemah *et al.*, 2001). The most important lepidopteran pests of grain crops in Africa are listed in table 1.1.

A high diversity of pest species was reported in maize by Ong'amo *et al.* (2006) in Kenya. The most dominant species in Kenya are *Chilo partellus* (Lepidoptera: Pyralidae) and *Busseola fusca* (Lepidoptera: Noctuidae), although they differ in dominance between the agro-climatic zones and between seasons. Since maize is grown in Kenya in highland tropics and moist transitional zones, it has a high potential to be attacked by these pests (De Groote, 2002). In Zambia, *B. fusca* prefers wet weather and cooler temperatures (Okech *et al.*, 1994). In Cameroon, *B. fusca* occurs mostly in the lowland and coastal forests (Ndemah *et al.*, 2007).

Table 1.1: Important lepidopteran pests of grain crops in Africa.

Region	Species	Host plant
East Africa (Seshu Reddy, 1998)	<i>Sesamia calamistis</i> Hampson (Noctuidae)	Maize and sorghum
	<i>Sesamia cretica</i> Lederer (Noctuidae)	
	<i>Busseola fusca</i> (Fuller) (Noctuidae)	
	<i>Eldana saccharina</i> Walker (Pyralidae)	
	<i>Chilo partellus</i> (Swinhoe) (Pyralidae)	
	<i>Chilo orichalcociliellus</i> (Strand) (Pyralidae)	
	<i>Coniesta ignefusalis</i> (Hampson) (Pyralidae)	
West Africa (Bosque-Pérez & Schulthess, 1998)	<i>B. fusca</i>	Maize, sorghum, millet, rice and sugar cane
	<i>S. calamistis</i> & <i>Sesamia</i> spp.	
	<i>Eldana saccharina</i>	
	<i>Chilo partellus</i>	
	<i>Coniesta ignefusalis</i>	
	<i>Mussidia nigrivenella</i> (Ragonot) (Pyralidae)	
Central Africa (Bosque-Pérez & Schulthess, 1998)	<i>B. fusca</i>	Maize
	<i>S. calamistis</i>	
	<i>C. partellus</i>	
	<i>C. orichalcociliellus</i>	
Southern Africa (Kfir, 1998)	<i>B. fusca</i>	Maize and sorghum
	<i>S. calamistis</i>	
	<i>C. partellus</i>	
	<i>C. orichalcociliellus</i>	
	<i>E. saccharina</i>	

1.2.2 Stem borer pests of maize and sorghum in southern Africa

Busseola fusca is one of the most important pests of maize and sorghum in South Africa (Kfir, 1998). Other important lepidopteran pests in South Africa are the spotted stem borer, *C. partellus* and the pink stem borer, *Sesamia calamistis* (Lepidoptera: Noctuidae), which also attack maize and sorghum (Kfir, 1998; Van den Berg & Drinkwater, 2000; Kfir *et al.*, 2002). Van Rensburg & Bate (1987) noted varying levels of yield loss between farms, ranging from no losses to total crop loss due to *B. fusca* in maize. In Zimbabwe, Sithole (1987) estimated yield losses of between 30 and 70% where no insecticides were applied, but less than 30% crop loss where insecticides were applied.

1.3 *Busseola fusca* as a pest of maize in Africa

1.3.1 Distribution

Busseola fusca is an indigenous pest species in tropical Africa (Mohyuddin & Greathead, 1970; Harris & Nwanze, 1992; Kfir *et al.*, 2002), attacking several grain crop species in Africa south of the Sahara (Harris, 1989). This species is usually the dominating species in high-altitude regions (Van Rensburg & Bate, 1987) but it also occurs at low altitudes in East Africa (Calatayud *et al.*, 2014) and Zimbabwe (Sithole, 1989). This species is therefore largely found in the cooler eco-zones of East and southern Africa and in mid-altitude and highland areas (Kfir *et al.*, 2002). While *B. fusca* has been reported at higher altitudes (>600 m a.s.l.) in East and southern Africa (Nye, 1960; Sithole, 1989), in West Africa it was reported to occur from sea level to >2000 m (Tams & Bowden, 1953). In southern Africa, *B. fusca* has been reported to occur at low-altitude elevations in coastal regions (Van Rensburg, 1997; Waladde *et al.*, 2002) up to the highlands of Lesotho (2131 m) (Ebenebe *et al.*, 1999a). In Eritrea, *B. fusca* mostly occurs at altitudes above 1500 m (Haile & Hofsvang, 2001).

The distribution of stem borer populations is likely to be influenced by temperature, rainfall and humidity, with temperature as the most important factor (Sithole, 1987). Elevation affects the physical environment such as temperature and relative humidity in an area, thus affecting the development and distribution of an insect (Sithole, 1987).

1.3.2 Pest status

Most of the damage done by *B. fusca* larvae is feeding on whorl leaves and stem tunnelling (Appert, 1970; Bosque-Perez & Mareck, 1991; Kfir, 1998). Stem tunnelling may have a weakening effect on the stem causing it to break and plants to lodge (Appert, 1970; Bosque-Perez & Mareck, 1991; Harris & Nwanze, 1992; Kfir, 1998). Stem damage interferes with translocation of nutrients and metabolites in the plant, thus having an effect on plant growth and also development of grains (Appert, 1970; Bosque-Perez & Mareck, 1991; Kfir, 1998). The growing points of very young maize plants may also be killed by larvae (Appert, 1970; Bosque-Perez & Mareck, 1991; Kfir, 1998) thus creating 'dead heart' symptoms (Harris & Nwanze, 1992). Maize ears are also directly damaged by larvae (Bosque-Perez & Mareck, 1991; Harris & Nwanze, 1992; Kfir, 1998) and damage can also cause malformation of the grains (Appert, 1970; Bosque-Perez & Mareck, 1991; Kfir, 1998) thus producing low quality maize for consumption. Infestations by stem borers increase the incidence and severity of stalk rots in maize (Bosque-Perez & Mareck, 1991). Yield loss estimates may vary with region, *B. fusca* infestation levels and plant developmental stages (Van Rensburg & Flett, 2008).

Yield reduction by stem borers depends on conditions such as the plant growth stage, number of larvae per plant and the reaction of the plant to feeding of the stem borer (Appert, 1970; Bosque-Perez & Mareck, 1991). Yield losses vary between different regions. In Kenya, Hassan *et al.* (1998) estimated the average loss due to stem borer infestations in low to medium potential areas to be between 26.8 and 27.9 % and for dry mid-altitude areas to be 18 %. Ong'amo *et al.* (2006) reported 10 % loss due to *B. fusca* in highland tropics and moist transitional and mid-altitude zones, but only 1 % loss in low potential zones (dry mid-altitude and lowland tropics) of Kenya. Dabrowski (1985) reported yield loss between 15 - 78 % in Kenya. Usua (1968) reported that one or two larvae per maize plant resulted in yield loss up to 25 % in south-western Nigeria.

1.3.3 Effect of climate on *Busseola fusca*

Climate change is described as a direct or indirect alteration of the environment through anthropogenic activities (IPCC, 2007). Climate change can lead to habitat destruction and fragmentation affecting population dynamics of insects in an area (Thomas *et al.*, 2004;

Dover & Settele, 2009). Insect herbivores are expected to suffer direct and indirect effects of climate change through the changes experienced by their host plants (Cornelissen, 2011).

The global mean annual temperatures have been estimated to increase by 1 °C by 2025 and 3 °C by the end of the next century (IPCC, 1990a, b, 2007). Increased temperatures may result in rapid growth and development of insects, thus resulting in rapid increase in pest populations over time. Rising temperatures may also lead to earlier infestations by pests and create new niches for insect pests (Sharma, 2010). Lower temperatures on the other hand may limit the geographical distribution of insects (Hill, 1987). According to Sharma (2010), global warming and climate changes will affect the following: geographical ranges of pests, diapause duration, population growth rate, changes in insect-host plant interactions, rates of invasion by non-native pests, changes in diversity, changes in synchrony between insect pests and their host plants, different host introductions, and reduced effectiveness of crop protection technologies.

Future climate change may therefore have a significant effect on the interactions between *B. fusca* and its host plants. Changing rainfall patterns and ambient temperatures, due to climate change, will lead to varying planting dates which in turn will affect the phenology of maize at the landscape level, thereby affecting current management practices (Hassan *et al.*, 1998; De Groote, 2002).

1.3.4 Control

Apart from environmental factors such as soil fertility and rainfall, the success of maize production also depends on the time of planting, maize genotype, fertiliser application and weed and pest control. When used properly, insecticides may be effective against pests. Application of pesticides is important but is not always practical on small farms (Warui & Kuria, 1983). Chemical control of stem borer larvae at advanced growth stages may not be effective because the larvae are protected within the stem. However, first instar larvae can be controlled effectively since they feed in plant whorls for a period of 7 - 14 days before they migrate to neighbouring plants or to feed in stems of plants (Critchley *et al.*, 1997).

Various host plant resistance and cultural control strategies have been implemented with partial or local success but none have been proven to provide really effective control of stem borers of maize (Van den Berg *et al.*, 1998; Kfir *et al.*, 2002). Crop residues play an important role in off-season survival of *B. fusca* and *C. partellus*. Mally (1920) suggested a

cultural control method of crop residue destruction by means of ploughing of maize stubble, in order to destroy overwintering larvae. Slashing maize and sorghum may result in a 70% reduction in *B. fusca* and *C. partellus* numbers while further ploughing and discing may destroy a further 19% in maize (Kfir *et al.*, 1989; Kfir, 1990). Adesiyun & Ajayi (1980) suggested that partial burning of stalks or spreading of stalks on the ground during the dry season may help to control stem borer larvae inside stalks.

Different planting dates have been used in pest management strategies with the aim to reduce stem borer numbers in maize (Van Rensburg *et al.*, 1985; Ebenebe *et al.*, 1999b). For example, earlier planting can ensure that plants are at their most susceptible stage (mid-whorl) when the moth flight activity of *B. fusca* is the lowest in South Africa (Van Rensburg *et al.*, 1985). Many farmers experience less stem borer damage in maize due to early planting dates (Van Rensburg *et al.*, 1988). Van Rensburg *et al.* (1987) reported that *B. fusca* infestation pressure was higher in late-planted maize, with the highest number of eggs laid on plants 3 - 4 weeks after seedling emergence. Even though moths prefer maize plants of a certain age they do lay eggs on plants of any age if the area is isolated and if no plants of other ages are available (Van Rensburg *et al.*, 1987). In Ethiopia, Gebre-Amlak *et al.* (1989) observed a positive correlation between crop loss due to *B. fusca* and late planting dates.

Biological control of *B. fusca* and *C. partellus* has been attempted with *Cotesia* spp. However, in Kenya *B. fusca* was not effectively parasitised by the parasitoid *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) and the exotic parasitoid, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), which were introduced in a classical biocontrol program. This parasitoid is only effective in certain areas of Kenya (Kfir, 1995).

Intercropping methods have helped to reduce numbers of eggs and larval populations of stem borers, thus resulting in a lower crop loss when different plants were used (Schulthess *et al.*, 2004; Chabi-Olaye *et al.*, 2005a, b). More recently, the push-pull management strategy has been deployed in East Africa and was shown to be effective against stem borers in that region (Khan *et al.*, 2000; Pickett *et al.*, 2013). The push-pull strategy uses plants that trap or repel pests and also attract predators or parasitic insects by means of semio-chemicals emitted by companion plants (Pickett *et al.*, 2013).

Another control method used for stem borers are genetically modified Bt-maize that contains the gene of the soil bacterium *Bacillus thuringiensis* (Bt). This method has been most effective on *B. fusca* larvae and was shown to have reduced the pest status of this species in South Africa since the release of Bt-maize in 1998 (Gouse *et al.*, 2005; Kruger *et al.*,

2012). *Busseola fusca* has, however, developed resistance to Bt-maize in South Africa (Van Rensburg, 2007; Kruger *et al.*, 2012).

1.4 Life cycle of *Busseola fusca*

The life cycle of *B. fusca* is shown in figure 1.1. The emergence of moths occurs usually around late afternoon since they are active at night (Unnithan, 1987; Harris & Nwanze, 1992). The males tend to emerge before the females as observed by Calatayud *et al.* (2007). After emergence, female moths release a pheromone to attract the males (Harris & Nwanze, 1992; Frérot *et al.*, 2006). Mating is quite simple and rapid and generally takes place during the first six hours of the night (Calatayud *et al.*, 2007). Males can mate several times, but fertilise the eggs with only one spermatophore per mating. Females mate only once per night (Unnithan & Paye, 1990) and disperse afterwards in search of a suitable host for oviposition. Eggs are laid under the inner surfaces of leaf sheaths in batches (Unnithan, 1987; Harris & Nwanze, 1992) and the number of eggs varies greatly. Reports of the number of egg batches as well as total number of eggs laid per female vary greatly, for example, 600 - 800 eggs per female in 30 - 100 batches (Unnithan, 1987); 1 - 140 egg batches with a maximum of 891 eggs per female (Mally, 1920), 30 - 100 egg batches and 1000 eggs per female (Harris, 1962); 70 egg batches and 568 eggs per female (Ingram, 1958) and 100 - 800 eggs per female (Van Rensburg *et al.*, 1987; Kruger *et al.*, 2014). Males tend to live slightly longer than the females (Unnithan, 1987).

Larvae generally take about a week to emerge after oviposition, thereafter dispersing over the leaves to settle on their host plants before they start to feed on whorl leaves. Most larvae up until the 4th instar will feed in the plant whorl after which they move to the stems (Van Rensburg *et al.*, 1987). They feed for about 3 - 5 weeks inside the stems and maize ears producing tunnels before entering the pupal stage (Harris & Nwanze, 1992). The duration of the larval stage is about 24 - 54 days depending on temperature (Calatayud *et al.*, 2007). Before pupation, larvae will create an exit hole for the moths by tunnelling towards the outside of the stem but leaving the outer epidermal layer intact (Harris & Nwanze, 1992).

Female pupae are bigger than the male pupae and can be distinguished by the genital scars on sternum 8 of females and sternum 9 of males (Harris & Nwanze, 1992). Male and female pupae are shown in figure 1.2. The pupation period is about 9 - 14 days depending on the temperature (Harris & Nwanze, 1992; Onyango & Ochieng'-Odero, 1994; Ratnadass *et al.*, 2001). The life cycle of *B. fusca* is completed within 7 - 8 weeks if the conditions are favourable (Harris & Nwanze, 1992). Not all of the larvae pupate, some of them enter

diapause. In South Africa, *B. fusca* overwinters as diapause larvae from April - October (dry winter season) inside the lower dry stalks just beneath the ground surface (Kfir, 1988; 1990; 1991; Kfir *et al.*, 1989).

The first seasonal flight of *B. fusca* moths starts in South Africa during early spring (September) (Van Rensburg *et al.*, 1985; Van Rensburg, 1997). The second seasonal moth flight occurs largely after flowering of early planted maize plants and more towards the end of the crop production season. There are usually only two seasonal flights per year but a third may also occur. This flight and the infestation that follows are not regarded as economically important (Van Rensburg *et al.*, 1985; Van Rensburg, 1997). Third-generation moths cannot find suitable host plants for oviposition due to desiccation of the old plants, decreasing temperature and humidity, and young larvae unable to feed on older plants (Van Rensburg, 1997).

1.5 Diapause

During unfavourable conditions, stem borer larvae are able to enter diapause. To ensure the survival of larvae during unfavourable conditions, mature larvae can enter diapause for six months or more during dry or cold periods before they pupate. Diapause larvae usually occur in stems and stubble (Harris & Nwanze, 1992). Most larvae will reside in the lower parts of stems just underneath the soil surface for protection against natural enemies and unfavourable climatic conditions (Kfir, 1988, 1990; Kfir *et al.*, 1989).

Lees (1955) indicated that the most important factors playing a role in inducing diapause in insects are photoperiod, temperature and diet. These factors may work together or alone, depending on how the insect react to unfavourable conditions, to induce diapause. Usually higher temperatures won't cause diapause in arthropods but lower temperature can slow down development or growth in an insect (Lees, 1955). Temperature may play a role in inducing diapause in arthropods but not for tropical insects.

According to Usua (1973), diapause can be induced in *B. fusca* through food, for example if the constituents of maize tissue have been altered through environmental conditions. Fewer larvae are reported to diapause when maize stems have a high water, high protein and low carbohydrate content (Usua, 1973). Because of the high carbohydrate content inside maize stems, larvae can store fat in the cells of the body. Usua (1973) also noted that factors such as maturity and food composition played an important role in initiating diapause. Okuda

(1990) indicated that soil moisture plays an important role in terminating diapause for *B. fusca* populations. Van Rensburg and Van Rensburg (1993) described how temperature, humidity and mainly photoperiod can be used to manipulate the diapause process in *B. fusca* larvae.

In Zimbabwe, Smithers (1960) has found *B. fusca* larvae in diapause in maize stems 25 - 60 cm above the soil surface. The reason they are found near the soil is a reaction to temperature found only in colder regions. It may also be that during the winter, the stem base may have a higher temperature inside than on the soil surface. During winter, the stem may have higher humidity than outside as well as the stem base also having a higher humidity level than the upper parts of stem. Since diapause may also occur as a drought-survival mechanism, diapause larvae may also use the base of the stem to avoid desiccation (Van Rensburg *et al.*, 1987).

Diapausing larvae lose their typical creamy-brown colour during the diapause stage. Kfir (1991) noticed that during diapause, borer larvae became less active and lost their pigmentation, therefore turning dirty white colour. A possible reason may be that before entering diapause, larvae accumulate large energy reserves (mostly fat) thus causing a loss of pigmentation (Kfir, 1991). Diapausing larvae also lose weight during the diapause stage through the consumption of energy reserves while in the diapause stage (Kfir, 1991).

During diapause the insect consumes its body fat thus causing weight loss which results in a decrease in body size. During normal conditions of growth the external skeleton becomes too small for the insect, therefore the insect needs to moult (Kfir, 1991). Insects moult because their exoskeleton (external skeleton) can't expand as the insect grows larger. Non-diapausing larvae normally have six moults but diapausing larvae have additional six or seven moults. Some larvae may therefore moult as many as 13 times before entering pupation (Kfir, 1991). To terminate diapause, a combination of temperature, humidity and photoperiod is needed in which photoperiod is the most important (Van Rensburg & Van Rensburg, 1993). When the conditions are favourable the diapause stage will end, followed by the pupal stage.

Female moths of diapause larvae have fewer eggs in their ovaries than moths originating from non-diapausing larvae. Van Rensburg *et al.* (1987) indicated that the egg batches of spring moths were smaller than those of summer moths. A possible reason might be that the body energy reserves of spring moths are smaller than that of the summer moths because

the energy reserves are used during diapause. During a study done by Kfir (1991), a positive correlation was observed between body mass and eggs in the ovaries of *B. fusca* moths.

1.6 Oviposition

Busseola fusca moths lay their eggs in batches underneath leaf sheaths, behind the vertical edges and also underneath outer husk leaves of maize ears (Mally, 1920). Moths show an oviposition preference for 3 - 5 week old maize plants (Van Rensburg *et al.*, 1987; Van Rensburg & Van Rensburg, 1993), but oviposition can occur on plants of other ages if plants of the preferred age are not available. These moths find the youngest unfolded leaf sheath of maize plants the most attractive with the tendency that the preferred oviposition site moves gradually upwards with the growth of the plant. Larger plants have more oviposition sites and may accommodate bigger egg masses and improved survival of larvae (Harris & Nwanze, 1992). Increased plant age is correlated with increasing occurrence of egg batches higher up on the plant (Van Rensburg *et al.*, 1987). With age, the leaves become looser around the stem, attracting ovipositional moths (Van Rensburg *et al.*, 1987).

1.7 Temperature dependent development and reproduction

Temperature has an effect on the development, survival and reproduction of insects. Studies on the effect of temperature on *B. fusca* are still inadequate. This study is important for pest management strategies and for the prediction of outbreaks of this species.

Temperature is an environmental condition that causes specific morphological and physiological responses in individuals of a species (Hallman & Denlinger, 1998; Huey & Berrigan, 2001; Begon *et al.*, 2006; Golizadeh *et al.*, 2007). Development occurs within a specific temperature range and is best performed at an optimum temperature. Development rate decreases as the temperature decreases or deviates from the optimum (Begon *et al.*, 2006). Changes in performance are caused by metabolic changes in an organism. For each 10 °C rise in temperature, the rate of biological enzymic processes nearly doubles until around 20 °C thereafter the rate will increase less rapidly with higher temperatures (an exponential curve on a plot of performance rate against temperature can show this relationship) (Begon *et al.*, 2006). The reason for increase in enzymic processes is the increased speed of molecular movement caused by high temperatures that speeds up chemical reactions in insects. Insects have a functional temperature range at which they can

live at their best. Yet, outside that temperature range the conditions are regarded as extremes that cause impaired function and ultimately death for insects (Begon *et al.*, 2006).

The effect of temperature on growth rate (increase in mass) and development (progression through life cycle stages), and on final body size drive the main ecological activities of survival, reproduction and growth. The relationship between growth rates, development and temperature are effectively linear, showing only slight deviations. The temperatures experienced by an organism, is indicated as degree-days (Begon *et al.*, 2006).

The final size of a fully grown organism will be determined by the rates of growth and development. If the rates of growth and development are rapid, the final size of the fully grown organism will be smaller than slower growing and developing organisms of the same species. Therefore, since an organism responds differently in terms of growth and development with varying temperatures, the full grown size of the organism will also be affected by temperature (Begon *et al.*, 2006).

Temperature affects the rate of survival, reproduction, population growth and development (Roy *et al.*, 2003). Many different models have been created to describe the relationship between temperature and insect development and growth (Briere & Pracros, 1998; Roy *et al.*, 2002; Golizadeh *et al.*, 2007). If the adaptation of insects to environmental conditions is known, pest management can predict the timing of development, reproduction, dormancy (diapause) and migration (Nechols *et al.*, 1999; Roy *et al.*, 2002). Thus, determining the relationship between temperature and rate of development and reproduction is important in studies of population dynamics of pests. Population studies have several applications such as analysing population stability and structure, estimating extinction probabilities, predicting life history evolution, predicting outbreaks of pest species, and examining the dynamics of colonising or invading species (Vargas *et al.*, 1997).

The temperature thresholds for *B. fusca* published differ between studies (Nye, 1960; Usua, 1968, 1973; Harris & Nwanze, 1992; Dixon *et al.*, 2009). Thermal requirements may, however, vary among different populations (Lee & Elliot, 1998, Gomi *et al.*, 2003) because of different geographical areas with variable climate conditions due to a gradient of latitude (Honék, 1996, Addo-Bediako *et al.*, 2000, Chen & Kang, 2004). Honék (1996) has shown that in subtropical and temperate zones the lower development threshold decreases with increasing geographical latitude. The thermal constants and lower and upper threshold temperatures of *B. fusca* determined for populations in different geographical areas will

enable the prediction of the impact of climate change on the distribution and population growth of this pest.

1.8 Objectives of this study

1.8.1 General objective

The main objective of the study is to evaluate the effect of temperature on the development and reproduction of *Busseola fusca* (Lepidoptera: Noctuidae).

1.8.2 Specific objectives

The specific objectives were to determine:

- the development rate of *B. fusca* at different constant temperatures
- to determine the number of degree-days (°D) required for each stage to complete development as well as for overall egg-to-adult development
- to determine the number of larval instars by measuring larval head capsule width and to develop criteria to determine the specific instar of *B. fusca*
- to determine the effect of different temperatures on reproduction of *B. fusca*

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

- Chapter 2: The effect of temperature on the development of *Busseola fusca* (Lepidoptera: Noctuidae)
- Chapter 3: The effect of temperature on reproduction of *Busseola fusca* (Lepidoptera: Noctuidae)

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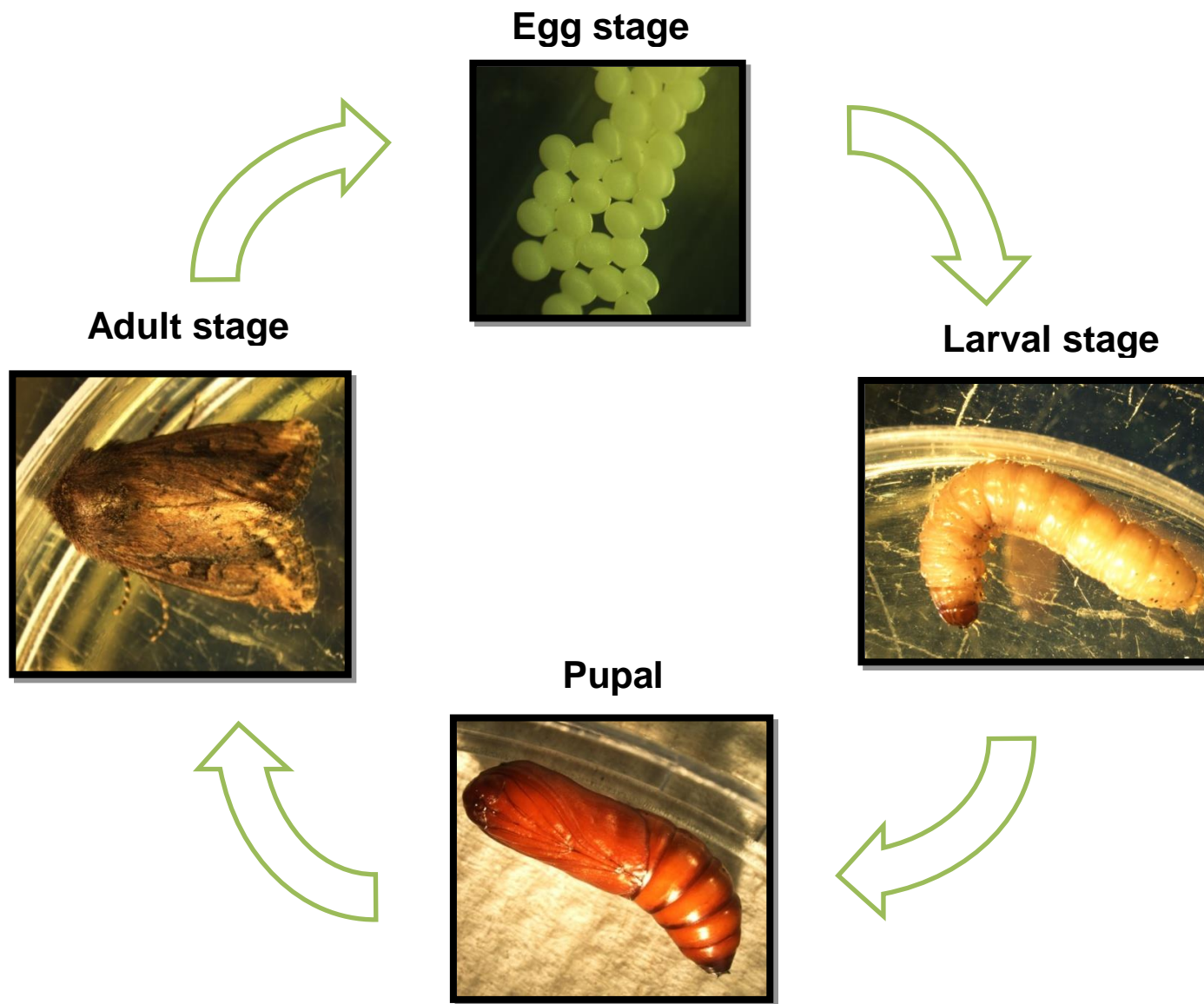


Figure 1.1: Life cycle of *Busseola fusca*.

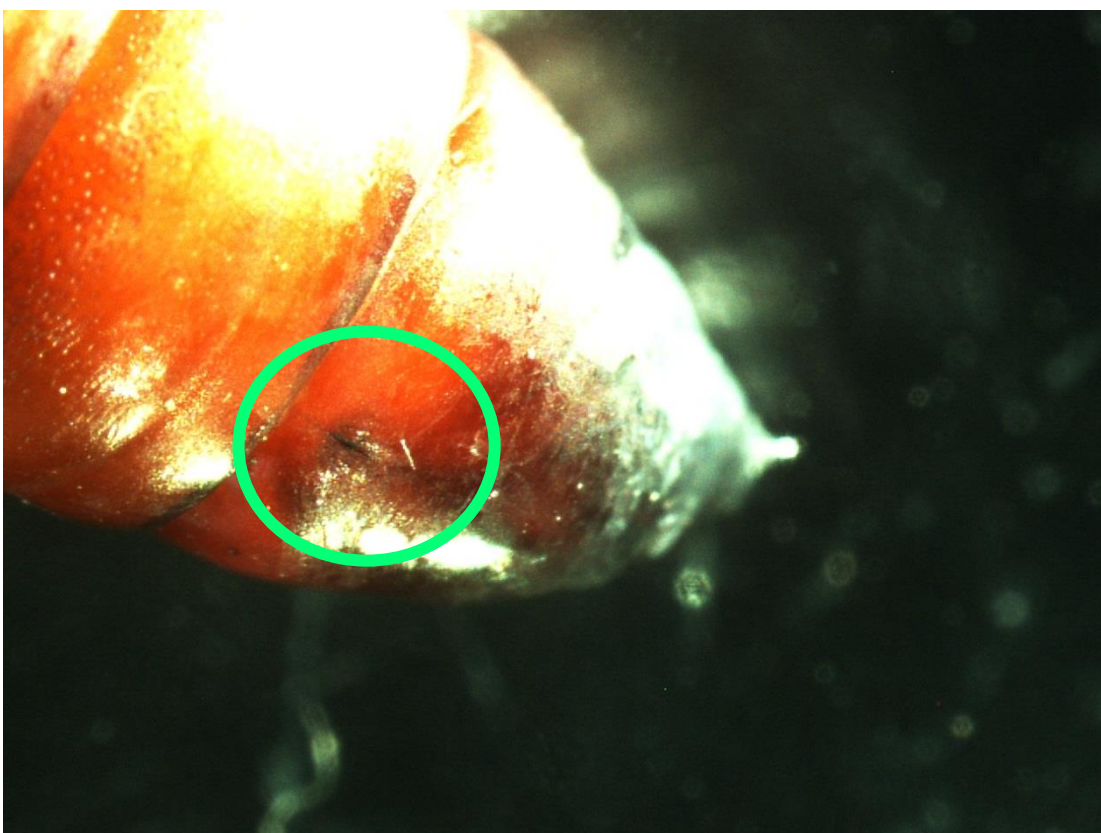


Figure 1.2: The male (top) and female (bottom) pupae of *Busseola fusca* distinguished by the genital scars on sternum 9 of males and sternum 8 of females.

CHAPTER 2

The effect of temperature on the development of *Busseola fusca* (Lepidoptera: Noctuidae)

2.1 Abstract

The African stem borer, *Busseola fusca*, is one of the major stem borer pests of maize throughout Africa. Climate change may in future affect the biology of this species. The effect of temperature on the development of *B. fusca* was studied at five different temperature regimes namely 15, 18, 20, 26 and 30 ± 1 °C. The number of instars for *B. fusca* was also determined. Development rate was inversely related to temperature within the range 15 - 26 °C, but development was reduced at 30 °C. The most favourable temperature as well as the upper threshold temperature for larval development was observed to be between 26 and 30 °C. There was a strong positive linear relationship between temperature and the rate of development of the egg, larval and pupal stages as well as total development. The total development period was 152.6 to 52.6 days, respectively, at 15 °C, and 26 - 30 °C. The thermal constants for *B. fusca* were 99.50, 536.48, 246.25 and 893.66 °D and lower temperature thresholds was 10.36, 8.14, 8.99 and 8.84 °C, for completion of the egg, larval, pupal, and egg-to-adult stages, respectively. The number of larval instars was determined by using head capsule widths that ranged from 0.31 - 2.68 mm. Overlapping occurred from instar 4 to 6. No distinction could be made between instars 7 and 8 in terms of head capsule width. All successive instars, except for instar eight, increased in size according to Dyar's ratio.

Key words: *Busseola fusca*, degree-days, development, head capsule widths, temperature

2.2 Introduction

2.2.1 The effect of temperature on insect development

Information on insect pest development under different environmental conditions is vital for forecasting, management and risk analysis (Calvo & Molina, 2005). Pest management is improved by knowledge of the life stages that cause damage, and an understanding of the conditions that slow or accelerate development (Wilson & Barnett, 1983; Higley & Peterson, 1994; Waldstein & Reissig, 2001).

Temperature plays an important role in the development and growth rates of an insect, the duration of its life cycle and survival (Roy *et al.*, 2003). Development occurs within a specific temperature range and is best performed at an optimum temperature. Development rate decreases as the temperature decreases or deviates from the optimum (Begon *et al.*, 2006). Temperature can also affect the physiological traits of insects by causing extra moults and larval stages (Ali & Gaylor, 1992). Other physiological effects of insects that can also be affected are for example change in body-size, metabolic rates, water relations and feeding habits (Speight *et al.*, 2008).

Making use of studies at constant temperature can provide a basis for prediction of phenological and seasonal development of insects in the natural environment (Mironidis, 2014). However, temperature in the natural environment fluctuates and will affect the life-time parameters of insects differently compared to constant temperatures. Determining development under constant temperature can, however, provide information on pest population dynamics and accurate scheduling of control techniques of pests (McFarland *et al.*, 1992; Shanower *et al.*, 1993).

2.2.2 Larval development of *Busseola fusca* (life cycle)

Busseola fusca (Lepidoptera: Noctuidae) is oligophagous (Le Ru *et al.*, 2006a, b; Ong'amo *et al.*, 2006; Ndemah *et al.*, 2007), which implies it feeds on a limited number of plant species (Bernays & Chapman, 1994). Duration of egg development of *B. fusca* is approximately 5 - 6 days depending on temperature. The eggs are yellow and turn black before hatching (black head stage). After emergence, first instar larvae disperse over the leaves to settle on their host plants before they start to feed on the leaves or whorls. Most

larvae up to the 4th instar will feed in the whorl part of the plant, thereafter moving to the stems (Van Rensburg *et al.*, 1987; Harris & Nwanze, 1992). Once there, they will feed for about 3 - 5 weeks in the stems and maize ears producing tunnels before entering the pupal stage (Harris & Nwanze, 1992; Onyango & Ochieng'-Odero, 1994; Ratnadass *et al.*, 2001). The larval stage is about 24 - 54 days long depending on the temperature (Calatayud *et al.*, 2007).

The duration of the pupal stage is about 9 - 14 days, depending on the temperature (Harris & Nwanze, 1992; Onyango & Ochieng'-Odero, 1994; Ratnadass *et al.*, 2001). The life cycle of *B. fusca* is completed within 7 - 8 weeks if the conditions are in their favour (Harris & Nwanze, 1992). Not all of the larvae pupate; some of them enter diapause which normally happens during winter time when the environment is dry and cold (Kfir, 1991; Kfir *et al.*, 2002).

2.2.3 Geographical distribution of *Busseola fusca*

Busseola fusca is an indigenous pest species in sub-Saharan Africa (Mohyuddin & Greathead, 1970; Kfir *et al.*, 2002; Le Ru 2006a, b). They attack grain crops (Harris, 1989) and compared to other stem borer species, normally dominates in the high altitude regions (Van Rensburg & Bate, 1987). Even though it appears as if *B. fusca* occurs only at high altitudes, reports have shown that they also occur at low altitudes in East Africa (Calatayud *et al.*, 2014) and Zimbabwe (Sithole, 1989). These species are also found in the cooler eco-zones of East and southern Africa, in mid-altitude areas and the highlands (Kfir *et al.*, 2002).

Although *B. fusca* is an important pest in Africa, its pest status varies among different regions. In East and southern Africa, *B. fusca* has been found at higher altitudes (>600 m) (Nye, 1960; Sithole, 1989), but in West Africa, it has been found from sea level to >2000 m (Tams & Bowden, 1953), as well as in the dry savannah zone (Harris, 1962). In South Africa and other African countries, *B. fusca* have been found at elevations 900m above sea level, at lower altitudes (Sithole, 1989; Kfir, 1998), at coastal regions (Van Rensburg, 1997; Waladde *et al.*, 2001), and in the mountains 2131 m a.s.l. in Lesotho (Ebenebe *et al.*, 1999). In Eritrea, *B. fusca* mostly occur at altitudes higher than 1500 m (Haile & Hofsvang, 2001).

2.2.4 Climate Change

Climate change has an impact on environmental conditions such as temperature and precipitation that may lead to extreme events, which may have an effect on the environment and the organisms living in it (IPCC, 2007). Determining the effect of temperature on development of insects will help to predict the life cycle duration and the timing when seasonal emergence occurs as changes in climate occurs (Angilletta & Dunham, 2003; Kingsolver & Huey, 2008). Climate determines the distribution and abundance of most insect species (Sutherst, 2000). Their habitats and survival strategies are dependent on the local weather conditions. Since insects are ectotherms and their body temperature varies with the environmental temperature, changes in temperature will have an effect on insects (Harrington *et al.*, 2001; Speight *et al.*, 2008).

2.2.5 Head capsules

Determination of larval instars can provide valuable information in pest management (Daly, 1985) providing basic information for the development of morphometric and ecological studies (Williams & McDonald, 1982; Fischbacher, 1996). The head capsules of larvae are sclerotised (Anderson, 2003), and does not grow or expand during each larval stage. Therefore, the width of head capsule is an important parameter in identifying larval stages (Daly, 1985) and has been used for many lepidopteran and coleopteran species (McClellan & Logan, 1994; Goldson *et al.*, 2001; Hammack *et al.*, 2003). Many mathematical models have been used to determine the different instars of insects. One of the best known models for distinguishing between instar stages is the Dyar's rule (Gaines & Campbell, 1935). This model explains that the head capsule of an instar stays more or less constant during one instar stage but increases with every moult. Other statistical methods assume that the head capsules widths are normally distributed for each instar and the peaks indicate each instar (Sokal & Rohlf, 1995; Panzavolta, 2007). Knowledge regarding pest population age and phenology contribute to improved timing of spray applications aimed at certain instars as well as explaining possible treatment failures (McClellan & Logan, 1994).

2.2.6 Objectives

The objectives of this study were to assess the development rate of *B. fusca* at different constant temperatures, to determine the number of degree-days (°D) required for each stage

to complete development as well as for overall egg-to-adult development, and to determine the number of larval instars by measuring larval head capsule widths and to develop criteria to determine the specific instar of *B. fusca*.

2.3 Materials and Methods

2.3.1 *Busseola fusca* stock colony

Maize stem borer larvae were collected from maize fields (F_0) in the North-West province at Brits (S25°23'22.7" E27°34'40.4"), Orkney (S26°56'59.7" E26°53'49.6") and Potchefstroom (S26°46'58.4" E27°08'17.1") as well as at the Vaalharts irrigation scheme (S27°44'43.6" E24°47'02.5") (Northern Cape province). These larvae were reared in plastic containers (40 x 20 x 15 cm) with aerated lids on conventional (non-Bt) maize stems which were replaced at four day intervals. They were kept in a rearing room at 26 ± 1 °C, 70% RH and 14L: 10D photoperiod until pupation. Male and female pupae were separated and kept individually in small plastic bottles (52 mm high and 30 mm in diameter) in the same rearing room as the larvae. Pupae were observed daily until the moths emerge.

After emergence of the moths, single male-female pairs were confined to oviposition chambers in a rearing room at 26 ± 1 °C, 70 ± 10 % RH and a 14L: 10D photoperiod. The chambers and method used are according to Kruger *et al.* (2012). A plastic bottle (22 cm high and 10 cm in diameter) was cut open at the top (Fig. 2.1a). The bottle was filled with crusher stones up to a height of 5 cm. One thick maize stem (25 - 30 mm diameter) and 18 cm in length with bases of leaves intact was placed in an upright position in the bottle. Stems were inserted 3 - 4 cm into the crusher stones to keep the maize stems upright. Water was added up to a level three-quarter of the height of the stones to provide humidity and to keep the stems fresh. The containers were covered with a fine gauze mesh to prevent escape of moths. The stems were observed daily for egg batches and replaced every second day.

2.3.2 Temperature-dependent egg development

The egg batches from the stock colony were removed from maize plants within 12 hours of oviposition. They were collected by cutting off the piece of leaf sheath to which the egg batches were attached. About 50 eggs were placed in a small plastic container (52 mm high and 30 mm in diameter) with a steel mesh infused lid. Thereafter, these plastic containers

were kept in a glass desiccator (150 mm) in which RH was maintained at 70 ± 10 % using a potassium hydroxide solution according to the method of Solomon (1951) (Fig. 2.1b). The desiccators were kept at the 15, 18, 20, 26 and $30^\circ \pm 1$ °C in incubators with a 14L: 10D photoperiod. The temperature and RH in each desiccator was recorded at 30-minute intervals using iButtons® from Coldchain Thermo Dynamics (Fairbridge technologies) as seen in figure 2.2. The eggs were checked daily until they hatched. The number of days for hatching was recorded until five days after the first larvae hatched.

2.3.3 Temperature-dependent larval and pupal development

Eggs were collected from moths kept at 26 ± 1 °C, 70 ± 10 % RH and a 14L: 10D photoperiod and transferred into small plastic containers (52 mm high x 30 mm in diameter) with a steel mesh infused lid and placed into a glass desiccator (150 mm) in which RH was maintained at 70 ± 10 % using a potassium hydroxide solution according to the method of Solomon (1951). The desiccators were kept under the same conditions as the abovementioned moths were kept. After hatching, fifteen neonate larvae (F_1) were placed on the whorl of a maize seedling in a plastic test tube (115 mm high x 29 mm in diameter) closed with a steel mesh infused lid (Fig. 2.1c). Larval and pupal development was studied under the same conditions of constant temperature and photoperiod as for the eggs in incubators. There were 15 - 20 test tubes containing 15 larvae each per temperature regime. A piece of filter paper (4 cm^2) was placed on the bottom of each test tube to avoid excessive wetness inside the test tube. The test tubes were cleaned daily.

The whorls were checked daily for head capsules and exuviae (shed cuticles). Head capsule and exuviae found were removed to avoid confusion. On moulting to the second instar, larvae were transferred individually to a test tube containing a piece of maize plant consisting of compact unfolded leaves above the growing tip (Fig. 2.3). Daily observations of the larvae were made and moulting as well as survival was recorded. Food was replaced every second day. From fifth instar onwards larvae were fed with a piece of maize stem cut from the base of the whorl. Later instar larvae were fed with stem pieces of older plants (Fig. 2.3 and 2.4). To provide for checking of larvae inside the stem, stems were cut longitudinally and kept together with an elastic band (Fig. 2.5). The temperature and RH in the maize stem at each temperature regime was recorded at 30-minute intervals using iButtons® from Coldchain Thermo Dynamics (Fairbridge technologies) as seen in figure 2.2.

When pupation occurred, the test tube was cleaned and the pupa was transferred back into the tube. Thereafter, it was placed in a plastic container (37.7 x 26.8 x 19.9 cm) enclosed with a steel mesh infused lid containing water in a small bowl to keep RH at a level of 75 ± 10 % at the respective temperatures (Fig. 2.1d). Pupae were checked daily until the emergence of the moths and the number of days to emergence was recorded. The temperature and RH in the container at each temperature regime was recorded at 30-minute intervals using iButtons[®] from Coldchain Thermo Dynamics (Fairbridge technologies) (Fig. 2.2).

2.3.4 Number of instars

First instar larvae were collected within 24 hours of hatching from small plastic containers in a glass desiccator which was kept at 26 ± 1 °C and 14L: 10D (see 2.3.3). These larvae were reared individually in plastic test tubes (115 mm high x 29 mm in diameter) closed with a steel mesh infused lid. After moulting, thirty larvae of each consecutive instar were fixed in 70% ethanol. Head capsules were measured under a stereo microscope (Nikon SMZ 1500) equipped with a camera and the NIS-Elements D 3.1, Microscope Imaging Software. The head capsule widths were measured as the distance between the most distant lateral sides of the head capsule margins.

2.3.5 Data analysis

Degree-day model

The relationship between temperature (x) and development rate (y) was determined by using a simple linear regression analysis. The lower threshold temperature (t) and number of degree-days (k) required to complete development for each of the stages, as well as their standard errors were calculated using the equations of Campbell *et al.* (1974). The lower threshold temperature was estimated by setting $y = 0$ and solving x for the regression equation, $y = a + bx$, where:

$$y = 1/\text{days}$$

$$x = \text{temperature}$$

$$a = \text{intercept}$$

$$b = \text{slope}$$

$$\text{the lower temperature threshold: } t = -a/b$$

$$\text{number of degree-days (°D): } k = 1/b$$

$$\text{S.E. of } t = \bar{y}/b \sqrt{(s^2/Ny^{-2})} + [\text{S.E. of } b/b]^2$$

$$\text{S.E. of } k = (\text{S.E. of } b)/b^2$$

The mean degree-days (°D) for the development of the egg, larval and pupal stage was estimated using the equation of Jackson & Elliot (1988): $^{\circ}\text{D} = T(c - T_{\min})$, where T is the number of days taken to complete development at a constant temperature (c) and T_{\min} is the minimum temperature for development. The thermal constant was used and the mean number of °D required for development of each life stage at the set constant temperatures were compared.

The effect of temperature on development was analysed by means of one-way ANOVA using STATISTICA 12 (Statsoft, Inc., 2013), followed by Tukey's HSD test ($P = 0.05$).

Head capsule width

The number of instars was reported by means of frequency distribution of head capsule width and ranges were assigned to individual instars based on measured larval instar head capsule width. The possibility of assigning specimens to instars by Dyar's rule (Dyar, 1890) as [postmoult size/premoult size (moult increment) = constant], was determined. Linear regression analysis was used to establish relationships between temperature and growth ratios.

2.4 Results

Busseola fusca completed development at all temperatures between 15 and 30 °C (Table 2.1). Development time for eggs and larval instars one to five was inversely related to temperature from 15 to 26 °C, with development for all life stages significantly delayed at 15 °C. Incubation time for eggs significantly decreased from 22.5 days at 15 °C to 5.9 days at 30°C ($F_{4,102} = 938.3$; $P < 0.001$). *Busseola fusca* larvae completed their life cycle the slowest at 15 °C (90.2 days) and the fastest at 26 °C (31.2 days) (Table 2.1). Development time was longer at 30 °C, although not significantly different from 26 °C. Total development of *B. fusca* took 152.6 days at 15 °C and 52.6 days at 26 and 30 °C. In general, development time decreased as temperature increased. However, at 30 °C, duration of development for fifth instar larvae onwards, as well as total larval development time increased (Table 2.1). The upper threshold temperature for larval development is therefore between 26 and 30 °C.

All larvae went through six larval instars, 70 of these went through seven instars and four larvae reached an eight instar before pupation. One larva moulted within one day from fifth to sixth instar at 30 °C and several larvae took two days to complete an instar at 26 and 30°C for consecutive instars from the second to sixth instars and from the second to fourth instars, respectively. The longest development time spent in an instar was 32 days at 15 °C (Table 2.1).

There was a significant difference in development time at 15, 18, 20 and 26°C for first to fifth instar larvae [(first instar: $F_{4,125} = 271.2$; $P < 0.001$), (second instar: $F_{4,125} = 133.5$; $P < 0.001$), (third instar: $F_{4,125} = 203.4$; $P < 0.001$), (fourth instar: $F_{4,125} = 122.6$; $P < 0.001$) and (fifth instar: $F_{4,125} = 102.1$; $P < 0.001$)]. Development time was also similar for instars one, three, four, five, six and seven at 26 and 30 °C (Table 2.1). Development time of second instar larvae differed significantly between all temperatures evaluated in this study (Table 2.1).

Development was significantly longer at 15 °C compared to the other temperatures. Sixth instar larvae developed at a similar rate at 18 and 20 °C, which was significantly longer than at 26 and 30 °C ($F_{4,125} = 23.7$; $P < 0.001$). Although sixth instar larvae developed slower at 30 °C compared to 26 °C, this difference in development time was not significant (Table 2.1). Development time of sixth instar larvae accounted for 22 - 32 % of the total larval development period and 14 - 19 % of the total development period. The duration of development time for larval instar six and onwards (instar 6 to 8, where applicable), accounted for 48 - 59 % of the total- and 80 - 96 % of the larval development period, respectively.

Larval survival was low at all temperatures evaluated with the highest percentage survival of 25 %, at 20 °C (Table 2.1). It was lower at 15, 18 and 30 °C compared to 20 and 26 °C. Almost no larvae (3.3%) survived and completed their development at 15 °C. The optimum temperature for *B. fusca* development, in terms of survival and development is therefore between 20 and 26 °C.

The relationship between temperature and developmental rate of *B. fusca* was linear between 15 and 26 °C and more rapid development was observed with increasing temperatures (Fig. 2.6 and Fig. 2.7). The highest rearing temperature of 30 °C was excluded from the least square linear regression analyses, since it did not fit the linear portion of the graph. The correlation (r-value) between temperature and development rate ranged between 0.93 and 0.96 for eggs, total larval development, pupae and egg-to-adult development (Table 2.2). Linear regression equations describing these relationships and estimates of the lower temperature threshold (t) and the number of degree-days (°D) for each life stage are summarized in table 2.2. *Busseola fusca* required a thermal constant (k) of 99.5, 536.5, 246.3 and 893.7 °D for completion of the egg, larval, pupal and egg-to-adult development, respectively (Table 2.2), which is comparable to the mean degree-days estimated as determined in this study (Table 2.3). The number of degree-days estimated to complete the life cycle of *B. fusca* at 15, 18, 20 and 26 °C were also similar (Table 2.3). Based on linear regression analysis of development rate at all temperatures, a minimum temperature threshold between 8 and 11 °C was estimated depending on the development stage. The minimum threshold temperature for the larval stage was lower than for the egg stage. Eggs will therefore hatch at temperatures which is also suitable for larval development.

Head capsule widths of *B. fusca* ranged from 0.31 mm to 2.68 mm (Fig. 2.8). The frequency distribution of head capsule widths of instar 1, 2 and 3 was absolute with no overlapping. The distribution of head capsule widths showed overlapping from the fourth instar onwards.

No distinction could be made between instars 7 and 8 in terms of head capsule width. All successive instars, except for instar eight, increased in size according to Dyar's ratio (Table 2.4). The head capsule width distribution of *B. fusca* was also confirmed by linear regression analysis indicating a relationship between larval instars and (log head capsule width) with the r-value of 0.975. Instar 8 did, however, not fit the linearity and the capsule widths overlapped with those of instar 6 and 7 larvae (Fig. 2.9). The head capsules of each instar (1 - 7) are shown in figure 2.10.

2.5 Discussion

Insects have a limited temperature range within which they can develop. There is an optimal temperature point where individual development occurs at its best, and the upper and lower lethal points which may lead to the death of the insect. Considering development time and survival in this study, the most favourable temperature range for *B. fusca* development was between 26 and 30 °C. Usua (1968) reported a suitable range for *B. fusca* development to be between 20 and 30 °C, with the optimum temperature range between 26 and 30 °C. Results from the current study is in concordance with this suitable range as suggested by Usua, (1968), but supports the optimal temperature range for *B. fusca* development which was also determined by Khadioli *et al.* (2014) to be between 25 and 28 °C.

Busseola fusca eggs, larvae and pupae did develop at a constant temperature of 15 °C, although very slowly and the percentage survival was low compared to higher temperatures. These results differ from those of Usua (1968), who reported that 22 °C was too cold for development. However, these results are in accordance with Khadioli *et al.* (2014) who reported development to occur at 22 °C. Usua (1968) also reported temperatures of 32 °C and above as lethal to *B. fusca*. This was supported by the findings of Khadioli *et al.* (2014) that no development occurred above 30 °C. During the present study, development was completed at 30 °C, but development rate was not linear with development at lower temperatures. Development was slower at 30 °C than at 26 °C, indicating that the maximum temperature for development was exceeded. Since mortality of larvae was also high at a constant temperature of 15 and 20 °C, continuous low temperatures, although not extreme, will reduce a population.

Development rate was inversely related to temperature up to 26 °C. Development time of eggs accounted for 11 - 15 % of the total development period at the respective temperatures. Development time of later instar *B. fusca* larvae (sixth instar onwards) accounted for 80 %

and more of the total larval development period, regardless of the temperature. Later instar larvae consume more plant material than earlier instars, because they are larger and approach pupation. In many insect species, the last instar of the immature stages needs to ingest large amounts of food to maximise the reproductive potential of the adult (Scriber & Slansky, 1981). Maize plants are therefore longer exposed to the later and thus more damaging instars of *B. fusca* compared to the less injurious instars.

A recent temperature study on *B. fusca* in Kenya, reported total development from 223.85 - 62.74 days at 15 - 28 °C, respectively (Khadioli *et al.*, 2014). The development time at 30°C increased to 81.26 days (Khadioli *et al.*, 2014). The Kenyan *B. fusca* population therefore had a longer development time than the South African population used in the present study. Photoperiod did, however, differ with a 12L: 12D photoperiod used in Kenya compared to 14L: 10D in South Africa. The incubation period of eggs decreased with increasing relative humidity. At 80 % and higher the incubation period was 5 - 6 days to hatching, at 70 % RH, 6 - 7 days and 60 % RH, 7 - 8 days (Usua, 1968). The difference in relative humidity used in the two studies compared, may therefore also account for some of the observed differences. Unnithan (1987) also reported temperature-dependent development of *B. fusca* from Kenya where larval to adult development time was an average of 40.8 days and the egg stage was between 5 - 6 days at 23 - 27 °C. Thus, complete development was between 45.8 - 46.8 days.

Development at constant temperatures was also studied for other lepidopteran stem borer species with similar temperature thresholds as with *B. fusca* during this study. The spotted stem borer, *Chilo partellus* (Lepidoptera: Crambidae), was studied under three temperature regimes, namely 22, 26 and 30 °C using different levels of RH of 40, 60 and 80%, and its development was found to be significantly affected by temperature and RH (Tamiru *et al.*, 2012). The mean number of days to complete its life cycle was 70.2 days at 22 °C and 80 % RH and 26.5 days at 30 °C and 40% RH. The most suitable temperature for development of *C. partellus* was reported to be between 26 - 30 °C (Tamiru *et al.*, 2012). Larval development time of *Sesamia nonagrioides* (Lepidoptera: Noctuidae) significantly declined as temperature increased between 14 - 25 °C (Andreadis *et al.*, 2013). The upper and lower development threshold temperatures ranged between 31.2 - 36.2 °C and 7.0 - 10.3 °C, respectively and the optimum temperature was reported between 28.1 - 30.3 °C (Andreadis *et al.*, 2013). Complete development rate of *Helicoverpa armiga* (Lepidoptera: Noctuidae) decreased from 57.2 - 23.4 days as temperature increased from 17.5 to 30.0 °C (Mironidis & Savopoulou-Soultani, 2008). Development for this species was not completed at 15 °C,

therefore having a higher, lower temperature threshold than 15 °C (Mironidis & Savopoulou-Soultani, 2008).

The larval stage of *B. fusca* reared on maize stems at temperatures between 26 and 30 °C in the current study lasted on average 32.1 and 33.4 days, respectively. However, Ratnadass *et al.* (2001) reported 50 days at 25 ± 1 °C and Onyango & Ochieng'-Odero (1994) an average of 46 days at temperatures between 25 and 30 °C. Khadioli *et al.* (2014) reported the larval stage duration to range from 50.7 at 25 °C to 58.1 at 30 °C. All these populations were from temperate regions and were reared on artificial diet compared to the South African *B. fusca* population of this study which originated from an area with sub-zero night temperatures during winter. The fact that larvae in this study were reared on maize tissue and not artificial diet may also have contributed to the observed differences. Development time of the eggs and pupae was similar when compared to the abovementioned studies. The average development time of *B. fusca* eggs from this study was 6.5 and 5.9 days and pupal development took 14.0 and 13.3 days, respectively at 26 and 30 °C. Eggs of *B. fusca* developed between five to six days at 23 - 27 °C (Unnithan, 1987). Onyango & Ochieng'-Odero (1994) reported 6 and 14 days respectively, for egg and pupal development at 26 - 30 °C. A recent study where *B. fusca* was also fed on an artificial diet reported the average development time for eggs and pupae as 7.2 and 14.7 at 25 °C and 6.5 and 16.6 at 30 °C (Khadioli *et al.*, 2014). The diet of larvae does, therefore not seem to affect development time of the pupae.

Although relative humidity is often reported in temperature-dependent development studies for insects, it reflects only the relative humidity inside of the incubator or rearing room. The actual temperature inside the container or exact place of rearing of the insect is often not recorded as was done in the present study. In this study, the relative humidity inside stems where the stem borer larvae were reared was always 100 %. The possibility of desiccation where larvae occur inside stems under field conditions where low humidity may prevail is therefore not possible, except during the natural dry off-season phase of the crop or towards the end of the season. Relative humidity is an important factor influencing the biology and development of insects (Gullan & Cranston, 2005). For example, low relative humidity can cause development rate to slow down (Gullan & Cranston, 2005). However, this will not be applicable to *B. fusca* occurring and developing in maize stems where 100 % humidity occurs during the active vegetative and reproductive growth stages of the plants.

The development rate of *B. fusca* was strongly related to temperature. The number of degree-days needed by *B. fusca* to complete the different life stages was 99.58, 543.58,

253.18 and 899.92 °D, and the lower temperature thresholds were 10.36, 8.99, 8.14 and 8.84 °C, respectively, for eggs, larvae, pupae and egg-to-adult. Survival of larvae was, however, still low at a temperature six degrees higher than the lower threshold temperature determined. This indicates that the optimum temperature for development should be well above the lower threshold temperature.

The thermal constants and development thresholds were also determined for other stem borer species. For *Eldana saccharina* (Lepidoptera: Pyralidae), the degree-days and thermal thresholds for the egg, larval and pupal stages were 119.0, 618.6 and 160.3 °D, above average thresholds of 5.3, 10.2 and 10.7 °C, respectively (Way, 1995). Total development period of *Sesamia calamistis* (Lepidoptera: Noctuidae), required 700 °D, eggs required 122 °D above a threshold of 9.7 °C, larvae, 383 °D above 12.2 °C and pupae 204 °D above 10.2 °C (Shanower *et al.*, 1993). The degree-days needed for the different development stages of *Chilo sacchariphagus* (Lepidoptera: Crambidae) are: eggs: 144 °D above 13.1 °C; larvae: 586 °D above 12.7 °C; pupae: 172 °D above 13 °C and egg-to-adult: 872 °D (Goebel, 2006). Variation in temperature thresholds is an important determinant in the distribution of insects (Cammell & Knight, 1992; Marco *et al.*, 1997). If a specific development stage cannot survive a certain temperature, then the distribution of the species is likely to be restricted by the temperature threshold of that stage (Cammell & Knight, 1992).

The geographical distribution of insect species can be ascribed to their adaptation to thermal conditions and their tolerances for these conditions (Bijlsma & Loeschcke, 2005; David *et al.*, 2005; Overgaard *et al.*, 2010). High temperatures may cause insects to avoid overheating through shade-seeking, avoidance behaviour, through increased evaporate cooling or biochemical reactions. They can create rapid biochemical protection such as heat shock proteins that will cause cell function to cease after the probability of cell damage (Denlinger & Lee, 2010; Terblanche, 2013). High temperatures also cause rapid response to hardening (inducible thermo-tolerance) when exposed to stressful conditions where the insect increase its tolerance level (Denlinger *et al.*, 1991; Loeschcke *et al.*, 1997; Dahlgaard *et al.*, 1998). Hardening in terms of high temperatures is more associated with rapid responses to short-term moderately stressful exposures (Denlinger *et al.*, 1991, Loeschcke *et al.*, 1997; Dahlgaard *et al.*, 1998). When insects are exposed to high temperatures, heat injury may occur depending on its severity. High temperature injury causes disruption of membrane function (Cossins & Bowler, 1987), changes in the cell microenvironment (pH), perturbation of protein structure, and DNA lesions (Somero, 1995; Feder, 1999). Therefore, these changes affect development, muscular contraction and other processes (Denlinger & Yocum, 1998). The membrane's responses to temperature cause changes in the

composition of cellular lipids (Gracey *et al.*, 1996; Somero *et al.*, 1996). Heat shock proteins are a response to stress such as thermal stress (Feder & Hofmann, 1999). Severe thermal stress can cause perturbation to the protein structure (Feder, 1996, 1999; Feder & Hofmann, 1999).

In temperate regions, there is a part of the year that is unsuitable for growth and development especially for insects (Nylin & Gotthard, 1998). Therefore, insects must have survival strategies in order to reach a certain size and development stage before the onset of winter. Consequently, development is time limited for temperate individuals. The time available for development can be estimated through variations in climatic variables such as temperature and photoperiod (Nylin & Gotthard, 1998).

Insects under cold temperatures below the optimum (sub-lethal and lethal temperatures) have more responses than at high temperatures (Lee, 1989). These responses to cold temperatures are cessation of activity and feeding, reduction in neuromuscular responses, chill coma or mortality (Chown & Nicolson, 2004), as well as responses to anti-freezing and to prepare itself against freezing temperatures (Lee, 1989). The rate at which insects cool, has an effect on their ability to survive low temperatures (Ramlov, 2000; Sinclair, 2001). Cold hardening is a slow process that gradually increases the insect's tolerance to cold temperatures increasing better survival in the nature (Denlinger & Lee, 1998; Kelty & Lee, 2001). Insects can survive extreme low-temperature environments through tolerating or avoiding freezing strategies that increases the probability to enter diapause at higher latitudes (Bale, 2002; Lee & Denlinger, 2010). The strategy used by *B. fusca* for survival during cold temperatures is to enter into diapause during the cold winter period (Kfir, 1991; Kfir *et al.*, 2002). During diapause, no activity occurs and this leads to profound drop in metabolic rate which is linked to an increase in cold hardiness (Bale *et al.*, 2002). Therefore, *B. fusca* can survive the cold winters of the South African maize producing region that often reach sub-zero temperatures at night. The thermo-biological scale given by Vannier (1994) shows that at temperature above and below the optimum, insects experience initially either heat or cold stupor, thereafter leading to either heat or chill coma, respectively, and ultimately death.

Temperature also affects the physiological traits of an insect. The body temperature of an insect affects their behaviour and physiology such as locomotion, immune function, sensory input, foraging ability, courtship and feeding and growth tempo (Angilletta *et al.*, 2002). Therefore, temperature determines the fitness of an insect (Akiyama & Nishida, 2013). Environmental conditions may play a role in the number of instars for certain insect species.

It may be more or less than the usual number of instars for a target species (Malusi & Okuku personal observation, 2013).

The maximum number of larval instars for *B. fusca* found during this study was eight. The head widths of larvae ranged from 0.31 mm in the first instar to 2.68 mm in the last larval instar prior to pupation. It is a known phenomenon that *B. fusca* usually has six instars, but the species are not restricted to six only. Eight instars were also reported by Unnithan (1987), Smithers (1960) and Calatayud *et al.* (2007). The head capsule widths measured in the current study for instars one to six ranged from 0.31 mm to 2.40 mm, and differed from the head capsule widths range of 0.32 - 2.2 mm for the respective instars reported by Unnithan (1987). Larvae kept for prolonged periods at 15 and 18 °C reached up to 10 larval instars determined by moulting only (J. Glatz, personal observation). Eight instars were also reported for the sugarcane stem borer, *C. sacchariphagus*, at temperatures from 17 - 35 °C (Goebel, 2006). For *E. saccharina*, five to seven instars were reported (Atkinson, 1980; Way, 1995; Bonato & Schulthess, 1998).

Some insects can exhibit catch-up growth when certain environmental conditions cause a delay in their development. Supernumerary moults by the African armyworm, *S. exempta* (Lepidoptera: Noctuidae) were needed to reach the same final body size when reared on poor quality grasses (Yarro, 1985). According to Stockoff (1993), female gypsy moths (*Lymantria dispar*, Lepidoptera: Lymantriidae) went through an extra larval instar to store more nutrients for oogenesis. *Sesamia nonagrioides* increases its weight during extra moults prior to diapause and during short day photoperiods of 10L: 14D (Eizaguirre *et al.*, 1994; Gadenne *et al.*, 1997). Larvae moult mainly to increase the size of its mouthparts (Esperk & Tammaru, 2004). Thus, during moults feeding may be limited that results in a loss of growing time. Growth slows down remarkably before moulting and stops completely when the process starts (Sehnal, 1985).

According to Dyar's rule, a straight line should result if the logarithm of the measurement of a sclerotised body part in different instars is plotted against the instar number, and any deviation from a straight line indicates missing instars (Gullan & Cranston, 2005). A linear progression therefore exists if the growth ratio is constant between the head capsule widths of each instar for a given species where the ratio ranges between 1.3 - 1.7 (Gullan & Cranston, 2005). The larval instars of *B. fusca* followed Dyar's rule up to the seventh instar, but the head width measurements indicated that there was no difference in head capsule widths of instar seven and eight even though these larvae moulted.

Malusi & Okuku (personal observation, 2013) reared *B. fusca* larvae under optimum environmental conditions (25 °C and 50 - 60% RH) on an artificial diet and reported only five instars within its life cycle that was completed at an average of 35 days. Under less favourable conditions such as a sub-optimal temperature or where larvae enter a diapause, additional instars were observed (Malusi & Okuku, personal observation 2013). Additional moultings were also reported for other lepidopteran species depending on the environmental conditions. An extra moult occurred for *Spodoptera exigua* (Lepidoptera: Noctuidae) with six instead of five instars at the extreme temperatures of 15 and 34 °C (Karimi-Malati *et al.*, 2014). The size of these larvae was, however, smaller at 33 - 34 °C than at 25 °C. Karimi-Malati *et al.* (2014) speculated that the extra moults may be needed for larvae to reach a critical size.

Insects can modify their responses to temperature effects, a phenomenon known as capacity adaptation. Resistance adaptations can also occur when insects are faced with lethal effects of temperature extremes (Cossins & Bowler, 1987). However, insects don't exactly adapt to the changes but the responses are mostly a consequence of phenotypic plasticity or flexibility (Pörtner, 2001). Insects can alter the relationship between temperature they experience and their survival probability, as well as the costs of these changes and the similarities and differences between the responses to upper and lower lethal temperatures (Chown & Nicolson, 2004). Each life history trait has benefits and costs. Increased development time can increase the risk of predation and high growth rates depend on rapid feeding rates (Bernays, 1997). High growth rates are expected to be associated with rise in temperatures but other factors may also play a role. Therefore, time can be a limiting factor leading individual larvae to increase their growth rates and thereby achieve shorter development times but at a cost in final body size when fully grown (Gotthard, 2004).

Insects experiencing changes in environmental temperatures have fluctuations in their metabolism, even when they are inactive. Energy is produced through the metabolism of substrates and a high rate of metabolism contributes to rapid growth rates as long as food resources are abundant (Speight *et al.*, 2008). Metabolic rate of insects has high fluctuations and is affected by environmental (e.g. temperature), developmental, behavioral and evolutionary factors (Waters & Harrison, 2012). Since energy consumption is low and metabolic rates are slow in cold environments, insects can survive longer without starving under such conditions. The same survival strategy can occur when food resources are scarce (Speight *et al.*, 2008).

Constant feeding is important to ensure optimal growth and development. The food quality and quantity consumed by insects determine its growth rate, development time, body mass and survival (Slanky & Scriber, 1985). For larvae, high protein amounts are required for rapid tissue growth (Bernays, 1986; Waldbauer & Friedman, 1991). Larvae have faster growth rates, and double the consumption and gut capacity of grasshoppers or cockroaches (Bernays, 1986). In this study, higher temperatures caused *B. fusca* to eat rapidly and grow faster. This will result in more generations per year under favourable environmental conditions. Nitrogen-rich proteins are the major building blocks of tissue. Therefore, young larvae need relatively large amounts of protein in their diets. Many plants have relatively low levels of organic nitrogen in their tissues so insects have developed many ways to increase the levels of protein in their diet (Speight *et al.*, 2008). One protein source utilized by insects is to eat its own exuviae (shed cuticle) (Mira, 2000). This has been observed for *B. fusca* larvae especially the older larvae (J. Glatz, personal observation). Other chemical components are also important for insects such as carbohydrates that provide the energy (fuel) and fats for fuel storage (Speight *et al.*, 2008).

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Figure 2.1: Containers used for rearing of *Busseola fusca*: a) oviposition chambers with one cut maize stem per moth pair; b) desiccator with small plastic containers for egg development; c) test tubes used for larval development; d) plastic container with water and test tubes containing pupae.



Figure 2.2: Temperature and RH recorded inside a maize stem with an iButton®.



Figure 2.3: *Busseola fusca* larvae where fed with compact unfolded leaves from the second to the fifth instar (above) and a piece of maize stem from the six instar onwards (bottom).



Figure 2.4: Damage by *Busseola fusca* larvae through tunnelling into maize stems.



Figure 2.5: Piece of maize plant cut longitudinally and kept together with an elastic band for daily observation of *B. fusca* larvae.

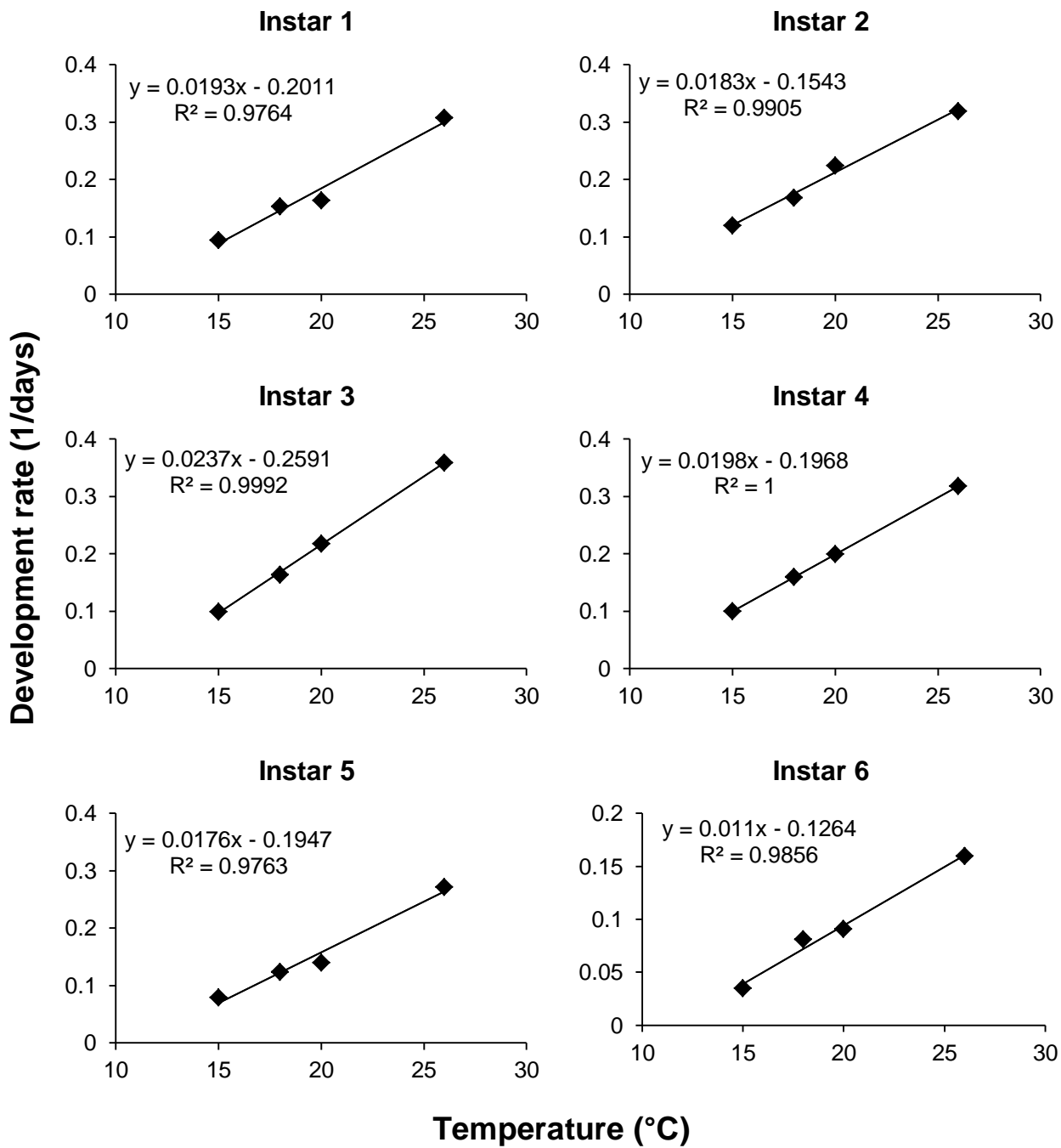


Figure 2.6: The relationship between *Busseola fusca* development rates and rearing temperature for larval instar one to six.

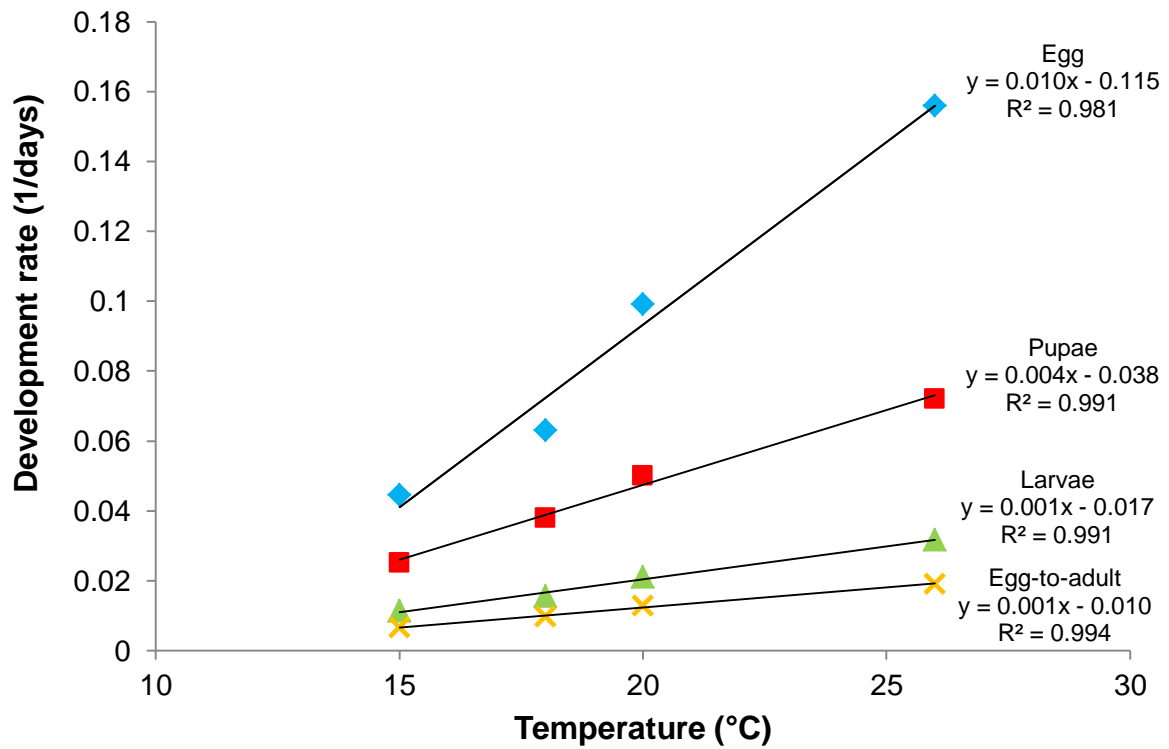


Figure 2.7: The relationship between development rates and rearing temperature for eggs, larvae, pupae and egg-to-adult stages of *Busseola fusca*. (Development rates for larvae and egg-to-adult stage include instars 7 and 8).

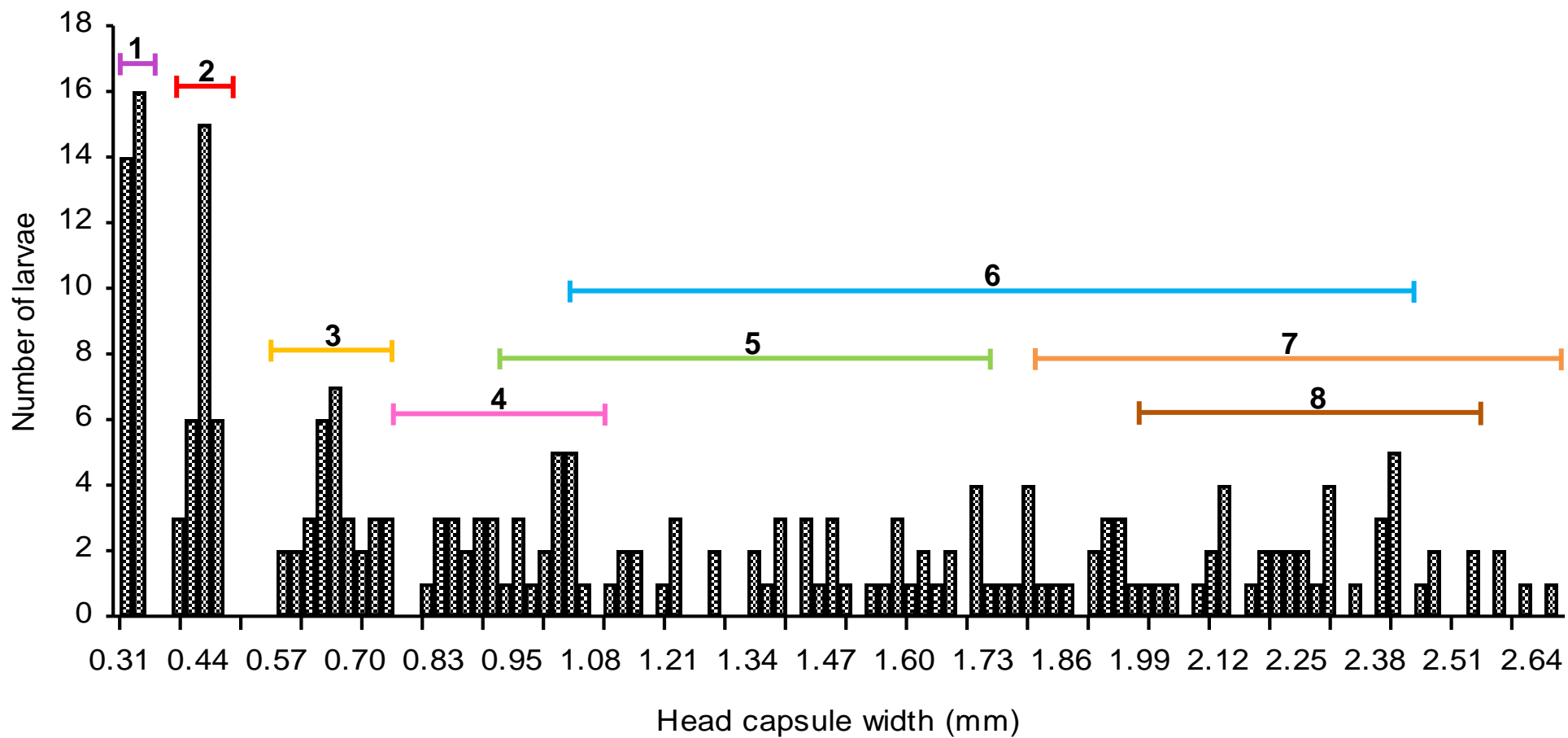


Figure 2.8: Frequency distribution of head capsule widths of *Busseola fusca* larvae. Each coloured line indicates the range of a specific instar.

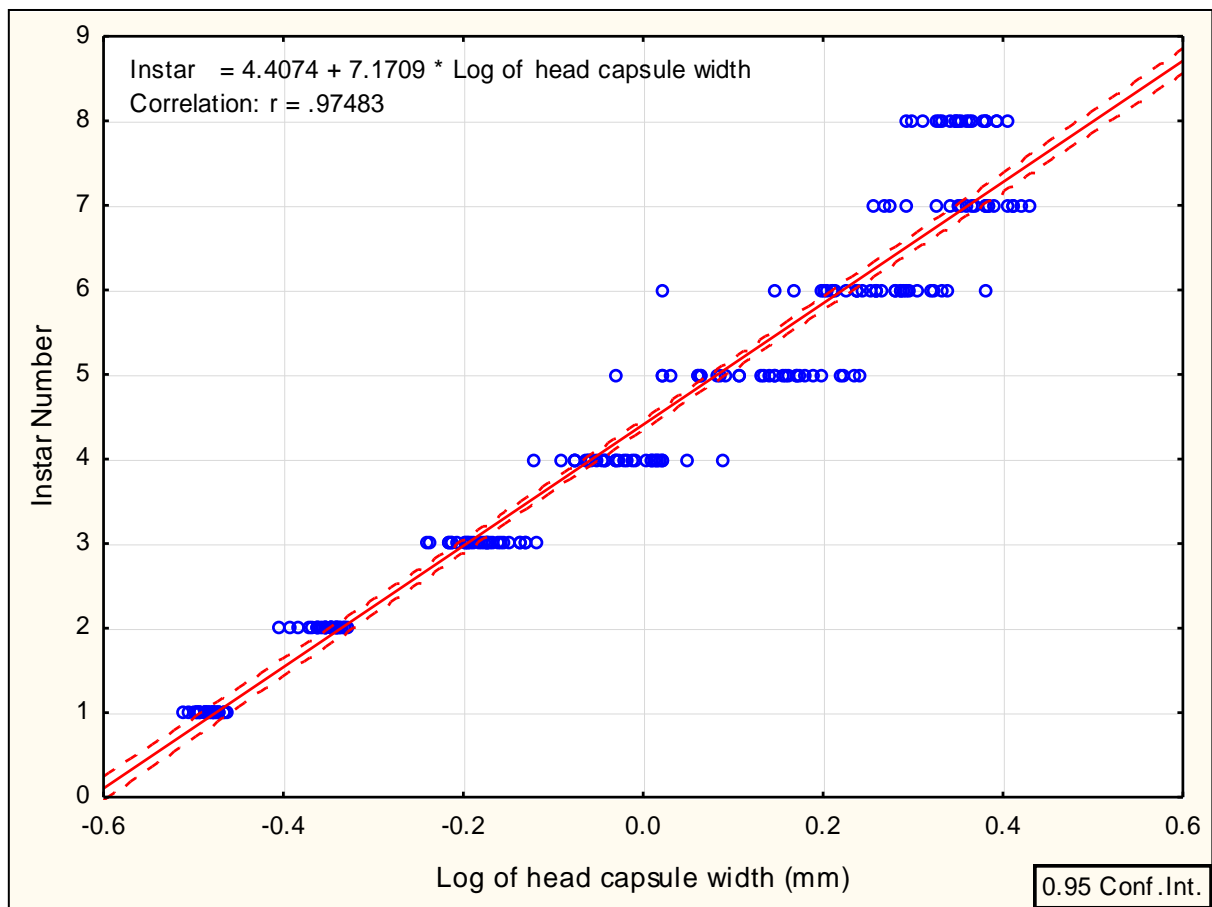


Figure 2.9: Relationship between head capsule width and instar of *Busseola fusca* larvae. The linear regression shows a straight line which fitted Dyar's rule.



Figure 2.10: Head capsules of *Busseola fusca* larvae indicating instars one to seven.

Table 2.1: Mean development time (days \pm S.E.) of different life stages and larval survival of *Busseola fusca* at constant temperatures. The range of days to develop is shown in brackets.

Temperature (± 1 °C)	15	18	20	26	30
Egg	22.5 \pm 0.2 a (21 - 24)	12.8 \pm 0.1 b (12 - 13)	10.2 \pm 0.2 c (9 - 12)	6.5 \pm 0.1 d (6 - 8)	5.9 \pm 0.04 e (5 - 6)
Instar 1	10.6 \pm 0.3 a (8 - 9)	6.8 \pm 0.4 b (5 - 10)	6.1 \pm 0.1 c (6 - 7)	3.3 \pm 0.1 d (3 - 5)	3.2 \pm 0.1 d (3 - 5)
Instar 2	8.4 \pm 0.3 a (8 - 9)	6.0 \pm 0.2 b (5 - 7)	4.5 \pm 0.1 c (4 - 5)	3.3 \pm 0.1 d (2 - 8)	2.6 \pm 0.1 e (2 - 4)
Instar 3	10.2 \pm 0.6 a (9 - 12)	6.3 \pm 0.3 b (5 - 9)	4.7 \pm 0.1 c (4 - 5)	2.9 \pm 0.1 d (2 - 4)	2.9 \pm 0.1 d (2 - 6)
Instar 4	10.2 \pm 0.7 a (9 - 13)	6.3 \pm 0.2 b (5 - 8)	5.2 \pm 0.2 c (4 - 8)	3.2 \pm 0.1 d (2 - 4)	3.1 \pm 0.2 d (2 - 6)
Instar 5	12.8 \pm 0.8 a (11 - 15)	8.5 \pm 0.6 b (6 - 12)	7.3 \pm 0.2 c (5 - 9)	3.9 \pm 0.2 d (2 - 9)	4.3 \pm 0.20 d (1 - 7)
Instar 6	29.2 \pm 1.4 a (24 - 32)	14.1 \pm 1.9 b (9 - 30)	13.7 \pm 1.0 b (4 - 23)	8.5 \pm 0.7 c (2 - 18)	9.5 \pm 0.8 c (3 - 23)
Instar 7	44.0 \pm 0.0 a (44)	20.9 \pm 1.3 b (14 - 27)	14.4 \pm 0.9 c (6 - 18)	11.2 \pm 0.5 cd (5 - 16)	12.7 \pm 0.7 d (8 - 22)
Instar 8*	-	17.0 \pm 0.0 (17)	17.0 \pm 0.0 (17)	11.0 \pm 0.0 (11)	9.0 \pm 0.0 (9)
Larvae	90.2 \pm 8.0 a (81 - 122)	65.1 \pm 2.0 b (55 - 75)	47.7 \pm 0.8 c (42 - 57)	32.1 \pm 0.6 d (27 - 47)	33.4 \pm 0.9 d (25 - 46)
Pupae	40.0 \pm 0.8 a (38 - 43)	26.4 \pm 0.4 b (25 - 29)	20.0 \pm 0.2 c (18 - 22)	14.0 \pm 0.2 d (12 - 18)	13.3 \pm 0.2 d (11 - 17)
Egg-to-adult	152.6 \pm 8.6 a (143 - 187)	104.3 \pm 2.1 b (93 - 114)	77.8 \pm 0.8 c (70 - 88)	52.6 \pm 0.7 d (46 - 67)	52.6 \pm 0.9 d (45 - 66)
Larval survival (%)	3.3	7.7	24.8	18.7	15.5

Means within the same row followed by the same letter do not differ significantly at $P = 0.05$ (Tukey's HSD).

*Data not included in ANOVA analysis due to small sample size.

Table 2.2: Linear regression equations describing the relationship between development rate (1/days) and temperature (15 - 26 °C) and the thermal requirements of different developmental stages of *Busseola fusca*.

Development stage	Regression model	k ± S.E.	t ± S.E.	r-value
Eggs	$y = 0.0101x - 0.1041$	99.50 ± 1.92	10.36 ± 0.33	0.98
First instar	$y = 0.0207x - 0.2368$	48.31 ± 2.27	11.44 ± 0.48	0.92
Second instar	$y = 0.0178x - 0.1400$	56.18 ± 3.47	7.78 ± 1.46	0.86
Third instar	$y = 0.0238x - 0.2600$	42.02 ± 2.61	10.92 ± 0.97	0.86
Fourth instar	$y = 0.0198x - 0.0197$	50.51 ± 2.76	9.92 ± 0.99	0.89
Fifth instar	$y = 0.0197x - 0.2408$	50.89 ± 3.70	12.26 ± 0.78	0.82
Sixth instar	$y = 0.0099x - 0.1049$	101.32 ± 19.79	10.63 ± 4.16	0.48
Pupal stage	$y = 0.0041x - 0.0331$	246.25 ± 7.96	8.14 ± 1.67	0.96
All Instar stages	$y = 0.0019x - 0.0168$	536.48 ± 22.83	8.99 ± 1.37	0.93
Egg-to-Adult	$y = 0.0011x - 0.0099$	893.66 ± 26.91	8.84 ± 1.08	0.96

t = estimated lower temperature threshold, k = estimated thermal requirement in degree-days

Table 2.3: Mean development time in days and degree-days (°D) for *Busseola fusca* at constant temperatures from 15 - 26 °C. Degree-days were calculated using the lower threshold temperature for development determined for each developmental stage (eggs = 10.36 °C, larvae = 8.99 °C, pupae = 8.14 °C and egg-to-adult = 8.84 °C).

Development Stage	Temperature (°C)	n	Development time (days ± S.E.)	Range	°D ± S.E.
Egg	15	5	22.47 ± 0.17	21 - 24	104.9 ± 1.9
	18	12	12.76 ± 0.07	12 - 13	97.4 ± 1.0
	20	31	10.17 ± 0.17	9 - 12	98.0 ± 1.6
	26	43	6.47 ± 0.12	6 - 8	101.1 ± 1.8
Mean					99.58 ± 0.98
Larvae	15	5	90.20 ± 7.97	81 - 122	542.1 ± 47.9
	18	12	65.08 ± 2.04	55 - 75	586.4 ± 18.3
	20	31	47.65 ± 0.75	42 - 57	524.6 ± 8.2
	26	43	32.07 ± 0.61	27 - 47	545.5 ± 10.4
Mean					543.58 ± 6.8
Pupae	15	5	40.00 ± 0.84	38 - 43	274.4 ± 5.7
	18	12	26.42 ± 0.38	25 - 29	260.5 ± 3.7
	20	31	20.00 ± 0.15	18 - 22	251.9 ± 5.8
	26	43	13.98 ± 0.19	12 - 18	249.6 ± 3.4
Mean					253.18 ± 2.7
Egg-to-adult	15	5	152.60 ± 8.61	143 - 187	940.0 ± 53.0
	18	12	104.25 ± 2.11	93 - 114	954.9 ± 19.3
	20	31	77.81 ± 0.84	70 - 88	868.3 ± 9.4
	26	43	52.61 ± 0.65	46 - 67	902.7 ± 11.2
Mean					899.92 ± 7.7

Table 2.4: Mean head capsule widths and ranges for each *Busseola fusca* larval instar stage and Dyar's ratio.

Instar	n	Range (mm)	Range Size	Mean \pm S.E.	Dyar's ratio
1	30	0.31 - 0.34	0.04	0.33 \pm 0.002	-
2	30	0.39 - 0.47	0.08	0.44 \pm 0.003	1.35
3	30	0.57 - 0.76	0.19	0.66 \pm 0.009	1.49
4	31	0.75 - 1.23	0.47	0.95 \pm 0.018	1.43
5	31	0.93 - 1.74	0.80	1.36 \pm 0.036	1.43
6	32	1.05 - 2.40	1.35	1.80 \pm 0.046	1.33
7	21	1.80 - 2.68	0.89	2.30 \pm 0.057	1.28
8	20	1.96 - 2.54	0.58	2.26 \pm 0.036	0.98

CHAPTER 3

The effect of temperature on reproduction of *Busseola fusca* (Lepidoptera: Noctuidae)

3.1 Abstract

Temperature is an important factor affecting reproductive parameters of insect species. Knowledge on the effect of temperature on reproduction will provide insight into population dynamics and contribute to prediction of pest outbreaks. The aim of this study was to determine the effect of temperature on reproduction parameters of *Busseola fusca*. A laboratory study was conducted using four different constant temperatures namely 15, 20, 26 and 30 \pm 1 °C, 70 \pm 30 % RH and 14L: 10D photoperiod. Oviposition occurred at all the temperatures evaluated, but eggs laid at a constant temperature of 30 °C were infertile. The total number of eggs laid by *B. fusca* females was 300 - 400 eggs at temperatures between 15 and 26 °C. Longevity of females also decreased at 30 °C. There was a longer pre- and post-oviposition period at 15 °C and the minimum number of eggs laid at 15 °C was fewer than at 20 and 26 °C. The total number of eggs laid per female at 15 °C was, however, not fewer than at 20 and 26 °C, but a high percentage of these eggs were infertile. In addition, most of the larvae that developed to the black head stage at 15 °C were unable to hatch. The lower threshold temperature for reproduction of *B. fusca* is therefore higher than 15 °C and the upper threshold temperature, below 30 °C. Results from this study indicate the optimum temperature for oviposition and fertility to be between 20 - 26 °C.

Key words: *Busseola fusca*, fertility, longevity, reproduction, temperature

3.2 Introduction

3.2.1 The effect of temperature on reproduction

Reproductive success is very important in evaluating the ecological fitness of an insect and will contribute to understanding the evolutionary biology of the insect (Fantinou *et al.*, 2004). Temperature is considered to be one of the most important abiotic factors affecting various life history parameters of insects (Hallman & Denlinger, 1998; He *et al.*, 2003). Reproduction parameters such as the length of oviposition period, overall fecundity and egg viability are directly affected by temperature (Kim & Lee, 2003; Son & Lewis, 2005; Ali & Rizvi, 2008).

Understanding the effect of temperature on reproduction of an insect will not only contribute to the prediction of oviposition period, but also population growth rate and pest status (Son & Lewis, 2005). Therefore, the impact of climate change on pest population growth can be predicted by determining the upper and lower temperature thresholds of the population. Oviposition models can include temperature-dependent elements such as overall fecundity and duration of age-specific oviposition and survival (Son & Lewis, 2005). Mathematical models can be used to predict population dynamics (Sporleder *et al.*, 2004) and species distribution (Hauptfleisch *et al.*, 2014). For example, in an assessment of the potential of *Busseola fusca* (Lepidoptera: Noctuidae) as a pest in sorghum and maize growing countries around the world by Hauptfleisch *et al.* (2014), it was indicated that if introduced, *B. fusca* may establish and become a pest in most of the maize growing regions globally. According to the model, it might do especially well along the east coast of Brazil, Madagascar, and in Indonesia and Thailand.

Improved knowledge on the effects of temperature on *B. fusca* biology will assist in modelling climate requirements of *B. fusca* in different regions where different clades with different ecological characteristics occur (Sezonlin *et al.*, 2006; Félix *et al.*, 2009). It is most likely that genotype-specific base temperatures for development of *B. fusca* occur (Hauptfleisch *et al.*, 2014).

Apart from temperature, photoperiod also has an effect on the development and reproduction of an organism. Factors such as intensity and quality of light directly affect development and reproduction (Philogene & McNeal, 1984).

3.2.2 *Busseola fusca* moths

The moths of *B. fusca* are seldom observed in maize fields as they are inactive during the daytime resting on plants or plant debris unless when disturbed (Harris & Nwanze, 1992). These moths emerge 13 - 14 days after pupation if the conditions are favourable and they usually emerge after sunset or at night (Onyango & Ochieng'-Odero, 1994; Calatayud *et al.*, 2007). Soon after emergence, the female releases a pheromone and starts calling to attract the males and mating starts soon after (Unnithan, 1987; Calatayud *et al.*, 2007). Hereafter, the female moth searches for a suitable host plant for oviposition. The females normally lay eggs between the youngest unfolded leaf sheaths of the host plant, (Unnithan, 1987; Harris & Nwanze, 1992). Females find 3 - 4 week old maize plants the most attractive for oviposition (Van Rensburg *et al.*, 1985; Unnithan, 1987). This species prefers to lay eggs on plants with a waxy surface (Haile & Hofsvang, 2002). Surface texture (smooth surfaces), plant size (stem thickness) and leaf sheath rigidity all play a role in *B. fusca* moths' hostplant suitability for oviposition (Calatayud *et al.*, 2008). The oviposition period takes about 3 - 4 successive nights when conditions are favourable (Harris & Nwanze, 1992; Calatayud *et al.*, 2007). The steps during the host-finding stage are searching, orientation, encounter, landing, surface evaluation and acceptance (Renwick & Chew, 1994). A detailed study on oviposition behaviour was done by Calatayud *et al.* (2008).

The seasonal flight patterns of *B. fusca* peak three times per annum (Van Rensburg *et al.*, 1985; Van Rensburg, 1997; Van Rensburg, 1999). The moths of the third seasonal flight are low in numbers because only a few larvae of the second generation can pupate because the host plants (maize) (late in the production season) are not suitable for oviposition or for feeding of young larvae (Van Rensburg *et al.*, 1985; Krüger, 2006). There are significantly more moths during the second flight than during the first. Maize plants from an early planting date (September to early November) are infested by moths from the first flights resulting in relatively small infestations and those of a late planting date (mid-November) are subject to more severe infestations (Van Rensburg *et al.*, 1985). *Busseola fusca* population numbers are therefore lower in early planting dates (Abu, 1986). In South Africa, the moth flight season starts from around October and ends around April just before the frost commences (Van Rensburg *et al.*, 1985; Kfir, 1998). Van Rensburg *et al.* (1985) described the number of *B. fusca* moths as low during the first flight (November), with the second flight that reaches a high peak between January and February and the third flight decreases again to low numbers around March and April. *Busseola fusca* moth flights were also noted by Jack (1917) who reported the seasonal moth flight to reach a peak during November in

Potchefstroom, while Du Plessis & Lea (1943) noted moth flights to start around December in Kroonstad, South Africa. The understanding of seasonal moth flight patterns provide knowledge in seasonal variation and can be used to predict stem borer outbreaks (Van Rensburg, 1997).

3.2.3 Monitoring of *Busseola fusca* moths

Busseola fusca is of economic importance since it causes a decrease in maize production (Kfir, 1998; Kfir *et al.*, 2002). In general, *B. fusca* is found in maize and sorghum but seldom in wild grasses such as Napier grass (Le Ru *et al.*, 2006a, b). Yield losses by *B. fusca* can range between 0 - 100% depending on conditions (Barrow, 1987; Van Rensburg & Bate, 1987).

Monitoring of moth numbers can provide early-warning of pest outbreaks and also contributes to developing chemical control strategies that rely on accurate timing of pesticide applications. Pheromones are used in integrated pest management programs to monitor population dynamics and to control by mass trapping and mating disruption (Van Rensburg, 1992). Light traps can also be used to determine the seasonal abundance of *B. fusca* populations. Although it is very effective, a three year period or more is needed to determine seasonal moth flight pattern of moths (Van Rensburg *et al.*, 1987). Seasonal rainfall also determines *B. fusca* flight patterns (Van Rensburg *et al.*, 1987) and distinctive patterns occur in areas with only one rainfall season (Van Rensburg, 1997). Planting dates play an important role in avoiding severe infestations by *B. fusca* or any other stem borer pest. Knowing the flight patterns of *B. fusca* can help to determine the optimum planting date to escape high infestation levels.

Accurate knowledge on the effects of temperature on the biology of a South African population of *B. fusca* will assist in modelling climate requirements of this species.

3.2.4 Objectives

The objective of this study was to determine the effect of different temperatures on reproduction of *B. fusca*. The longevity, oviposition period, total number of eggs laid, egg fertility and mortality were determined for each temperature regime, evaluated.

3.3 Materials and Methods

3.3.1 *Busseola fusca* collection sites

The collection sites are those described in Chapter 2 (see 2.3.1).

3.3.2 Fecundity and longevity of female moths

This study was conducted in a laboratory at the North-West University, Potchefstroom.

After emergence of the moths, single male-female pairs were confined to oviposition chambers (Fig. 2.1a) within 24 hours of emergence. There were 30 oviposition chambers per temperature regime. The temperature regimes used were 15, 20, 26, and 30 ± 1 °C, RH of $70 \pm 30\%$ and a 14L: 10D photoperiod. The temperature and RH at each regime was recorded at 30-minute intervals using iButtons[®] from Coldchain Thermo Dynamics (Fairbridge technologies) as seen in figure 2.2.

The chambers and method used are as described in Chapter 2 (see 2.3.3). The pre- and post-oviposition periods, days without oviposition, longevity and fertility, number of egg batches oviposited and egg production of each adult female were determined. Eggs were counted using a stereo-microscope (Nikon SMZ 1500) and kept in a small plastic container (52 mm high and 30 mm in diameter) with a steel mesh infused lid. These containers with eggs were kept in glass desiccators (150 mm in diameter) in which RH were maintained at $70 \pm 5\%$ using a potassium hydroxide solution according to the method of Solomon (1951). The eggs were observed daily until larvae hatched. To determine fertility, the number of eggs that developed to the black head stage was counted and expressed as a percentage of the total number of eggs per female. Eggs that did not develop were regarded as infertile. Larvae that developed to the black head stage were regarded dead, if they had not hatched three weeks after oviposition.

3.3.3 Statistical analysis

The effect of temperature on pre-, post-, and oviposition period, longevity of female moths and egg production were analysed by means of one-way ANOVA using STATISTICA 12 (Statsoft, Inc., 2013), followed by Tukey's HSD test ($P = 0.05$).

3.4 Results

Temperature had a significant effect on fecundity and longevity of *B. fusca* moths (Table 3.1). Moths laid eggs at all the temperatures evaluated during this study. There was, however, a significant difference in total number of eggs laid between these temperatures ($F_{3,121} = 12.02$; $P < 0.001$). The total number of eggs laid at 15, 20 and 26 °C was similar, with no significant difference between them. The total number of eggs laid at 30 °C was approximately a third of those laid at 15, 20 and 26 °C and differed significantly from them (Table 3.1).

Pre-oviposition and oviposition periods were inversely related to temperature (Table 3.1). The pre-oviposition ($F_{3,121} = 8.68$; $P < 0.001$) and oviposition ($F_{3,121} = 29.06$; $P < 0.001$) periods were significantly longer at 15 °C than at 30 °C, with no significant difference in the duration of these two reproductive periods between 20 and 26 °C. The post-oviposition period ($F_{3,121} = 10.72$; $P < 0.001$) was the longest at 15 °C and similar at 20, 26 and 30 °C. Moths died within a day of their last oviposition at 20, 26 and 30 °C, but lived between two and three days after oviposition at 15 °C (Table 3.1). Longevity of female moths differed significantly between the different temperatures ($F_{3,121} = 59.6$, $P < 0.001$). Longevity of female moths was significantly longer at 15 °C compared to 20, 26 and 30 °C. However, females lived significantly shorter at 30 °C than at 20 and 26 °C (Table 3.1).

Moths laid significantly more eggs per day at 26 °C compared to 15, 20 and 30 °C ($F_{3,121} = 10.5$; $P < 0.001$). The mean number of eggs laid per day at 20°C was also significantly higher than at 15 °C (Table 3.1). The minimum number of eggs laid per female per day was significantly fewer at 15 °C compared to 26 and 30 °C ($F_{3,121} = 6.6$; $P < 0.001$). The minimum number of eggs laid daily per female moth, was more than 20 eggs at 26 and 30 °C during their oviposition period (Table 3.1).

The percentage infertile eggs at all temperatures evaluated were high and differed significantly ($F_{3,121} = 21.79$, $P < 0.001$) although the number of infertile eggs showed no significant difference between the four temperatures evaluated ($F_{3,121} = 2.0$; $P = 0.1$) (Table 3.2). Since there was no fertilisation at 30 °C, fertility was compared at 15, 20 and 26 °C only. There was no significant difference in fertility between the different temperatures (number of fertile eggs and unhatched larvae), although fertility increased from 15 to 26 °C ($F_{2,90} = 1.5$; $P = 0.2$) (Table 3.2). Differences do, however, exist if it is expressed as percentage fertility ($F_{2,90} = 3.91$; $P > 0.01$). Fertility was significantly lower at 15 °C than at 20

°C, but it did not differ from percentage fertility at 26 °C. The highest percentage fertility was at 20 °C and no fertility (as a result of no fertilisation) was recorded at 30 °C. There was also a significant difference in the number ($F_{2,90} = 9.25$; $P < 0.001$) and percentage ($F_{2,90} = 11.64$; $P < 0.001$) eggs with larvae in the blackhead stage that failed to hatch between the four temperatures evaluated. Since there was no fertilisation at 30 °C, the percentage larvae that were unable to hatch, was compared at 15, 20 and 26 °C only. Percentage hatch was significantly higher at 15°C than at 20 and 26°C. There was therefore a tendency that the failure to hatch was reduced with increasing temperature between 15 and 26 °C, although the percentage unsuccessful hatchings at 20 and 26 °C did not differ significantly.

3.5 Discussion

The reproductive parameters of female *B. fusca* moths were significantly affected by temperature, and oviposition commenced sooner at temperatures higher than 15 °C. The pre- and post-oviposition periods were long, when compared to temperatures between 20 and 30 °C. Energy is therefore used for physiological processes rather than reproductive effort. Although female moths lived longer at 15 °C, the number of days without oviposition was also higher than at the increased temperatures. The mean number of eggs laid per day at 15 °C, was lower than at temperatures between 20 and 26 °C. The total number of eggs laid per female at 15 °C was not fewer than at 20 and 26 °C, but a high percentage of these eggs were infertile. Most of the larvae that developed to the black head stage at 15 °C were unable to hatch. Fitness of these larvae was therefore negatively affected by a constant temperature of 15°C. Hatching success of neonate larvae increased with increasing temperature between 15 and 26 °C. Although not too cold for oviposition, a constant temperature of 15 °C proved to be too cold for fertility of *B. fusca*. The lower threshold temperature for *B. fusca* reproduction is therefore higher than 15 °C. Goebel (2006) reported a temperature of 15 °C also to be too low for *Chilo sacchariphagus* (Lepidoptera: Crambidae) mating and oviposition, with no mating and eggs laid at 15 °C.

The upper limit for *B. fusca* reproduction is below 30 °C because fewer eggs were laid at 30 °C compared to 26 °C, 100% infertility of eggs occurred and female moths had a short life span at 30 °C. Many infertile eggs (>100) were recorded at all temperature regimes, but the highest egg production and fertility rate of eggs were at 20 and 26 °C. Female longevity was also similar at these two temperatures. These results are in accordance with observations by Fantinou *et al.* (2004) on *Sesamia nonagrioides* (Lepidoptera: Noctuidae) where the number of eggs laid and fertility increased from 15 °C to 27.5 °C but declined significantly at 30 °C.

However, *Chilo sacchariphagus* (Lepidoptera: Crambidae) had a higher upper fertility temperature where the mean number of eggs laid increased from 17 °C to 30 °C but reached a low number only at 35 °C (Goebel, 2006). Fecundity usually increases as temperature increases, between a lower and upper threshold, up to the optimum point (Milonas & Savopoulou-Soultani, 2000). The lower threshold for both these stem borer species is therefore above 15 °C and the upper threshold is in the mid-twenties for *B. fusca*. The best suited temperature for adult fecundity of the stem borer *Chilo partellus* (Lepidoptera: Crambidae), is between 26 - 30 °C (Tamiru *et al.*, 2012).

During this study, the females of *B. fusca* laid about 300 - 400 eggs at temperatures between 15 and 26 °C. This was more than the average of 243 - 299 eggs between 18 and 25 °C reported by Khadioli *et al.* (2014), but in accordance with Harris (1962) who reported an average of 400 eggs per female and Kruger *et al.* (2012) who reported summer moths to lay an average of 374 eggs. However, Unnithan (1987) reported a high average fecundity number of 855 when reared in the laboratory. In this study, the highest number of eggs laid by a single moth was 976. Although *B. fusca* moths can lay many eggs and the potential offspring may be many, the realised offspring may be few. Depending on the temperature that prevails, not all eggs will be fertilised resulting in reduced fertility and thus low larval numbers. The more eggs are laid, the higher the probability that some of the offspring survives and reproduce (Speight *et al.*, 2008). The total number of eggs laid at 30 °C, decreased by more than half the number of eggs laid at the other temperatures. This significant reduction indicates that 30 °C is not suitable for oviposition by *B. fusca* moths. Although the quantity of eggs is important, high quality of eggs will provide strong offspring. The quality of the eggs is determined by energy and resources available from the adults collected during their larval stage (Riddick, 2006; Speight *et al.*, 2008).

Longevity of female moths decreased as the temperature increased (14.9 - 5.2 days) at a 14L: 10D photoperiod. Khadioli *et al.* (2014) reported similar results for female moth longevity ranging from 12.4 - 4.7 days from 15 - 30 °C and at 12L: 12D photoperiod. Unnithan (1987) reported an average longevity of 7.47 days for female moths of *B. fusca* reared in a laboratory. Similar results were recorded for *S. nonagrioides* moths at different temperatures (15 - 30 °C) and 16L: 8D photoperiod with longevity declining from 7.6 to 3.9 days as the temperature increased (Fantinou *et al.*, 2004). For *C. partellus*, longevity was also negatively affected by increasing temperature from 22 - 30 °C (Tamiru, 2012), and for *S. calamistis* it ranged from 6.7 - 9.9 days between 15 - 30 °C at 12D: 12L photoperiod (Khadioli *et al.*, 2014).

Studies of pest development at a range of constant temperatures can give valuable insight on the population dynamics of a species and help with pest management strategies (McFarland *et al.*, 1992; Shanower *et al.*, 1993). These conditions differ from environmental conditions in natural habitats where the temperatures fluctuate and the host plants are less abundant. Fecundity under natural conditions will therefore be determined by the amount of time that females spend on mating and host finding behavior. Oviposition behaviour involves sexual behaviour, calling and mating during the scotophase, and oviposition which occurs in the dark over a period of several days (Calatayud *et al.*, 2007). Successful oviposition behaviour ensures better survival for the offspring. Night temperatures in South Africa are usually not high and will not be a limiting factor for oviposition. The lifespan of female moths may, however, be inhibited by high temperatures exceeding 30 °C that may prevail during daytime.

Busseola fusca have three distinctive flight patterns per annum in South Africa, of which the second is the most severe (Van Rensburg *et al.*, 1985). There exists a temperature and rainfall gradient from east to west in the maize production area of South Africa (Van Rensburg *et al.*, 1985). The number of *B. fusca* moths increases significantly over this gradient from east to west especially during the third flight season (Van Rensburg *et al.*, 1985). First moth flights commences earlier in the east than the west causing larvae to enter diapause earlier in the eastern areas than in the west (Walters, 1979), thus leading to no third moth flight in the east but increasing moth flights to the west (Van Rensburg *et al.*, 1985). The pattern in south-western Nigeria is the opposite since spring rains start earlier in the east than in the west (Usua, 1968). This leads to earlier planting dates in the east and also earlier dates for diapause initiation in *B. fusca*.

Knowledge on thermal limits for *B. fusca* reproduction and understanding their response to changes in temperature can contribute to modelling of climate requirements of *B. fusca* in different regions and assist in predicting pest outbreaks. Changes in climate may cause extinction of species or severe outbreaks, depending how they react to these changes. Although insects can adapt to environmental changes, extreme conditions may be too harsh to survive.

Development studies such as those described in this study provide information on the optimal and lethal temperatures for specific species. The effect of fluctuating temperatures on *B. fusca* reproduction and development is, however, not known. A study on *Adoxophyes orana* (Lepidoptera: Tortricidae) reported that fluctuating temperatures causes a shorter life

span than constant temperatures (Mironidis & Savopoulou-Soultani, 2008), and should therefore be investigated for *B. fusca* too.

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Table 3.1: Mean fecundity and longevity (\pm S.E.) of *Busseola fusca* moths at constant temperatures.

Temperature (± 1 °C)	n	Total no. of eggs laid	Pre- oviposition (days)	Oviposition (days)	Post- oviposition (days)	Longevity of adult female (days)	No. of days during which no eggs were laid	Mean no. of eggs laid per day	Max no. of eggs laid per day	Min no. of eggs laid per day	Mean number of egg batches per female
15	30	336.7 \pm 37.3 a	3.3 \pm 0.3 c (1-9)	8.9 \pm 0.7 c (1-16)	2.7 \pm 0.5 b (0-15)	14.9 \pm 0.8 c	1.5 \pm 0.3 b	39.0 \pm 4.5 a	119.2 \pm 14.3 ab	4 \pm 1.5 a	6.73 \pm 0.79 ab
20	36	345.0 \pm 28.1 a	2.5 \pm 0.2 ab (1-6)	5.2 \pm 0.4 b (1-10)	0.8 \pm 0.2 a (0-3)	8.5 \pm 0.4 a	0.5 \pm 0.1 a	68.5 \pm 5.2 b	182.4 \pm 15.7 bc	11.1 \pm 2.9 ab	7.97 \pm 0.69 b
26	27	396.1 \pm 57.9 a	2.7 \pm 0.3 bc (1-7)	4.5 \pm 0.9 ab (2-11)	0.9 \pm 0.2 a (0-4)	8.2 \pm 0.4 a	0.3 \pm 0.1 a	100.0 \pm 16.3 c	206.2 \pm 33.9 c	27.0 \pm 7.3 c	7 \pm 0.6 ab
30	32	118.7 \pm 13.9 b	1.7 \pm 0.1 a (1-3)	2.9 \pm 0.2 a (1-6)	0.6 \pm 0.2 a (0-4)	5.2 \pm 0.3 b	0.2 \pm 0.1 a	43.9 \pm 4.3 ab	70.6 \pm 7.4 a	22.6 \pm 3.4 bc	5.22 \pm 0.58 a

Values in brackets represent the range in days.

Means in each column followed by the same letter are not significantly different (Tukey's HSD, P = 0.05).

Table 3.2: Fertility (\pm S.E.) of *Busseola fusca* eggs at constant temperatures.

Temperature (± 1 °C)	n	Total no. of eggs laid	Infertile eggs	% Infertile eggs	#Fertility	% Fertility	Unhatched larvae	% Unhatched larvae
15	30	336.7 \pm 37.3 a	161.2 \pm 20.7 a	63.7 b	175.5 \pm 36.5 a	36.3 \pm 6.6 a	127.5 \pm 29.4 a	26.3 \pm 5.5 a
20	36	345.0 \pm 28.1 a	103.4 \pm 17.3 a	37.3 a	241.6 \pm 27.7 a	62.8 \pm 5.8 b	71.8 \pm 12.3 ab	18.1 \pm 2.5 a
26	27	396.1 \pm 57.9 a	110.5 \pm 21.1 a	51.0 ab	285.6 \pm 65.6 a	49.1 \pm 8.6 ab	10.3 \pm 3.4 b	1.8 \pm 0.5 b
30	32	118.7 \pm 13.9 b	118.7 \pm 13.9 a	100 c	-	-	-	-

Means in each column followed by the same letter are not significantly different (Tukey's HSD, $P = 0.05$).

#Fertility = number of fertile eggs

CHAPTER 4

Conclusion

Busseola fusca is the most important insect pest of maize in South Africa. It is also one of the major stem borer species found in other African countries. The density of stem borers was reported to be much lower in wild host plants than in cultivated crops (Gounou & Schulthess, 2004). These results were confirmed for *B. fusca* by Moolman *et al.* (2014). This emphasises the importance of maize as the primary host plant of this species in South Africa. Survival on maize is therefore essential for the existence of *B. fusca*. There are three peaks in the *B. fusca* moth flight pattern in South Africa (Van Rensburg *et al.*, 1985; Van Rensburg, 1997). The moth flight season is from October until April each year (Van Rensburg *et al.*, 1985; Kfir, 1998), which coincides with the maize production season in South Africa.

Temperature is considered as one of the most important abiotic factors affecting various life history parameters of insects (Hallman & Denlinger, 1998; He *et al.*, 2003). This study provides information about the effect of temperature on the development and reproduction of *B. fusca*. This study was conducted at five constant temperatures, viz. 15, 18, 20, 26 and 30 ± 1 °C and a 14L: 10D photoperiod. Temperature affected both development and reproduction of *B. fusca* significantly. Development rate of larvae was inversely related to temperature from 15 to 26 °C. The most favourable temperature range for development was between 26 and 30 °C. Most of the development time of *B. fusca* larvae (80 % and more) is spent in instar six onwards and the maize crop is therefore exposed to the more damaging stage of this pest species for long periods which contributes to its pest status in terms of damage to maize plants. Development time of the egg stage varied between 22.5 and 6.5 days at 15 and 26 °C; the larval stage between 90.2 and 32.1 days; the pupal stage between 40.0 and 14.0 days, and the duration of egg-to-adult was 152.6 and 52.6 days, at the two respective temperatures. The lower development threshold temperatures were calculated for eggs (10.36 °C), larvae (8.99 °C), pupae (8.14 °C) and the total life cycle (egg-to-adult) (8.84 °C). The lower development threshold for larvae and pupae was higher than for eggs, indicating the eggs to be the more sensitive stage in the life cycle. The degree-days needed to complete development were calculated using the abovementioned lower threshold temperatures. For total development from egg-to-adult at constant temperatures of 15 and 26 °C, 1348.98 and 465.03 degree-days are needed, respectively. The very slow development rate at 15 °C and almost no survival indicated that a constant temperature of

15 °C is too low for *B. fusca* development. Most of the eggs laid by *B. fusca* moths at a constant temperature of 15 °C were also infertile or larvae that developed to the black head stage failed to hatch. A constant temperature of 15 °C is therefore too cold for fertility of *B. fusca*. The percentage infertile eggs laid at a constant temperature of 30 °C, was 100 % and females had a short life span. A constant temperature of 30 °C is therefore too warm for *B. fusca* reproduction. The optimum temperature range for *B. fusca* oviposition and fertility was determined to be between 20 - 26 °C.

Climate change results in changes in global temperatures which in turn result in weather extremes. It is predicted that climate change will cause a rise in global temperature of between 1.4 - 5.8 °C by 2100 (IPCC, 2007). The development, survival and abundance of insects will, as a result, be affected. Climate change will not only affect insects directly by impacting on their physiology and behaviour, but also indirectly, through changes that may be affected in their host plants (Bale *et al.*, 2002).

For insects, synchronised growth and development between host plant and specific insect stages is important, especially in temperate regions where the host plant as food resource is only available for a limited period of time within the growing season. As climate becomes less favourable, synchronisation becomes more critical and fewer insects are successful at completing their life cycle (Bale *et al.*, 2002). The impact that climate change will have on *B. fusca* in South Africa is unknown. However, the effect of increasing temperature on maize production will also have an impact on the pest. The strategy used by *B. fusca* for survival during cold temperatures is to enter into diapause during the cold winter period (Kfir, 1991; Kfir *et al.*, 2002). During diapause, no activity occurs and this leads to a profound drop in metabolic rate which is linked to an increase in cold hardiness of larvae (Bale *et al.*, 2002). It can therefore be speculated that if winter temperatures rise as a result of climate change, commencement of the first moth flight of *B. fusca* may be earlier in the year and may result in most moths not finding suitable host plants to oviposit on. However, higher soil temperatures may also result in earlier planting by farmers, especially under irrigation, which then may coincide with the earlier moth flights. This in turn may result in an additional generation and a higher peak in the third moth flight resulting in more damage to maize crops planted later.

Head capsule widths are frequently used to determine the number of instars for insects. It was also used in this study and head capsule widths for *B. fusca* ranged from 0.32 - 2.64 mm up to instar eight. Head capsule widths for instar one to four did not overlap, but overlapping occurred from instar 5 onwards. There was no difference in head capsule width of instars seven and eight. Dyar's rule proved to be applicable for instar one to seven, but

not for instar eight. This information can be used in the field to identify instars and determine the age of a population during the growing season (Calvo & Molina, 2008). More than eight instars were also found for *B. fusca* under less favourable conditions during this study. Head capsule widths of these extra instars did, however, not differ from the previous instars.

Determining the effect of fluctuating temperatures on the development of *B. fusca* may improve on the accuracy of the parameters determined in this constant temperature study. Data from this study do, however, have significant prediction and modelling value.

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