Future distribution and life history traits of three major insect pests of Arabica coffee (*Coffea arabica* L.) in East Africa: risk assessment in light of global warming

By

Abdelmutalab Gesmalla Ahmed Azrag

Submitted in partial fulfilment of the requirements for the degree

PhD Entomology

In the Faculty of Natural & Agricultural Sciences

University of Pretoria

Pretoria

February 2019

Declaration

I, Abdelmutalab Gesmalla Ahmed Azrag declare that the thesis, which I hereby submit for the degree Doctor of Philosophy in Entomology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: 

DATE: 28/02/ 2019

Publications and thesis outline

This thesis consists of a series of chapters that have been prepared as stand-alone publications or manuscripts submitted to different scientific journals. Consequently, unavoidable overlaps and/or repetitions may occur.

Publications

Chapter Two: Azrag, A.G.A., Yusuf, A.A., Pirk, C.W.W., Niassy, S., Mbugua, K. K., & Babin, R. (2020). Identifying temperature thresholds using life cycle modelling and their implications for developmental times and survival of the coffee berry borer *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae). *Bulletin of Entomological Research*, 110 (2): 207-218.

Chapter three: Azrag, A.G.A., Yusuf, A.A., Pirk, C.W.W., Niassy, S., Guandaru, E., & Babin R. (2019). Modelling the impact of temperature on the demographic parameters of the African coffee white stem borer, *Monochamus leuconotus* (Pascoe) (Coleoptera: Cerambycidae. *Journal of Thermal Biology*, 89, 102534

Chapter four: Azrag, A.G.A., Yusuf, A.A., Pirk, C.W.W., Niassy, S., Guandaru, E., & Babin R. (2019). Factors affecting the distribution and seasonal variation of two stink bugs on Arabica coffee, *Antestiopsis thunbergii* and *Antestiopsis facetoides*. *Manuscript*.

Chapter five: Azrag, A.G.A., Pirk, C.W.W., Yusuf, A.A., Pinard, F., Niassy, S., Mosomtai, G. & Babin, R. (2018). Prediction of insect pest distribution as influenced by elevation: Combining field observations and temperature-dependent development models for the coffee stink bug, *Antestiopsis thunbergii* (Gmelin). *PLoS One* 13(6): e0199569. https://doi.org/10.1371/journal.

Outline of the thesis

Chapter One presents a general introduction and overview of literature in the field thermal biology of coffee insect pests and modelling approaches. **Chapter two** presents the thermal thresholds for immature stages development and survival of coffee berry borer, *Hypothenemus hampei* (Ferrari). It developed an original observation method that allowed the direct observation of *H. hampei* immature stages from egg to adult for the first time and precisely studied its life history at different constant temperatures. It also provided basic data on the pest life history as impacted by temperature and determined the thermal thresholds for the *H. hampei* development and survival. **Chapter three** looks at the detailed life history traits of African coffee white stem borer, *Monochamus leuconotus* (Pascoe) reared at different constant temperatures on an artificial diet and provided data on the biology and thermal thresholds of this pest using life cycle modelling. The fitted linear and nonlinear models for the development and survival of immature, as well as fecundity and adult senescence for *M. leuconotus* were compiled and used to simulate the population growth parameters that determine *M. leuconotus'*s population growth rate, using stochastic simulations. **Chapter four** assessed the the seasonal variations and distribution of antestia bug species**,** *Antestiopsis thunbergii* and *A. facetodies* in coffee plantation over elevation gradients. This chapter analysed and identified factors that affect the distribution and seasonal variations of both species in coffee plantations. **Chapter five** elucidates the relationship between *A. thunbergii* populations and elevation using the knowledge of the pest's thermal biology and predicted its future distribution in a changing climate (raising temperatures). **General**

conclusions looked at the findings from all chapters and proposed recommendations and highlighted areas for future research.

Future distribution and life history traits of three major insect pests of Arabica coffee (*Coffea arabica* L.) in East Africa: risk assessment in light of global warming

Student: Abdelmutalab Gesmalla Ahmed Azrag

Supervisors: Dr. Abdullahi A. Yusuf

Prof. Christian W. W. Pirk

Dr. Régis Babin (CIRAD/*icipe* Nairobi, Kenya)

Department : Department of Zoology and Entomology

Degree : Doctor of Philosophy in Entomology

Thesis summary

Arabica coffee *Coffea arabica* L. is an important cash crop supporting millions of households in East Africa. However, the crop faces challenges of infestation by some insect pests that lead to substantial economic loss and lower quality of beans. The Antestia bugs, *Antestiopsis* spp. (Hemiptera: Pentatomidae), the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) and the African coffee white stem borer, *Monochamus leuconotus* (Pascoe) (Coleoptera: Cerambycidae) are major insect pests threatening coffee production in Africa. Nevertheless, the developed control measures against these pests are not effective to control their damages in coffee plantations. This is a consequence of the insufficient knowledge of their biology, ecology and distribution as influenced by environmental factors. Therefore, the objectives of this study were to: i) provide the thermal requirements for the development of immature stages of *H. hampei* and *M. leuconotus* by modelling their life history traits as influenced by temperature, ii) describe the seasonal variation of *Antestiopsis thunbergii* and *A. facetoides* populations and identify the factors that affect their populations over time and space, and iii) predict future distribution of *A. thunbergii* as influenced by temperature rising over elevation gradients using field observations and temperature-dependent development models. Life history traits of both *H. hampei* and *M. leuconotus* were studied at seven constant temperatures in the range 15-35°C. Linear and nonlinear models were fitted to the development and survival data for both pests in order to calculate their thermal thresholds. In addition, nonlinear models were fitted to *M. leuconotus* fecundity and adult senescence, compiled and used to simulate the population growth. The seasonal variation of both *A. thunbergii* and *A. facetoides* was assessed for two years on coffee

farms located on the Aberdare range, in Kenya. Future distribution for *A. thunbergii* up to the year 2055 was predicted over elevation gradients in the range 1000-1700 m asl, on Mt. Kilimanjaro, Tanzania using the life history traits of the pest and climatic data. Thermal thresholds (T_{min} and T_{max}) for *H. hampei* immature stages development ranged between 10.5 and 35.2°C, with development time ranges of 4.6-16.9, 11.6-39.5 and 3.0-13.6 days for the egg, larva and pupa, respectively. On the other hand, the thermal window for *M. leuconotus* immature development was estimated between 10.0 and 40.0°C, with an optimum temperature for survival in the range 23.0-23.9°C. The highest fecundity for *M. leuconotus* was 97.8 eggs per female at 23°C. Simulated life table parameters showed the highest net reproductive rate R_o of 11.8 per female at 26°C. The intrinsic rate of increase r_m was higher between 26 and 28°C, with a value of 0.008. The seasonal variation of *A. thunbergii* and *A. facetoides* showed that both species had a similar trend with the highest infestation being reported between the months of June and August. The availability of food (green berries) was that most crucial factor that determined these seasonal variations. Future prediction of *A. thunbergii* showed that the distribution of the risks of infestation by this pest will be higher at the highest elevations (1500-1800 m asl) and *A. thunbergii* will follow the shift of the coffee production areas to the highest elevations. These findings will help to better understand coffee pests' distribution and dynamics in coffee plantations and inform decision makers on the risks of these pests under changing climate. This will contribute to a more efficient and sustainable management strategy for coffee insect pests in Africa.

Dedication

With love to my mother, father, brothers and sisters for their love, support and encouragement.

Above all I extend my sincere gratitude to the Almighty Allah for making this journey possible.

Acknowledgements

I would like to express my deep gratitude to my supervisors, Dr. Abdullahi Yusuf and Professor Christian Pirk of the University of Pretoria and Dr. Régis Babin of *icipe*, for their constant expert guidance, enthusiastic encouragement, support and useful critiques through all the stages of this research work. I have learned a lot by being a student under their supervision, grown immensely as a scientist, and will be forever grateful for the advice and mentoring that they provided. Special thanks to Dr. Régis Babin who accepted me to work on his projects, I am extremely lucky to have this chance and learned a lot from him. I also highly acknowledge Dr. Saliou Niassy and Dr. Fabrice Pinard for the intellectual support in this work. More specifically, I am grateful to the members of the coffee laboratory at *icipe*, Dickson Mwenda, Ephantus Guandaru and Kelvin Mbugua, and the driver Charles Kanyi for their support during the data collection. I am also grateful to the coffee farmers of the Kilimanjaro region, Tanzania and Murang'a, Kenya for allowing me access to their farm for surveys.

My sincere appreciation goes to the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), who financed this study and German Academic Exchange Service (DAAD), who gave me the scholarship under the African Reginal Postgraduate Programme in Insect Science (ARPPIS), and the International Centre of Insect Physiology and Ecology (*icipe*) for hosting me to pursue this study. Special mention of the University of Pretoria for registering me to carry out this research work, I am very grateful. I wish to express my great appreciation to the University of Gezira (Sudan) for granting me study leave to pursue this study. Finally, I wish to thank

my family members, friends and colleagues at *icipe* for their support and encouragement throughout my study.

Table of contents

List of Figures

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

List of Tables

CHAPTER TWO

CHAPTER THREE

List of abbreviations

AI	Activity Index
AIC	Akaike Information Criterion
AR5	Fifth Assessment Report
ARPPIS	African Reginal Postgraduate Programme in Insect Science
CHIESA	Climate Change Impacts on Ecosystem Services and Food Security in Eastern Africa
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
DD	Degree Days
DEM	Digital Elevation Model
ERI	Establishment Risk Index
GI	Generation Index
GLM	Generalized Linear Model
GPS	Global Positioning System
icipe	International Centre of Insect Physiology and Ecology
ILCYM	Insect Life Cycle Modelling
IPCC	Intergovernmental Panel on Climate Change
m asl	meters above sea level
PTD	Potato Taste Defect
QGIS	Quantum Geographical Information Systems
RCPs	Representative Concentration Pathways scenarios

This thesis is partly based on the following publications:

Note: Chapters 2-5 were written for separate publication in appropriate journals.

Chapter Two

Azrag, A.G.A., Yusuf, A.A., Pirk, C.W.W., Niassy, S., Mbugua, K. K., & Babin, R. (2018). Identifying temperature thresholds using life cycle modelling and their implications for developmental times and survival of the coffee berry borer *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae). *Bulletin of Entomological Research*,110 (2): 207-218.

Chapter Three

Azrag, A.G.A., Yusuf, A.A., Pirk, C.W.W., Niassy, S., Guandaru, E., & Babin R. (2019). Modelling the impact of temperature on the demographic parameters of the African coffee white stem borer, *Monochamus leuconotus* (Pascoe) (Coleoptera: Cerambycidae. *Journal of Thermal Biology*, 89, 102534.

Chapter Four

Azrag, A.G.A., Yusuf, A.A., Pirk, C.W.W., Niassy, S., Guandaru, E., & Babin R. (2019). Factors affecting the distribution and seasonal variation of two stink bugs on Arabica coffee, *Antestiopsis thunbergii* and *Antestiopsis facetoides*. *Manuscript*.

Chapter Five

Azrag, A.G.A., Pirk, C.W.W., Yusuf, A.A., Pinard, F., Niassy, S., Mosomtai, G. & Babin, R. (2018). Prediction of insect pest distribution as influenced by elevation: Combining field observations and temperature-dependent development models for the coffee stink bug, *Antestiopsis thunbergii* (Gmelin). PLoS ONE 13(6): e0199569. https://doi.org/10.1371/journal.

CHAPTER ONE

General Introduction

Introduction

Coffee belongs to the family Rubiaceae under genus *Coffea*, which is grown in tropical and sub-tropical highlands (DaMatta, 2004; DaMatta and Ramalho, 2006). It is the second most internationally traded commodity after oil (Vega, 2007; Figueroa et al., 2016; Malara et al., 2018). The livelihoods of about 100 million people depend on coffee production worldwide, many of whom are in developing countries and vulnerable to climate change (Bunn et al., 2015). In 2016/2017, worldwide production was estimated at 153.9 million bags (132.28 pounds per bag) with a price of 130 US cents per pound (ICO, 2017). East African production was 17.12 million bags which represented 11% of world production (UNCTAD, 2018).The revenue generated in coffee trade in 2016 was estimated at US$30.7 billion worldwide (UNCTAD, 2018).

Among the 124 species within the genus *Coffea* (Davis et al., 2006), only two are economically dominant in the world trade, representing 99% of the world coffee production, namely, Arabica coffee *Coffea arabica* L. and Robusta coffee *C. canephora* Pierre (DaMatta and Ramalho, 2006). Robusta coffee is more vigorous and productive, however, beverages derived from its beans are of lower quality compared to Arabica coffee. In contrast, Arabica coffee is associated with high quality drink and it represents 70% of the world production (Dussert et al., 1997; DaMatta, 2004; Bunn et al., 2015). It is native to Afromontane rainforests of Ethiopia at elevations between 1,200 and 2,800

meters above sea level (m asl) (Meyer, 1965). At this elevation, the average annual temperature ranges between 18-20°C, with a little seasonal fluctuation and rainfall between 1,600 and 2,000 mm, which is optimal for coffee production (DaMatta and Ramalho, 2006).

In the highlands of eastern Africa, the livelihood of millions of people depends on incomes from the cultivation, processing, transportation and marketing of Arabica coffee. Coffee provides employment to about 22 million people in five of the main producing countries of the region, namely Ethiopia, Rwanda, Tanzania, Burundi and Kenya (Agwanda et al., 2008; Van Asten et al., 2011; Gathura, 2013; Craparo et al., 2015). Ethiopia is the fifth largest global exporter of Arabica coffee and the main producer in Africa (Davis et al., 2012). However, coffee producers in the region are mainly small scale farmers, with little on investment in farm inputs, improved coffee varieties, pesticides or fertilisers. As a consequence, they often face low and erratic yields that can lead them out of the coffee farming (Baffes, 2005).

Insect pests, diseases, decreasing soil fertility and susceptibility of coffee cultivars to climate variability are the major constraints threatening Arabica coffee production in east Africa (Ribas et al., 2006; Ovalle-Rivera et al., 2015). Among the biotic factors, coffee berry disease and leaf rust caused by the fungi *Colletotrichum kahawae* (Bridge and Waller), and *Hemileia vastatrix* (Berkeley and Broome), are the most dangerous diseases of coffee worldwide, accounting for 30-52% of the yield loss (Maia et al., 2013; Garedew et al., 2017). In addition, insect pests including Antestia bugs, *Antestiopsis* spp. (Hemiptera: Pentatomidae), coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) and the African coffee white stem borer,

Monochamus leuconotus (Pascoe) (Coleoptera: Cerambycidae), being the most important pests in east Africa (Jaramillo et al., 2011; Ahmed et al., 2016; Gichuhi et al., 2017).

Stink bugs (*Antestiopsis* spp.)

The stink bug in the genera *Antestiopsis* spp. (Hemiptera: Pentatomidae) consist of a species complex, that are widely distributed in Africa (Greathead, 1966). They have a distinctive morphology of striking patterns and markings in black, white and orange on their body (Greathead, 1966; Babin et al., 2018). The similarity in colour makes it difficult to distinguish between species (Figure 1.1). *Antestiopsis* spp. have six immature stages, namely egg and five nymphal stages (Mendesil and Abebe, 2004; Ahmed et al., 2016). The complete life cycle from egg to adult stages takes about three to six months depending on the environmental conditions (Kirkpatrick, 1937; Mendesil and Abebe, 2004; Azrag et al., 2017). All species from this genus were found on a wide range of plants belonging to different families (Babin et al., 2018). However, the Rubiaceae family, especially coffee is a primary host for those have been found in east Africa. Both nymphs and adults of *Antestiopsis* spp. feed on coffee berries, leaves, flower buds and shoots by sucking sap leading to serious damage to coffee plantations.

Greathead (1966) gave the taxonomy and distribution of *Antestiopsis* spp. in Africa, which includes *Antestiopsis thunbergii* (Gmelin) (known as *A. orbitalis*), *A. intricata* Ghesquière and Carayon, *A. facetoides* Greathead, *A. clymeneis* Kirkaldy, *A. cruciate* F., *A. falsa* Schouteden, *A. crypta* Greathead, *A. lepelleyi* Greathead and *A. littoralis* Greathead (Greathead, 1966). Nevertheless, the most important species that cause economic damage to coffee plantations are *A. thunbergii* (Figure 1.1A), *A. intricata*

(Figure 1.1B), and *A. facetoides* (Figure 1.1C), with *A. thunbergii* being the most dangerous in eastern Africa (Greathead, 1966; Azrag et al., 2018). These three species have different distributions with *A. thunbergii* being found in eastern and southern Africa. On the other hand, *A. intricata* is mostly found in western Africa and some part of east Africa in countries like Ethiopia and Uganda. However, the distribution of *A. facetoides* is limited to Kenya and Tanzania, where the species is usually found mixed with *A. thunbergii* at elevations between 1,000 and 1,600 m asl (Babin et al., 2018).

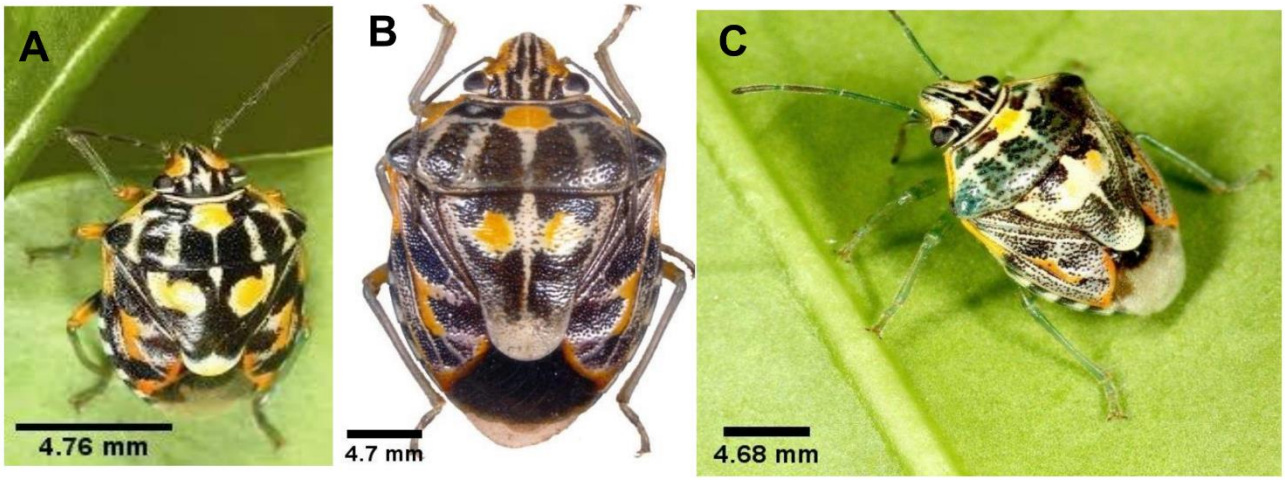


Figure 1.1 Three most important *Antestiopsis* spp. found in eastern Africa with A: *Antestiopsis thunbergii*, B: *Antestiopsis intricata*, and C: *Antestiopsis facetoides*.

Coffee berry borer (*Hypothenemus hampei*)

The coffee berry borer, *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae) is a small beetle of about 1.4-2.0 mm in length, with males being smaller than females. It is believed that the pest is native to central Africa and spread throughout the

worldwide due to coffee trade (Jaramillo et al., 2011). The female of *H. hampei* penetrates coffee berries for feeding and makes galleries where she lays her eggs. Infested berries can be easily recognised by the round entrance holes close to the apex of the berries (Jaramillo et al., 2005). According to Jaramillo et al. (2009), a single female can oviposit her entire lifespan about 300 eggs in the infested berry. Egg incubation period ranges between 3.3 and 12.0 days depending on the environmental conditions (Jaramillo et al., 2009). The duration of the larval stage ranges between 11 and 28 days under the temperature between 20 and 30°C (Jaramillo et al., 2009). However, some authors reported conflicting data on the development time for immature stages of *H. hampei*, even when the pest was reared at the same temperature. For example, the development times reported for the larval stage at 27°C, were 12, 13, 17, and 25 days (Brun et al., 1993; Barrera, 1994; Fernández and Cordero, 2007; Jaramillo et al., 2009). This mainly is due to the cryptic nature and small size of the beetle, which make it difficult to study its life history traits under controlled conditions. After immature stages develop to adult, the mating occurs inside the berry between the siblings, then, the females exit to infest new berries, while the males, which are flightless, usually die inside. The sex ratio is biased towards females in the ratio 10:1 (females: male) (Brun et al., 1995).

Coffee white stem borer (*Monochamus leuconotus*)

The African coffee white stem borer *Monochamus leuconotus* (Pascoe) is known as an indigenous pest of Arabica coffee in eastern and south Africa for more than a century (Knight, 1939; Schoeman et al., 1998). It was first recorded in south Africa by Pascoe in 1869 (Tapley,1960; Schoeman et al., 1998). Later, in 1930's, it was reported in east Africa (Kenya) on Arabica coffee at elevations below 1,600 m asl (Knight, 1939). *Monochamus*

leuconotus feeds on the coffee stems leading to phloem and wood destruction, yellowing of the leaves, low productivity, and dieback of the trees (Tapley, 1960). As *H. hampei*, *M. leuconotus* has a cryptic nature inside the coffee wood with a long-life cycle (a proximately 2 years), which make it difficult to study its life history traits. In fact, most studies on *M. leuconotus* described life cycle traits under field conditions or room temperatures inside the coffee wood (Tapley, 1960; Schoeman et al., 1998). However, a recent study by Gichuhi et al. (2017) developed an artificial diet and studied the life cycle of the pest at a constant temperature of 25°C, which opens a new door for further studies on the thermal biology of *M. leuconotus*.

In the field, the life cycle begins when the female deposits eggs under the bark of the lower part of the coffee stem (Jonsson et al., 2015; Kutywayo et al., 2013). The size of the egg is about 4-5 mm long and 1.5-2.0 mm in diameter (Tapley, 1960). The incubation period takes between 15 and 26 days at 25-26°C (Schoeman et al., 1998; Gichuhi et al., 2017). After egg hatching, the larva starts feeding on the bark of the coffee stem and destroys the phloem; this damage is known as ring barking (Figure 1.2A). The ring barking stage takes between three to four months after which the larva bores into the wood (wood boring phase) (Figure 1.2B and 1.2C). It feeds on the coffee stem for 17 to 20 months before pupation (Tapley, 1960; Schoeman et al., 1998). The pupation occurs in the pupa chamber, and it takes between 21 to 32 days before the adult emerges (Gichuhi et al., 2017). After emerging, the adult remains in the pupal chamber for about one month to become mature (Tapley, 1960). Thereafter, it starts chewing through the wood and makes a small circular exit hole (Figure 1.2D), after which mating between males and females occurs and egg laying resumes (Hamburg et al., 1998).

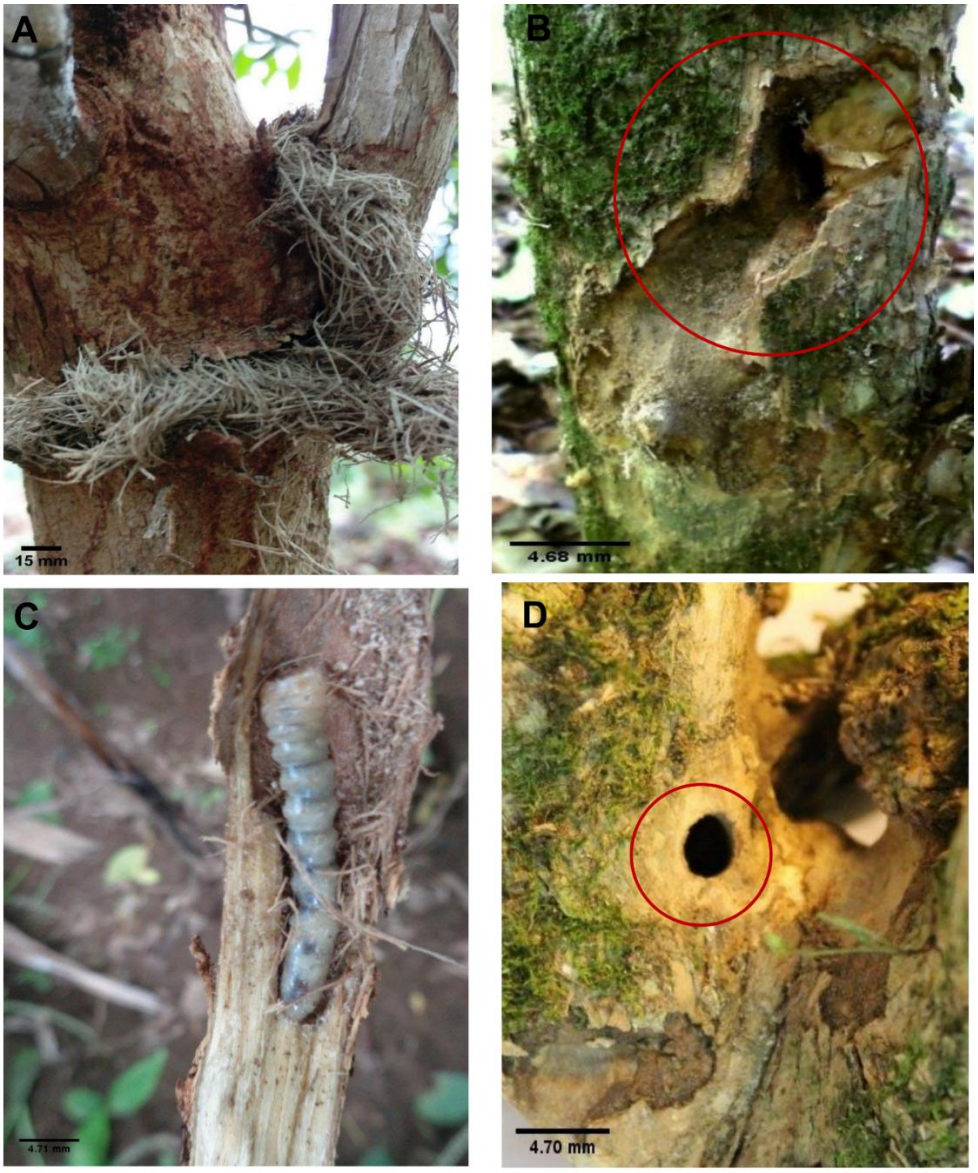


Figure 1.2 Signs of infestation by the coffee white stem borer *Monochamus leuconotus* on the stem of a coffee tree A, ring barking stage (frass); B, wood boring phase (entry hole of the larva into the coffee stem); C, larva feeding inside the coffee stem; and D, adult exit hole from the coffee stem.

Antestiopsis spp. and *M. leuconotus* cause significant yield loss of about 25 and 45% of total production and affect the quality of coffee beans (Gichuhi et al., 2017; Azrag et al., 2017). On the other hand, *H. hampei* causes yield loss estimated up to U$500 million

worldwide annually (Vega et al., 2002), with high infestation levels between 80 and 90% reported in east Africa (Jonsson et al., 2015). Yet, an increase of infestation levels by the three insect pests of coffee as well as, changes in their distribution in east Africa is forecasted with warming climate, which might lead to profound consequences on coffee farming (Jaramillo et al., 2011; Kutywayo et al., 2013).

In the last decades, responses of agricultural insect pests to temperature fluctuations in east Africa have gained much attention (Jaramillo et al., 2011; Khadioli et al., 2014; Mwalusepo et al., 2015; Ngowi et al., 2016). Temperature is the most important environmental factor that has a direct influence on insect development and distribution (Bale et al., 2002). In the context of global warming, an increase in temperature may have a direct effect on incidence of insect pests on crops, which might lead to severe damages. Pest population density, geographical distribution and population dynamics will be impacted by temperature changes through insect physiology and behaviour (Estay et al., 2009). Moreover, indirect effects are also expected due to the impact of temperature on host plants and biological control agents such as the natural enemies (Bale et al., 2002). Therefore, estimating the relationship between temperature and development rate, survival and reproduction of insect pests of coffee is crucial for calculating their thermal thresholds (Tonnang et al., 2013). This will allow the prediction of areas that are most suitable for the species establishment, which could help governments and farmers to adapt to changes in pest populations by developing adequate management strategies aimed at preventing crop losses and consequences of such.

To understand the relationship between species distribution and climatic factors, modelling has been widely used as a tool to predict insects' life histories and distribution

within the global warming scenario (Estay et al., 2009; Tonnang et al., 2013; Kutywayo et al., 2013). Two modelling approaches, namely inductive and deductive were adopted for evaluating and understanding the ecological niche of pests in different agroecological zones (Pearson and Dawson, 2003; Tonnang et al., 2013). Inductive approach is based on the environment in which the insect has been found in the region (present/absent data). In this method, long term meteorological data with interpolation are used in programs such as BIOCLIM and CLIMEX to predict the distribution of a species based on climatic factors (Pearson and Dawson, 2003; Beaumont et al., 2005; Feilhauer et al., 2012).

On the other hand, deductive method relies on phenology models (temperature-dependent development models) that describe basic insect life history parameters such as development time, development rate, mortality, fecundity and adult life span using linear and nonlinear functions (Sporleder et al., 2004; Tonnang et al., 2013; Fand et al., 2015). This approach is based on life table studies under laboratory conditions at different constant temperatures for the insect species. Then, the resultant life table parameters are simulated based on daily temperature data, in order to generate and map three main indices measuring the risks of the pest under climate warming: i) the risk index which assesses the probability of the pest to survive and establish in a particular area, in the current and future climatic situations, ii) the generation index, which estimates the mean number of generations that may be produced within a given year and iii) the activity index, which is related to the finite rate of increase of the pest population and assesses the damage that can be caused by the pest (Tonnang et al., 2013).

Based on the fact that temperature is the most important environmental factor that affects insect development and distribution, it is necessary to understand the impact of

temperature on the life history traits of insect pests in order to predict their current and future distributions on coffee plantations. Thus, the prediction of the potential impact of climate change on the distribution and abundance of coffee insect pests become more relevant for developing effective control measures to minimise the risk. Therefore, in this thesis, the deductive approach of modelling was used to predict the life history traits and distribution of three major insect pests of Arabica coffee in east Africa.

Rationale of the study

Climate projection in east Africa showed an increase in temperature between 1.9 and 3.0°C by the year 2055 (Platts et al., 2015). This rise is expected to have a serious effect on coffee flowering and fruiting pattern leading to yield reduction. In addition, insect pests, especially *H. hampei*, *M. leuconotus*, and *Antestiopsis* spp. cause high damage to coffee plantations in the region (Jaramillo et al., 2011; Ahmed et al., 2016; Gichuhi et al., 2017). The yield loss is estimated to be between 25 and 45% for *Antestiopsis* spp. and *M. leuconotus* (Gichuhi et al., 2017; Azrag et al., 2017). While *H. hampei* is responsible for economic loss estimated at U$500 million worldwide annually (Vega et al., 2002), with high infestation level between 80 and 90% being reported in east Africa (Jonsson et al., 2015). Due to climate change, the rising temperature may have a potential impact on the geographical distribution, abundance, and population dynamics of insect pests in coffee plantations. As a consequence, it may increase the extent of crop losses in eastern Africa which will be complemented with direct effects of temperature on coffee production (Jaramillo et al., 2011; Kutywayo et al., 2013). Interestingly, the control measures developed for *Antestiopsis* spp, *H. hampei* and *M. leuconotus* are not sufficient to control their damages in coffee plantations. This is mainly due to the insufficient knowledge of

the thermal biology, ecology and distribution of these pests. In particular, the impact of temperature on the life history traits and distribution has major gaps in knowledge, which need to be addressed in order to develop effective management strategies. Understanding the biology, ecology and distribution of the pest in the context of global warming is a key component to developing an effective and ecologically sound pest management strategy (Tonnang et al., 2013). The work presented in this thesis was conducted in order to better understand the role that temperature plays on the life histories of major coffee pests as well as, the effect of major agro-ecological factors on the distribution and seasonal variation of these pests.

References

Agwanda, C. O., Musebe, R., & Owaga, T. O. (2008). Improving coffee quality in East and Central Africa through enhanced processing practices (Rwanda and Ethiopia) final technical report July 2004–February 2008.https://commonfunds.sharepoint.com/Projects/ICO/ICO-22/Final%20Technical%20Report%20CFC-ICO-22.pdf.

Ahmed, A. G., Murungi, L. K., & Babin, R. (2016). Developmental biology and demographic parameters of antestia bug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae), on *Coffea arabica* (Rubiaceae) at different constant temperatures. *International Journal of Tropical Insect Science*, *36*, 119–127.

Azrag, A. G., Murungi, L. K., Tonnang, H. E., Mwenda, D., & Babin, R. (2017). Temperature-dependent models of development and survival of an insect pest of African tropical highlands, the coffee antestia bug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae). *Journal of Thermal Biology*, *70*, 27–36.

Azrag, A. G., Pirk, C. W., Yusuf, A. A., Pinard, F., Niassy, S., Mosomtai, G., & Babin, R. (2018). Prediction of insect pest distribution as influenced by elevation: combining field observations and temperature-dependent development models for the coffee stink bug, *Antestiopsis thunbergii* (Gmelin). *PloS One*, 13, e0199569.

Babin R., Mbondji Mbondji, P., Mendesil, E., Mugo, H. M., Lee, J-H., Serracin, M., Rukazambuga, N. D. T. M., & Miller, T. A. (2018). The antestia bug complex in Africa and Asia. In: McPherson, J. E, editor. Invasive stink bugs and related

species (Pentatomoidea): biology, higher systematics, semiochemistry, and management. *CRC Press*. USA. pp. 465–493.

Baffes, J. (2005). Tanzania's coffee sector: constraints and challenges. *Journal of International Development, 17*, 21–43.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., & Good, J. E. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, *8*, 1–16.

Barrera, J. F. (1994). Dynamique des populations du scolyte des fruits du caféier, *Hypothenemus hampei* (Coleoptera: Scolytidae), et lutte biologique avec le parasitoide *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae), au Chiapas, Mexique. PhD thesis, *Université Paul-Sabatier*. Toulouse III, France. p.301.

Beaumont, L. J., Hughes, L., & Poulsen, M. (2005). Predicting species distributions: use of climatic parameters in BIOCLIM and its impact on predictions of species' current and future distributions. *Ecological Modelling*, *186*, 251–270.

Brun, L. O., Gaudichon, V., & Wigley, P. J. (1993). An artificial diet for continuous rearing of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). *International Journal of Tropical Insect Science, 14*, 585–587.

Brun, L. O., Stuart, J., Gaudichon, V., Aronstein, K., & French-Constant, R. (1995). Functional haplodiploidy: a mechanism for the spread of insecticide resistance in an important international insect pest. *Proceedings of the National Academy of Sciences*, *92*, 9861–9865.

Bunn, C., Läderach, P., Rivera, O. O., & Kirschke, D. (2015). A bitter cup: climate change profile of global production of Arabica and Robusta coffee. *Climatic Change*, *129*, 89–101.

Craparo, A. C. W., Van Asten, P. J. A., Läderach, P., Jassogne, L. T. P., & Grab, S. W. (2015). *Coffea arabica* yields decline in Tanzania due to climate change: global implications. *Agricultural and Forest Meteorology*, *207*, 1–10.

DaMatta, F. M. (2004). Exploring drought tolerance in coffee: a physiological approach with some insights for plant breeding. *Brazilian Journal of Plant Physiology*, *16*, 1–6.

DaMatta, F. M., & Ramalho, J. D. C. (2006). Impacts of drought and temperature stress on coffee physiology and production: a review. *Brazilian Journal of Plant Physiology*, *18*, 55–81.

Davis, A. P., Govaerts, R., Bridson, D. M., & Stoffelen, P. (2006). An annotated taxonomic conspectus of the genus Coffea (Rubiaceae). *Botanical Journal of the Linnean Society*, *152*, 465–512.

Davis, A. P., Gole, T. W., Baena, S., & Moat, J. (2012). The impact of climate change on indigenous Arabica coffee (*Coffea arabica*): predicting future trends and identifying priorities. *PloS One*, *7*, e47981.

Dussert, S., Chabrillange, N., Engelmann, F., Anthony, F., & Hamon, S. (1997). Cryopreservation of coffee (*Coffea arabica* L) seeds: importance of the precooling temperature. *Cryo-Letters*, *18*, 269–276.

Estay, S. A., Lima, M., & Labra, F. A. (2009). Predicting insect pest status under climate change scenarios: combining experimental data and population dynamics modelling. *Journal of Applied Entomology*, *133*, 491–499.

Fand, B. B., Sul, N. T., Bal, S. K., & Minhas, P. S. (2015). Temperature impacts the development and survival of common cutworm (*Spodoptera litura*): simulation and visualization of potential population growth in India under warmer temperatures through life cycle modelling and spatial mapping. *PloS One*, *10*, e0124682.

Feilhauer, H., He, K. S., & Rocchini, D. (2012). Modeling species distribution using niche-based proxies derived from composite bioclimatic variables and MODIS NDVI. *Remote Sensing*, *4*, 2057–2075.

Fernández, S., & Cordero, J. (2007). Biología de la broca del café *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) en condiciones de laboratorio. *Bioagro*, *19*, 35– 40.

Figueroa, G. A., Homann, T., & Ravel, H. M. (2016). Coffee production wastes: potentials and perspective. *Austin Food Sciences*, *1*, 1014.

Garedew, W., Lemessa, F., & Pinard, F. (2017). Assessment of berry drop due to coffee berry disease and non-CBD factors in arabica coffee under farmers fields of Southwestern Ethiopia. *Crop Protection*, *98*, 276–282.

Gathura, M. N. (2013). Factors affecting small-scale coffee production in Githunguri District, Kenya. *International Journal of Academic Research in Business and Social Sciences*, *3*, 132–149.

Gichuhi, J. M., Ndegwa, P. N., Mugo, H. M., Guandaru, E. K., & Babin, R. (2016). Rearing method and developmental biology of the African coffee white stem borer, *Monochamus leuconotus* (Coleoptera: Cerambycidae). *Journal of Economic Entomology, 110*, 1120–1126.

Greathead, D. J. (1966). A taxonomic study of the species of *Antestiopsis* (Hemipteea, Pentatomidae) associated with *Coffea arabica* in Africa. *Bulletin of Entomological Research, 56*, 515–554.

ICO. (2017). Coffee prices increase as concerns about Brazil's 2017/18 crop emerge. http://www.ico.org/documents/cy2016-17/cmr-0717-e.pdf.

Jaramillo, J., Bustillo, A. E., Montoya, E. C., & Borgemeister, C. (2005). Biological control of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae) by *Phymastichus coffea* (Hymenoptera: Eulophidae) in Colombia. *Bulletin of Entomological Research, 95*, 467–472.

Jaramillo, J., Chabi-Olaye, A., Kamonjo, C., Jaramillo, A., Vega, F. E., Poehling, H. M., & Borgemeister, C. (2009). Thermal tolerance of the coffee berry borer *Hypothenemus hampei*: predictions of climate change impact on a tropical insect pest. *PloS One, 4*, e6487.

Jaramillo, J., Muchugu, E., Vega, F. E., Davis, A., Borgemeister, C., & Chabi-Olaye, A. (2011). Some like it hot: the influence and implications of climate change on coffee berry borer (*Hypothenemus hampei*) and coffee production in East Africa. *PloS One, 6*, e24528.

Jonsson, M., Raphael, I. A., Ekbom, B., Kyamanywa, S., & Karungi, J. (2015). Contrasting effects of shade level and altitude on two important coffee pests. *Journal of Pest Science, 88*, 281–287.

Khadioli, N., Tonnang, Z. E. H., Muchugu, E., Ong'amo, G., Achia, T., Kipchirchir, I., Kroschel., J., & Le Ru, B. (2014). Effect of temperature on the phenology of *Chilo partellus* (Swinhoe) (Lepidoptera, Crambidae); simulation and visualization of the potential future distribution of *C. partellus* in Africa under warmer temperatures through the development of life-table parameters. *Bulletin of Entomological Research, 104*, 809–822.

Kirkpatrick, T. W. (1937). Studies on the ecology of coffee plantations in East Africa. II. The autecology of *Antestia* spp. (Pentatomidae) with a particular account of a strepsipterous parasite. *Transactions of the Royal Entomological Society of London*, *86*, 247–343.

Knight, C. D. (1939). Observations on the life-history and control of white borer of coffee in Kenya. *The East African Agricultural Journal*, *5*, 61–67.

Kutywayo, D., Chemura, A., Kusena, W., Chidoko, P., & Mahoya, C. (2013). The impact of climate change on the potential distribution of agricultural pests: the case of the coffee white stem borer (*Monochamus leuconotus* P.) in Zimbabwe. *PloS One*, *8*, e73432.

Maia, T. A., Maciel-Zambolim, E., Caixeta, E. T., Mizubuti, E. S. G., & Zambolim, L. (2013). The population structure of *Hemileia vastatrix* in Brazil inferred from AFLP. *Australasian Plant Pathology*, *42*, 533–542.

Masumbuko, L. I., & Bryngelsson, T. (2006). Inter simple sequence repeat (ISSR) analysis of diploid coffee species and cultivated *Coffea arabica* L. from Tanzania. *Genetic Resources and Crop Evolution, 53,* 357–366.

Mendesil, E., & Million, A. (2004). Biology of antestia bug *Antestiopsis intricata* (Ghesquiême & Carayon) (Hemiptera: Pentatomidae) on *Coffea arabica* L. *Journal of Coffee Research, 32,* 30–39.

Meyer, F. G. (1965). Notes on wild *Coffea arabica* from Southwestern Ethiopia, with some historical considerations. *Economic Botany, 19,* 136–151.

Malara, A., Paone, E., Frontera, P., Bonaccorsi, L., Panzera, G., & Mauriello, F. (2018). Sustainable exploitation of Coffee silverskin in water remediation. *Sustainability, 10,* 3547.

Mwalusepo, S., Tonnang, H. E., Massawe, E. S., Okuku, G. O., Khadioli, N., Johansson, T., Calatayud, P., & Le Ru, B. P. (2015). Predicting the impact of temperature change on the future distribution of maize stem borers and their natural enemies along East African mountain gradients using phenology models. *PloS One, 10,* e0130427.

Naranjo S. E., & Ellsworth P. C. (2005). Mortality dynamics and population regulation in *Bemisia tabaci*. *Entomologia Experimentalis et Applicata, 116,* 93–108.

Ngowi, B. V., Tonnang, H. E., Mwangi, E. M., Johansson, T., Ambale, J., Ndegwa, P. N., & Subramanian, S. (2017). Temperature-dependent phenology of *Plutella xylostella* (Lepidoptera: Plutellidae): Simulation and visualization of current and future distributions along the Eastern Afromontane. *PloS One, 12,* e0173590.

Ovalle-Rivera, O., Läderach, P., Bunn, C., Obersteiner, M., & Schroth, G. (2015). Projected shifts in *Coffea arabica* suitability among major global producing regions due to climate change. *PloS One*, *10*, e0124155.

Pearson, R. G., & Dawson, T. P. (2003). Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful?. *Global Ecology and Biogeography*, *12*, 361–371.

Régnière, J., Powell, J., Bentz, B., & Nealis, V. (2012). Effects of temperature on development, survival and reproduction of insects: experimental design, data analysis and modeling. *Journal of Insect Physiology*, *58*, 634–647.

Ribas, A. F., Kobayashi, A. K., Pereira, L. F. P., & Vieira, L. G. E. (2006). Production of herbicide-resistant coffee plants (*Coffea canephora* P.) via agrobacterium tumefaciens-mediated transformation. *Brazilian Archives of Biology and Technology*, *49*, 11–19.

Schoeman, P. S., Van Hamburg, H., & Pasques, B. P. (1998). The morphology and phenology of the white coffee stem borer, *Monochamus leuconotus* (Pascoe) (Coleoptera: Cerambycidae), a pest of Arabica coffee. *African Entomology*, *6*, 83–89.

Sporleder, M., Kroschel, J., Quispe, M. R. G., & Lagnaoui, A. (2004). A temperature-based simulation model for the potato tuberworm, *Phthorimaea operculella* Zeller (Lepidoptera; Gelechiidae). *Environmental Entomology*, *33*, 477–486.

Tapley, R. G. (1960). The white coffee borer, *Anthores leuconotus* Pasc., and its control. *Bulletin of Entomological Research*, *51*, 279–301.

Tonnang, E. Z. H., Juarez, H., Carhuapoma, P., Gonzales, J. C., Mendoza, D., Sporleder, M., Simon, R. & Kroschel, J. (2013). ILCYM–Insect Life Cycle Modeling. A software package for developing temperature-based insect phenology models with applications for local, regional and global analysis of insect population and mapping. *International Potato Center*. Lima, Peru. pp.193.

UNCTAD. (2018). Commodities at a glance Special: issue on coffee in East Africa. https://unctad.org/en/PublicationsLibrary/ditccom2018d1_en.pdf.

Van Asten, P. J. A., Wairegi, L. W. I., Mukasa, D., & Uringi, N. O. (2011). Agronomic and economic benefits of coffee–banana intercropping in Uganda's smallholder farming systems. *Agricultural Systems*, *104*, 326–334.

Vega, F. E., Franqui, R. A., & Benavides, P. (2002). Scientific note: The presence of the coffee berry borer, Hypothenemus hampei, in Puerto Rico: fact or fiction?. *Journal of Insect Science*, *2*, 1–3.

Vega, F. E., Ebert, A. W., & Ming, R. (2007). Coffee germplasm resources, genomics and breeding. *Plant Breeding Reviews*, *30*, 415–447.

CHAPTER TWO

Identifying temperature thresholds using life cycle modelling and their implications for developmental times and survival of the coffee berry borer *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae)

Abstract

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) is the most destructive insect pest of coffee worldwide. It feeds on coffee berries at all maturation stages, leading to significant losses of both quantity and quality of coffee beans. This study aimed to provide the thermal requirements of immature stages of *H. hampei* in the laboratory, using a new observation method. Larval development and survival were monitored daily on fresh Arabica coffee seeds, under seven constant temperatures in the range 15-35°C, with 80 ± 5% RH and 12:12 L: D photoperiod. Linear and non-linear functions were fitted to the development data plotted against temperature, using Insect Life Cycle Modelling software (ILCYM). Temperature significantly affected the development time of all immature stages. Egg incubation period ranged 4.6-16.8 days, under temperature between 15 and 30°C. No development occurred at 35°C, and the larva stage did not develop to pupa at 15°C. The minimum temperature threshold (T_{min}) estimated from linear regressions was 10.5, 13.0, 15.0 and 13.0°C, for egg, larva, pupa, and the total development from egg to adult, respectively. The maximum temperature threshold (T_{max}) estimated from the Sharpe and DeMichele function was 32°C for egg to adult development. The thermal constant (*k*) was estimated at 78.1, 188.7, 36.5, and 312.5 degree days, for egg, larva, pupa, and for egg to adult, respectively. Our results will help understand the relationships between *H. hampei* life cycle and temperature and the pest population dynamics and distribution in coffee plantations as impacted by climate.

Key words: *Coffea arabica*, ILCYM, life cycle, development time, development rate.

Introduction

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) is the most devastating insect pest of coffee worldwide (Damon, 2000; Jaramillo et al., 2006; Vega et al., 2009). The tiny beetle feeds on coffee berries at all maturation stages, leading to losses of both quantity and quality of coffee beans (Le Pelley, 1968; Vega et al., 2003; Jaramillo et al., 2010). Feeding damage is of two types: adult female feeding lesions on developing fruits lead to berry drop, and offspring feeding galleries in the berry endosperm (coffee seeds) lead to bean weight loss and higher vulnerability to infection by diseases. Economic losses due to *H. hampei* infestations globally are estimated at US$500 million annually (Vega et al., 2002). In eastern Africa, infestation level can be as high as 80-90% in medium to low-elevation coffee plantations (<1,500 m asl), causing serious economic loss to small scale farmers (Jonsson et al., 2015). Its high reproductive rate, short life cycle (Jaramillo et al., 2009) and cryptic behaviour that makes chemical spraying inefficient (Brun et al., 1989) may account for the success of *H. hampei* as a devastating pest and a threat to coffee growers.

Reproductive females of *H. hampei* are small in size (1.4-2.0 mm long) and they bore galleries in the coffee seeds for egg laying; usually, a single female attacks a berry (Baker et al., 1992; Jaramillo et al., 2010; Jaramillo et al., 2011). The dry matter content of the seeds is the most crucial factor that determines egg-laying initiation. When females enter berries with dry matter content <20%, they usually exit them shortly or stay inside waiting that the seeds become hard enough to allow oviposition and offspring development (Jaramillo et al., 2010). After adults emerged, the new females mostly leave the berry after having mated with their male siblings. In some cases, mothers lay their eggs in the

same berry where they were born (Baker et al., 1992; Jaramillo et al., 2010), leading to generation overlapping and populations of hundreds of individuals in one single berry. Due to this mode of reproduction, the wingless males of *H. hampei*, which are smaller than females, usually do not leave the berry where they were born. In fact, the the sex ratio is female-biased, with a ratio estimated at 1:10 male to females (Brun et al., 1994).

Despite the economic importance of *H. hampei* in the coffee industry, there are still major gaps in the knowledge of its thermal biology, especially since the thermal requirements for its development are poorly documented. In addition, literature reports inconsistent data on the pest's development as influenced by temperature. For example, Barrera (1994) reported a larval development time of 17 days at 27°C, while it was 13 days at the same temperature in the study by Fernández and Cordero (2007). The reason for this may be the use of different methodologies for rearing and observation. *Hypothenemus hampei* immature stage development happens inside coffee berries and is therefore difficult to observe without disturbing it. In the present study, we developed a new observation method to overcome this difficulty.

As poikilotherms, insects depend on the temperature of their environment to develop (Régnière et al., 2012). The ability of an insect to develop under a wide range of temperatures is an important adaptive mechanism for enhanced survival and growth in different climatic conditions (Bale et al., 2002). Knowing this range of temperatures is crucial for predicting distribution, population dynamics and outbreaks of insect pests (Tonnang et al., 2013; Azrag et al., 2017). Linear models are usually used to describe the relationship between development rate and temperature. They allow the calculation of thermal requirements for development, such as minimum temperature threshold (T_{min})

and the thermal constant *k*, in degree days (DD), which represents the amount of energy needed to complete development (Wagner et al., 1991; Kontodimas et al., 2004; Nielsen et al., 2008). At the highest temperatures, however, the relationship between development and temperature is rarely linear, and nonlinear models are preferably adopted for the calculation of the maximum temperature threshold (T_{max}) and optimum temperature for development (Hilbert and Logan, 1983; Wagner et al., 1991; Worner, 1992). In this chapter, we adopted a similar approach for *H. hampei* immature stages. First, we developed an original observation method for monitoring the development of immature stages on a daily basis under seven constant temperatures. Then, we adapted linear and nonlinear models to development and survival data plotted against temperature in order to provide the thermal requirements for development of this major pest of coffee.

Materials and Methods

Insect field collection for colony initiation

Experiments started with the collection of *H. hampei* adult females from the field. Initial sampling was done in small holding coffee farms located on the Aberdare range, in Murang'a County, Kenya (sampling area between 0.710°S – 37.083°E and 0.695°S – 36.923°E, with elevation range ≈ 1,300-1,800 m asl). In this area, annual rainfall ranges ≈ 1,200-1,800 mm, with two rainy seasons, from mid-March to May and from October to mid-December, the former being the most important (Ovuka and Lindqvist, 2000). The annual mean temperature varies according to elevation, with ≈ 20°C at 1,500 m asl. In the area, land use is dominated by small scale coffee farms, mixed with food crops such as maize, beans and banana, with trees such as grevillea, *Grevillea robusta* (A. Cunn. ex

R. Br.) and macadamia, *Macadamia* spp. that provide shade to coffee trees. Coffee berries infested by *H. hampei* were collected from 13 coffee farms. Infested berries are easily detected by the holes the females drill, almost always at the apex of the berry, to penetrate the fruit. Berries were kept in 0.5-L plastic containers (Foodmate, Kenpoly Manufacturers Ltd, Kenya), measuring 10.4 cm in mean diameter and 6.0 cm deep, for transportation to the coffee pest laboratory at International Centre of Insect Physiology and Ecology (*icipe*), Kenya. Container lids had a 4 cm diameter opening, covered with a fine mesh tissue for aeration. Enough infested berries were collected to fill 20 of these containers. In the laboratory, the containers were kept for three weeks in an incubator (SANYO MIR-553, Sanyo Electrical Ltd., Tokyo, Japan) set at 25 ± 0.5°C, with 80 ± 5% RH and 12:12 L: D photoperiod (Jaramillo et al., 2010). Afterwards, the berries were dissected with a scalpel under a stereo microscope using the ×10 magnification and females were gently collected with a mouth aspirator. Approximately 12,000 *H. hampei* adult females were collected this way for subsequent experiments.

Egg production

Mature berries of *Coffea arabica* var. Ruiru 11 were collected from the same coffee farms where *H. hampei* were sampled for colony initiation. The berries were carefully checked for infestation and only non-infested fruits were collected and transported to the laboratory. Here, berries were washed with detergent (Teepol, Sudi Chemical Industry Ltd, Kenya), then thoroughly rinsed with water and finally placed on a paper towel for 2 h to dry at room temperature (≈25 ± 2°C). Afterwards, the berries were distributed in 60 containers of the same type as those used for field collection (0.5-L aerated plastic

containers), with each container having 80 berries. Then, approximately 200 reproductive *H. hampei* females obtained from field infested berries were introduced into each container. After 18 h, newly infested berries were collected and transferred into new containers (each container had between 50 and 70 infested berries) of the same type as previous (0.5-L aerated plastic containers) but, lined with a humidified mixture of plaster of Paris and activated charcoal to maintain a high level of humidity (Jaramillo et al., 2010). These containers were incubated (as previously described) at 25 ± 0.5°C, with 80 ± 5% RH and 12:12 L:D photoperiod. Five days after infestation, the berries were dissected under a stereo microscope using the ×10 magnification and the eggs were carefully collected with the aid of a fine camel-hair brush and thereafter placed on discs made of paper towels in small plastic Petri dishes (3.5 cm wide, 1 cm deep). Each Petri dish contained 10-12 eggs to ease observation under the stereo microscope. We assumed that most of eggs were ≤ 24 h old at that time (all of them were < 48 h), since *H. hampei* females usually start laying eggs 4 to 6 days after they have penetrated the berry (Jaramillo et al., 2010).

Development and survival of immature stages

Eggs obtained with the method described above (egg production section) were incubated in laboratory incubators of the same model as previously mentioned, but now set at the following seven constant temperatures: 15, 18, 20, 23, 25, 30 and 35°C (± 0.5°C), at 80 ± 5% RH and 12:12 L:D photoperiod. For each temperature, between 100 and 200 eggs were observed daily for a month under a stereo microscope (×10 magnification) to detect

hatching and assess the incubation period. The eggs that did not hatch during this period were observed for two additional weeks and then, if not hatched, recorded as dead.

After emerging, the larvae were reared individually on fresh coffee seeds. Mature coffee berries were dissected to extract the two seeds from inside. Then, a slit approximately 1.5 mm deep and 2 mm wide was made into the seed using a sharp scalpel, where the larva was carefully placed using a fine camel hair brush. The seed with the larva was gently wrapped with aluminium foil in order to maintain the larva in conditions similar to those inside the berry and to prevent it from escaping. Each seed was labelled and placed in a well (2.5 cm in diameter and 2 cm deep) of a 12-well plate (Costar, Corning Inc., NY, USA). Larvae were transferred to new fresh seeds every four (4) days to prevent them from boring deeply in the seed. The larvae were examined daily under a stereo microscope to record pupation and mortality. After pupation, the pupae were carefully extracted from the seeds and kept in the same well plates lined with paper towel and monitored daily until adults emerged. This rearing method enabled us to directly observe the development and survival of all immature stages and follow the same individuals from egg to adult.

Model parameterisation

The impact of temperature on the development and mortality of immature stages in *H. hampei* were described with linear and non-linear functions using the Insect Life Cycle Modelling software (ILCYM, version 3.0) (Tonnang et al., 2013). ILCYM includes a model builder that facilitates the fitting of non-linear functions to observed data. These models allow the calculation of the thermal requirements of the insect by describing the

temperature dependency of development time, development rate, and mortality rate for each life stage. Best-fitted models were thereafter selected based on their coefficient of determination (R^2) and Akaike's Information Criterion (AIC) (Tonnang et al., 2013).

Modelling the development time distribution

The frequency distributions of insect development time are usually skewed, and it is assumed that the distributions have the same shape at different constant temperatures (Sharpe and DeMichele, 1977). In a first step, cumulative frequencies of development times were plotted, for each life stage and temperature, against ln-transformed development times (normalised development time). Then, common binary distribution models were fitted to observed data in a parallel approach to estimate the development time. The estimated development time was the median of the distribution, in other words the time required for 50% cumulative frequency of the individuals in each life stage to complete the development. The best-fitted models were complementary log-log (CLL) for the egg stage and the complete development from egg to adult, and probit for the larva and pupa stages. The mathematical expressions of the CLL and probit functions are given in Table 2.2 (Tonnang et al., 2013).

Modelling the effect of temperature on the development rate

The development rate was calculated at each constant temperature, for each immature stage, and for the complete development from egg to adult as the inverse of the median development time (development rate = 1/ development time). We chose median development time because all distributions of the development times have the same

shapes and use of median times yield one standard curve for all temperatures (Wagner et al., 1984). In addition, the median is less sensitive to outliers compared to the mean times, especially when the distributions of development skewed to the longer times (Wagner et al., 1984). Calculated development rate was plotted against temperature and fitted to linear models following the formula:

$r(T) = a + bT$

Where, $r(T)$ is the development rate at temperature T; a is the intercept and b is the slope of the regression line. The minimum temperature threshold (T_{min}) at which the development rate = 0, was estimated using the intercept and slope of the regression line: $T_{min} = -a/b$; while the thermal constant k (in degree days) was estimated using $k = 1/b$.

In our study, the relationship between development rate and temperature fitted well to linear regressions for all temperatures. However, this relationship is usually not linear for the highest temperatures of development as demonstrated for other insect species (Sharpe and DeMichele, 1977). Therefore, non-linear models were also used to describe this effect for each immature stage. In addition, non-linear models allow the assessment of the maximum temperature threshold (T_{max}). Logan model (Logan et al., 1976) predicted well the effect of temperature on development rates for egg and pupa stages, while the modified version with five parameters of the biophysical Sharpe and DeMichele model (Sharpe and DeMichele, 1977) gave the best fit to the larva stage and for the complete development period from egg to adult. The mathematical expressions of the models are presented on Table 2.4 (Tonnang et al., 2013).

Modelling the effect of temperature on mortality rates

Mortality rates were calculated for each life stage at given temperatures from the number of surviving individuals relative to mortalities. Then, a modified version of the Wang model (Wang et al., 1982) was applied to describe the effect of temperature on mortality rate of each immature stage, while a second order polynomial function was used for the mortality rate for the period from egg to adult. The mathematical expressions of these models are presented on Table 2.5 (Tonnang et al., 2013).

Statistical analyses

The effect of temperature on *H. hampei* developmental time (in days) was assessed for each development stage and for the complete development from egg to adult. Data for egg incubation period, larva and pupa development time, and egg to adult development were separately subjected to Generalised Linear Model (GLM) with a Poisson distribution as recommended by O'Hara and Kotze (2010). R programming environment (R Core Team, 2016) was used for calculations with temperature as independent variable. Once significant differences were detected, data were submitted to post hoc analysis for mean comparison using Tukey test at $\alpha = 0.05$.

Results

Development time

Development occurred between 15 and 30°C for egg and between 18 and 30°C for larva and pupa (Table 2.1). The effect of temperature on the observed development times was significant for all *H. hampei* immature stages, as well as for the complete development

time from egg to adult (egg: $\chi^2 = 436$, $df = 848$, $P < 0.0001$; larva: $\chi^2 = 416.9$, $df = 745$, $P < 0.0001$; pupa: $\chi^2 = 317.98$, $df = 920$, $P < 0.0001$; egg to adult: $\chi^2 = 106.26$, $df = 81$, $P < 0.0001$). The mean observed development time for egg ranged between 4.6 ± 0.1 and 16.8 ± 0.5 days at 30 and 15°C, respectively. The longest mean development time for the larva was 39.5 ± 0.6 days at 18°C, while the shortest was 12.5 ± 0.2 days at 30°C. To complete its development to adult, the pupa stage took an average of 13.6 ± 0.3 days at 18°C and 3.0 ± 0.1 days at 30°C. Mean total development time from egg to adult was 63.4 ± 0.8 days at 18°C and, 18.0 ± 0.2 days at 30°C (Table 2.1).

The distribution of development times for egg stage and for the complete development from egg to adult was well described by a CLL model ($R^2 = 0.94$-95, AIC = 515.75-1030.1) (Figure 2.1; Table 2.2). By contrast, the distributions of development times for larva and pupa stages fitted well a probit distribution model (larva: $R^2 = 0.96$, AIC =710.4; pupa: $R^2 = 0.97$, AIC = 339.8). Simulated values for development time (median development time of the distribution) obtained from these models were consistent with observed development times (mean development times) (Table 2.1), attesting the quality of model fitting.

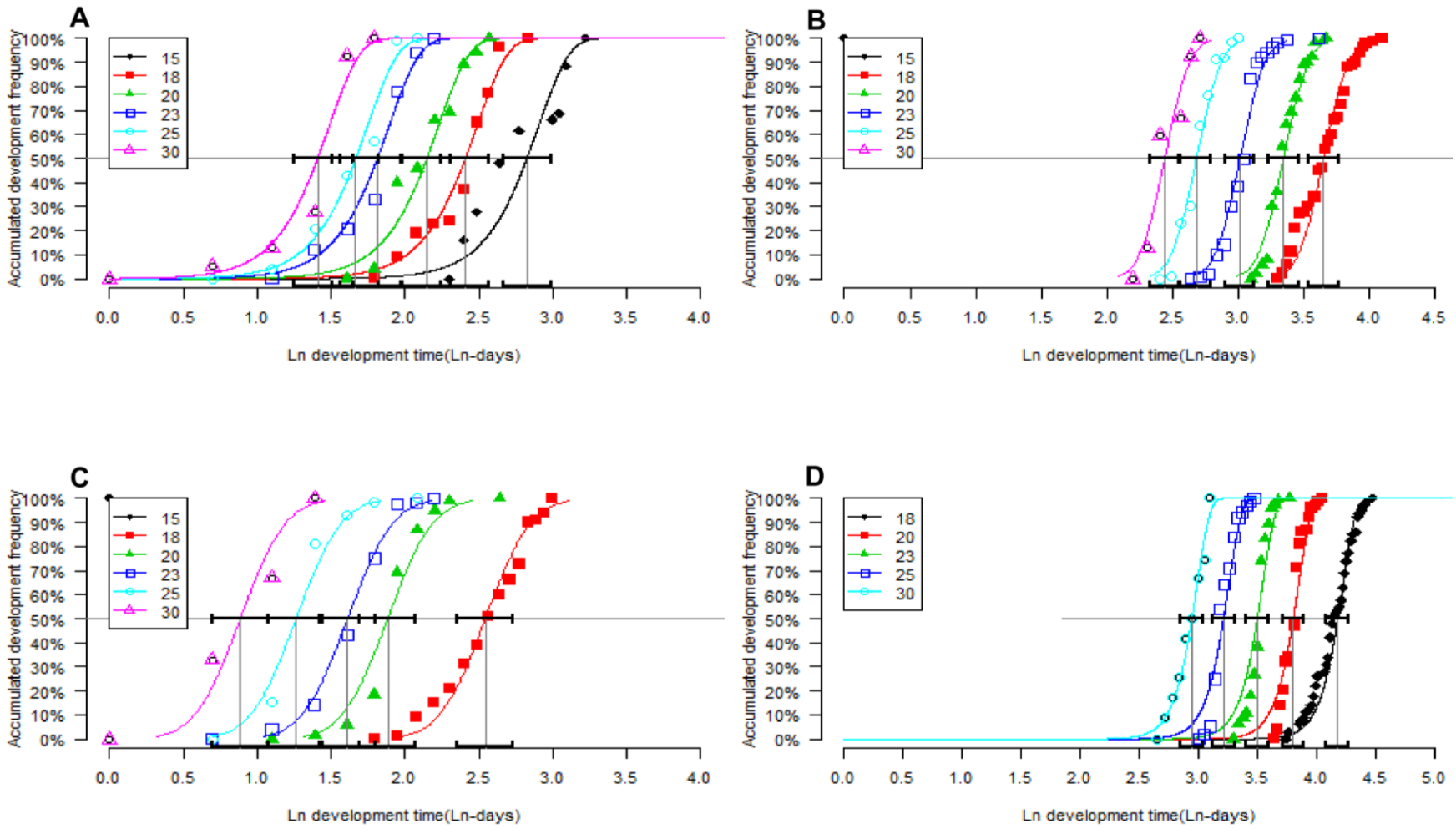


Figure 2.1 Cumulative distribution of developmental times of *Hypothenemus hampei* egg (A), larva (B), pupa (C), and egg to adult (D). Curves are fitted models: complementary log-log (CLL) model for egg stage and complete development from egg to adult, and probit model for the larva and pupa stages. Bars indicate 95% confidence intervals for median development times estimated from the models.

Table 2.1 Observed mean development times and development times simulated from the models (median of the distribution) for immature stages of *Hypothenemus hampei* reared in the laboratory at different constant temperatures. Means are in days with SE and *n* the initial number of eggs observed for each temperature.

T (°C)		Egg		Larva		Pupa		Egg to adult	
	n	Observed	Simulated	Observed	Simulated	Observed	Simulated	Observed	Simulated
15	112	16.86 ± 0.46a	16.83 ± 0.38a	-	-	-	-	-	-
18	200	11.58 ± 0.18b	11.09 ± 0.28b	39.45 ± 0.55a	38.34 ± 0.54a	13.58 ± 0.26a	12.71 ± 0.28a	63.38 ± 0.76a	62.79 ± 0.79a
20	167	8.93 ± 0.16c	8.55 ± 0.18c	29.12 ± 0.28b	28.26 ± 0.39b	7.29 ± 0.11b	6.57 ± 0.20b	46.75 ± 0.32b	44.19 ± 0.50b
23	161	6.57 ± 0.11d	6.12 ± 0.14d	21.09 ± 0.23c	20.32 ± 0.30c	5.80 ± 0.10c	5.01 ± 0.12c	33.71 ± 0.21c	32.82 ± 0.42c
25	191	5.76 ± 0.10e	5.29 ± 0.12e	15.24 ± 0.14d	14.58 ± 0.20d	4.15 ± 0.07d	3.53 ± 0.10d	26.00 ± 0.18d	24.42 ± 0.28d
30	110	4.62 ± 0.09f	3.48 ± 0.10f	12.09 ± 0.17e	11.46 ± 0.16e	3.00 ± 0.08e	2.42 ± 0.06e	18.00 ± 0.24e	18.57 ± 0.20e
35	100	-	-	-	-	-	-	-	-

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

Table 2.2 Parameters (a = y-intercept, b = common slope) and goodness of fit estimators (R^2 and AIC) of models fitted to cumulated frequency distributions of development times of *Hypothenemus hampei* immature stages reared at 6 constant temperatures.

	y-intercept (a)						Slope (b)	R^2	AIC
Life stage	15°C	18°C	20°C	23°C	25°C	30°C			
Egg[1]	-13.76 ± 0.30	-11.78 ± 0.25	-10.55 ± 0.23	-8.96 ± 0.20	-8.26 ± 0.19	-7.04 ± 0.17	4.74 ± 0.10	0.94	515.75
Larva[2]	-	-24.19 ± 0.37	-22.17 ± 0.34	-19.98 ± 0.31	-17.78 ± 0.28	-16.18 ± 0.26	6.63 ± 0.10	0.96	710.44
Pupa[2]	-	-10.46 ± 0.23	-7.74 ± 0.18	-6.63 ± 0.16	-5.19 ± 0.132	-3.64 ± 0.13	4.11 ± 0.09	0.97	339.76
Egg to adult[1]	-	-37.55 ± 0.58	-34.19 ± 0.53	-31.48 ± 0.49	-28.93 ± 0.45	-26.54 ± 0.41	8.91 ± 0.14	0.95	1030.14

Models[*]
[1]CLL distribution: $f(x) = 1 - \exp(-\exp(a_i + b\ln x))$
[2]Probit distribution: $f(x) = \emptyset(a_i + b\ln x)$

[*]CLL and probit distributions: $f(x)$ is the probability to complete development at time x, $\ln x$ is the natural logarithm of the days observed, a_i is the intercept of the regression line corresponding to temperature i, and b is the common slope of the regression line in all cases.

Development rate

Temperature had a significant effect on the development rate of immature stages of *H. hampei* as well as on the total development from egg to adult ($P < 0.001$) (Tables 2.3 and 2.4). Linear models showed that the minimum temperatures required for immature stage development (T_{min}) were 10.5, 13.1, 15.0 and 13.0°C, for egg, larva, pupa, and egg to adult respectively (Figure 2.2; Table 2.3). The thermal constants *k* was estimated at 78.1, 188.7, 36.5, and 312.5 DD for egg, larva, pupa and complete development from egg to adult, respectively. The Logan model gave the best fit for the egg and pupa stages (R^2 = 0.89-99 and AIC = −45.87 -2.31) (Figure 2.2; Table 2.4), while for the larva stage and for total development from egg to adult, modified version (five parameters) of the Sharpe and DeMichele model gave a best fit (R^2 =94-0.95 and AIC = −26.09 to −20.3). The maximum temperature threshold (T_{max}) was estimated at 35.2, 34.4, 33.9 and 32°C for egg, larva, pupa and from egg to adult, respectively (Figure 2.2; Table 2.4).

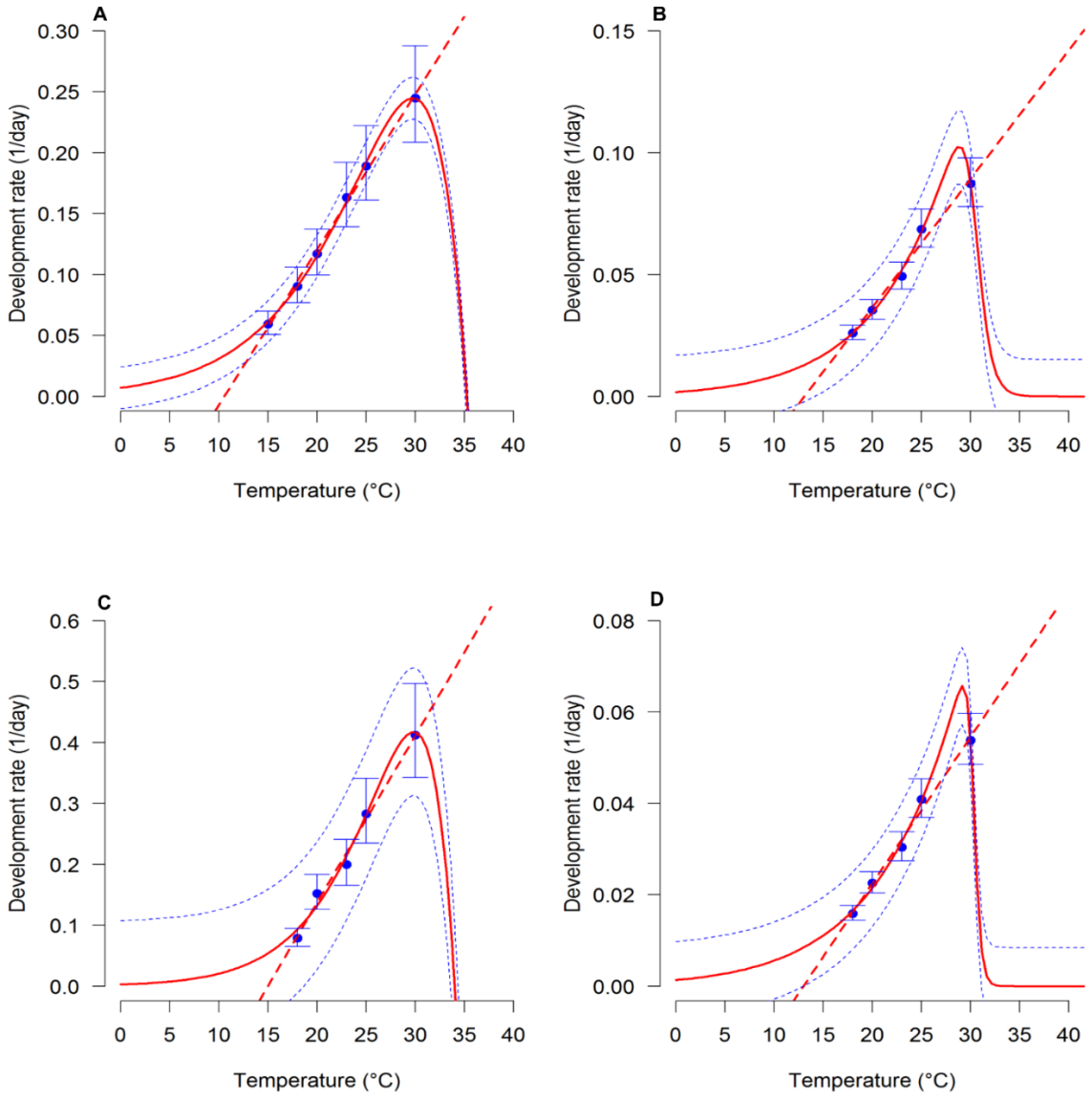


Figure 2.2 Temperature-dependent developmental rate of *Hypothenemus hampei* egg (A), larva (B), pupa (C), and egg to adult (D). Observed values are the black points, with bars representing standard deviation of the mean. Fitted models are the dashed straight lines for linear regression and solid lines for the Logan model (egg & pupa) and Sharpe and DeMichele model (larva & egg to adult). Dashed lines above and below represent the upper and lower confidence bands.

Table 2.3 Estimates of the linear regression describing the effect of temperature on *Hypothenemus hampei* development rate (1/day), *k*: thermal constant in degree days (DD), T_{min}: minimum temperature threshold, R^2: coefficient of determination, and AIC: Akaike's Information Criterion

Life stage	Linear regression equation	*F*	*df.*	*P*	R^2	AIC	T_{min}(°C)	*k* (DD)
Egg	$r(T)$ = -0.1349 + 0.0128 × T	947.29	1, 4	< 0.0001	0.99	43.08	10.54	78.13
Larva	$r(T)$ = -0.0692 + 0.0053 × T	153.70	1, 3	0.001	0.98	−37.66	13.06	188.68
Pupa	$r(T)$ = -0.4107 + 0.0274 × T	276.78	1, 3	< 0.0001	0.98	−24.13	14.99	36.49
Egg-adult	$r(T)$ = -0.0417 + 0.0032 × T	295.06	1, 3	< 0.0001	0.99	−45.89	13.03	312.5

Table 2.4 Model parameters of Logan and Sharpe and DeMichele models describing the effect of temperature on *Hypothenemus hampei* immature stage development rate (1/day), with for Logan models, Υ, ρ and v: model parameters (mean ± SE), and T_{max}: maximum temperature threshold (in °C); and for Sharpe and DeMichele model, P, T_o, H_A, T_L and H_L: model parameters (mean ± SE; R is the universal gas constant (1.987 cal degree^{-1} mol^{-1}), F: F-test statistic, *df:* degree of freedom, P: probability value, R^2: coefficient of determination, and AIC: Akaike's Information Criterion.

Life stage	Model	Parameters		F	*df*	P	R^2	AIC
Egg	Logan $r(T) = \Upsilon\left\{\exp(\rho T) - \exp(\rho\, T_{max} - \frac{(T_{max} - T)}{v})\right\}$	Υ T_{max} ρ v	0.018 ± 0.001 35.232 ± 0.001 0.175 ± 1.097 5.288 ± 0.010	490.91	2,3	0.002	0.99	−45.87
Larva	Sharpe and DeMichele $r(T) = \frac{P.\frac{T}{T_o}.\exp\left[\frac{\Delta H_A}{R}\left(\frac{1}{T_o} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{T_L} - \frac{1}{T}\right)\right]}$	P T_o H_A T_L H_L	0.179 ± 0.059 303.459 ± 0.057 -232290.3 ± 0.000 303.657 ± 0.168 -255315.2 ± 0.000	40.62	2,3	< 0.001	0.94	−20.30
Pupa	Logan $r(T) = \Upsilon\left\{\exp(\rho T) - \exp(\rho\, T_{max} - \frac{(T_{max} - T)}{v})\right\}$	Υ T_{max} ρ v	0.004 ± 0.003 33.993 ± 0.041 0.227 ± 7.865 3.920 ± 0.084	29.37	1,3	< 0.001	0.89	−2.31
Egg-adult	Sharpe and DeMichele $r(T) = \frac{P.\frac{T}{T_o}.\exp\left[\frac{\Delta H_A}{R}\left(\frac{1}{T_o} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{T_L} - \frac{1}{T}\right)\right]}$	P T_o H_A T_L H_L	0.013 ± 0.001 289.44 ± 0.000 21589.23 ± 0.000 303.48 ± 0.069 517475.3 ± 0.000	47.31	1,3	< 0.001	0.95	−26.09

Effect of temperature on the mortality rate

Temperature had a significant effect on the mortality rate of the egg and larva stages and for the total development from egg to adult (egg: $F = 68.04$, $df = 2, 3$, $P < 0.05$; larva: $F = 141.45$, $df = 2, 3$, $P < 0.01$; egg to adult: $F = 59.76$, $df = 2, 4$, $P < 0.05$) (Table 2.5). However, the tested temperatures did not have a significant effect on the mortality of the pupa stage ($F = 14.73$, $df = 1, 3$, $P = 0.18$). For all immature stages, the best-fitted model was the Wang 2 (R^2 = 0.98-0.99 and AIC between −22.92 and −21.20), while second order polynomial functions gave a good fit to mortality for the total development from egg to adult ($R^2 = 0.98$, AIC = −14.9) (Figure 2.3; Table 2.5). Larva stage had the highest mortality rate at the tested temperatures with 100%, 50%, 38%, 28%, 33% and 80% at 15, 18, 20, 23, 25 and 30°C, respectively (Figure 2.3). The thermal window for *H. hampei* survival from egg to adult was estimated from the second order polynomial function between 16.1 and 30.3°C, and the optimum temperature for survival was estimated at 23.2°C (Figure 2.3).

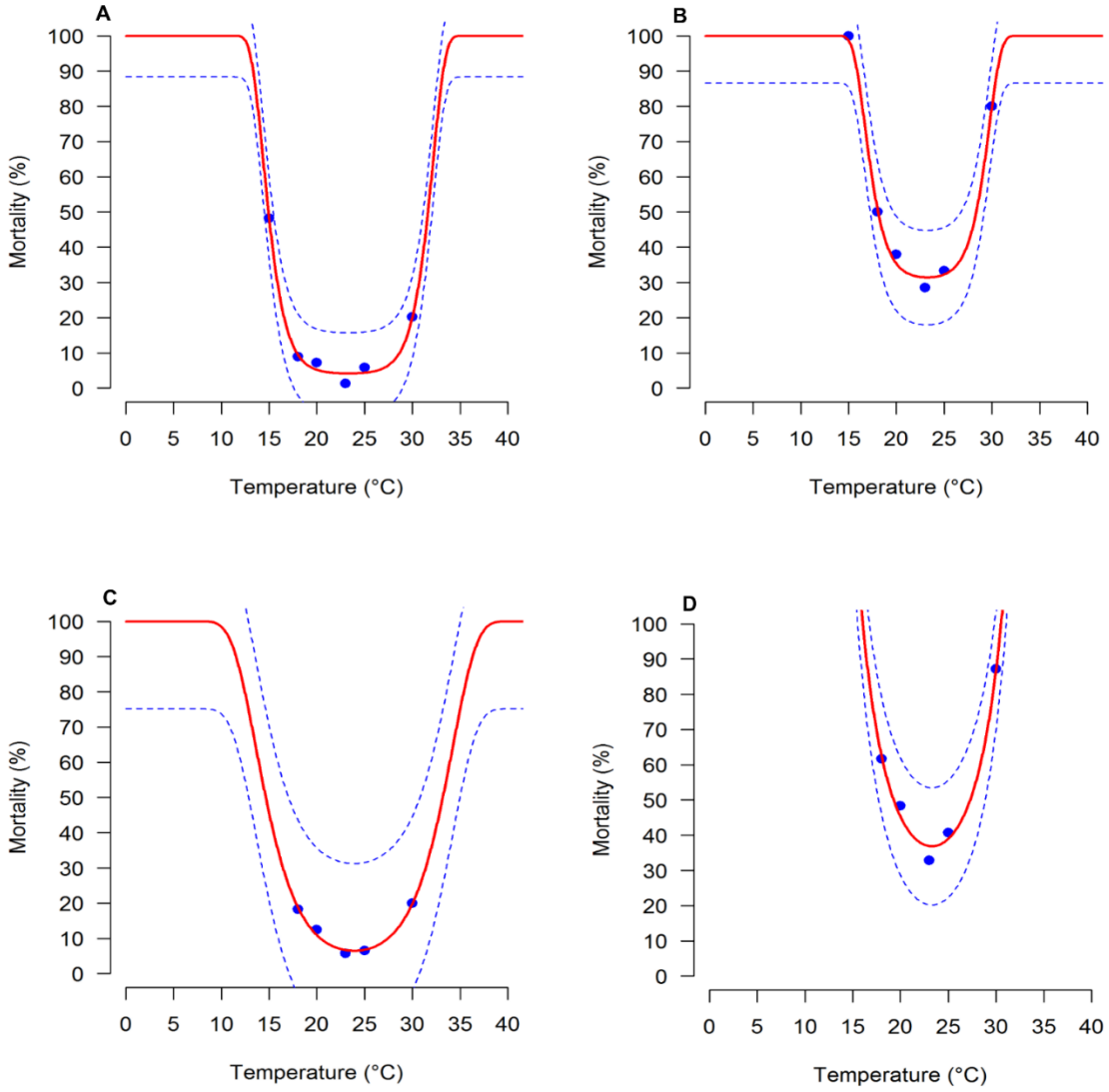


Figure 2.3 Temperature-dependent mortality rate of *Hypothenemus hampei* immature stages fitted to Wang 2 function for egg (A), Larva (B) and Pupa (C), and to second order polynomial function for the complete development from egg to adult (D). The points are observed values and the solid curves are the selected model output. Dashed lines above and below represent the upper and lower 95% confidence bands of the models.

Table 2.5 Model parameters of Wang 2 function (Tl, h, B and H ± SE) and second order polynomial function (b_1, b_2 and b_3 ± SE) testing temperature effect on *Hypothenemus hampei* immature stage mortality rate, *F*: F-test statistic, *df:* degree of freedom, *P*: probability value, R^2: coefficient of determination, and AIC: Akaike's Information Criterion.

Life stage	Model	Parameters		*F*	*df*	*P*	R^2	AIC
Egg	Wang 2 $m(T) = 1 - \frac{1}{\exp\left(\left(1+\exp\left(-\frac{T-Tl}{B}\right)\right)\times\left(1+\exp\left(-\frac{Th-T}{B}\right)\right)\times H\right)}$	Tl	18.516 ± 1.548	68.04	2,3	0.015	0.99	−22.92
		Th	28.031± 1.278					
		B	1.289 ± 0.358					
		H	0.040 ± 0.021					
Larva		Tl	28.492 ± 0.000	141.45	2,3	0.007	0.99	−21.20
		Th	17.933 ± 0.000					
		B	1.234 ± 0.000					
		H	1e-04 ± 0.000					
Pupa		Tl	23.911 ± 0.233	14.73	1,3	0.188	0.98	−23.23
		Th	23.912 ± 0.233					
		B	2.521 ± 0.281					
		H	0.017 ± 0.003					
Egg-adult	Second order polynomial function $m(T) = \exp(b_1 + b_2 T + b_3 T^2)$	b_1	9.329 ± 0.001	59.76	2,4	0.016	0.98	−14.91
		b_2	-0.887 ± 0.007					
		b_3	0.0191 ± 0.000					

Discussion

Observation method

Here, we developed and validated a new observation method that allows for direct monitoring of the complete development from egg to adult of a large number of *H. hampei* individuals, whilst maintaining rearing conditions similar to those found in a coffee berry. This is the recommended approach for accurate assessment of the impact of temperature on insect demography (Tonnang et al., 2013). Due to the cryptic nature of the pest, life cycle observation has been challenging in the past. Different methods and approaches were used in both field and laboratory that include those in Baker et al. (1992), where coffee berries on trees in the field were artificially infested with *H. hampei* and sampled after every 3-4 days for dissection to observe immature stages. Fernández and Cordero (2007) used parchment coffee beans moistened for 24 h to feed the larva stage in the laboratory. By contrast, Brun et al. (1993) reared *H. hampei* on artificial diets in the laboratory, which made the observation easier. However, artificial diets are but proxies to natural food for *H. hampei* and thus might affect the development process of the pest. In the recent past, Jaramillo et al. (2009) developed an observation technique based on artificial infestation of coffee berries in the laboratory, where berries were dissected on a daily basis to observe the development of groups of individuals. One of the unique improvements of our method is that it allows the easy monitoring of larva development on fresh coffee seeds and the same individuals can be monitored from egg through to adult stage. On one hand, packing the fresh coffee seed hosting the larva in an aluminium foil allowed us to mimic the living conditions inside the berry, especially darkness and

high level of humidity. On the other hand, aluminium package was easily opened without damaging or disturbing the larva, which is not ensured when berries are dissected.

Temperature-dependant development models

Although insects do not develop at constant temperature in nature, development models obtained from laboratory studies provide useful information on the thermal biology of the pest, such as its thermal thresholds. As such, they help us to understand and predict the pest's distribution and population dynamics in plantations according to temperature (Tonnang et al., 2013; Azrag et al., 2018). Jaramillo et al. (2009) studied the thermal tolerance of *H. hampei* at different constant temperatures and provided the thermal thresholds for all immature stages using a combination of linear regression and the modified version of the Logan model. On the other hand, Giraldo-Jaramillo et al. (2018) predicted the minimum temperature thresholds for *H. hampei* immature stages using linear model and estimated the number of generations per year for the pest in the State of São Paulo, Brazil. In our study, ILCYM software was used to fit the linear regression and to select the best nonlinear model from the fifty-eight models used to describe this relationship in insects (Tonnang et al., 2013).

Linear model well predicted the development rate for all the tested temperatures, confirming earlier findings for a number of insect species, including *H. hampei* (Sporleder et al., 2004; Jaramillo et al., 2009; Azrag et al., 2017). The Logan and five parameters of the Sharpe and DeMichele models (Logan et al., 1976; Sharpe and DeMichele, 1977) were the best fitted nonlinear models. The Sharpe and DeMichele biophysical model have a biological significance in predicting insect development rate

(Sharpe and DeMichele, 1977). Because it includes thermodynamic parameters associated with the developmental that is controlled by enzymes, as well as enthalpy of activation of the development process at minimum, optimum and maximum temperatures (Sharpe and DeMichele, 1977). In addition, the model can be fitted in different forms using four, five or six parameters, which make it flexible in fitting different temperature ranges (Tonnang et al., 2013). On the other hand, the model by Logan et al. (1976) has restricted number of parameters (four), and it considers enzyme-catalysed biochemical reactions rate at the optimum temperature. However, apriori, there is no valid reason to prefer one model over the other until further experimental studies indicate otherwise.

For mortality rate, the best model was that of Wang 2 model (Wang et al., 1982). This model gave a very good fit to the data over wide range of temperatures (Tonnang et al., 2013). It showed that, mortalities of immature stages of *H. hampei* was around 100% at 13°C but, decreased with an increase in temperature, and reached the minimal level at 23°C. Thereafter, the mortality increased again and reached 100% at around 34°C for all immature stages. This model was previously used to predict the relationship between temperature and mortality of immature stages for tropical pests such as the mealybug *Phenacoccus solenopsis* (Tinsley) (Fand et al., 2014) and the leaf miner *Liriomyza huidobrensis* (Blanchard) (Mujica et al., 2017).

Life cycle

The complete life cycle of *H. hampei* was obtained under constant temperatures between 18 and 30°C, with a total developmental time of about 63 days at 18°C and 18 days at 30°C. The egg incubation period ranged from 4.6-16.9 days under temperature range of

30-15°C. Our method might have underestimated this by a day less. Jaramillo et al. (2009) reported an egg incubation period of 4.7-12.0 days under the temperature range 33-20°C with no oviposition recorded by females at 15°C. Our result at 25°C is similar to that reported by Brun et al. (1993), who obtained an incubation period of 5 days at the same temperature for a population reared on an artificial diet. On the other hand, Ruiz et al. (1996) found an incubation period shorter than ours (3.3 days) at 26°C. The variation in incubation period between these studies may be linked to the methods used to produce eggs for the experiments and to the incubation conditions, such as observation settings, relative humidity and photoperiod, which might have played a role.

For larval stage, development times obtained here are in agreement with findings by Jaramillo et al. (2009) for all tested temperatures, with the exception of those obtained at 23°C. These authors reported a larval development time of 17 days, which differs from 21 days we reported here. In fact, in our study, only 12 (8.2%) individuals completed larval stage in 17 days, which the lowest value at 23°C. By contrast, 80% of the individuals completed this stage between 20 and 23 days. The larva development time assessed by Chami (2003) at 25°C (28.1 days) was much longer than ours (15.2 days) at the same temperature. The development time of pupa we obtained at 25°C was similar to those recorded by Bergamin (1943) and Chami (2003).

Thermal requirements

The minimum temperature thresholds (T_{min}) we obtained are similar to those reported by Giraldo-Jaramillo et al. (2018) who recorded 14.8, 13.4, 14.9 and 13.9°C for the egg, larva, pupa and egg to adult, respectively. The same was true for those obtained by

Jaramillo et al. (2009), with the exception of those for the egg stage, for which we found 10.5°C compared to 16.7°C. The thermal constant for the complete development from egg to adult in our study (312.5 degree days) is also comparable to 299 and 262.5 degree days reported by Giraldo-Jaramillo et al. (2018) and Jaramillo et al. (2009), respectively. However, thermal constant in our study was lower compared to 386.9 degree days reported by Costa et al. (1998). Again, these differences show the impact that experimental conditions and observation methods on development time. Another explanation may be the fact that *H. hampei* individuals used in ohher sudies came from different regions, with a different climate. We cannot rule out that populations from different geographical areas have slightly different thermal requirements.

Our models gave a thermal window of 13-32°C for *H. hampei* to complete its development from egg to adult. On the other hand, the thermal window for survival obtained from mortality models are narrower with about 16-30°C, with an optimal temperature for survival around 23°C. This is in line with other studies that established that the development rate response of insect is different from survival response due to some factors such as diet and manipulations that contribute to the mortality rate beside temperature (Sporleder et al., 2004; Mujica et al., 2017). For example, the survival response of the whitefly *Bemisia tabaci* (Gennadius) and the leaf miner *L. huidobrensis* (Blanchard) to temperature differed from developmental rate responses, especially in relation to optimum and extreme minimum and maximum temperature thresholds (Bonato et al., 2007; Mujica et al., 2017).

Relationships between thermal requirements and ecological traits

Hypothenemus hampei is a tropical species, which prefers coffee plantations at low elevation (below 1,300 m asl), where the climate is warm (Jaramillo et al., 2011). According to our models, constant temperature of 23°C is optimal for the survival of all immature stages. This temperature matches the mean annual temperatures for elevations between ≈1,100-1,300 m asl, where *H. hampei* causes the highest damage to Arabica coffee in east Africa (Jaramillo et al., 2011). In other parts of the world, like in Latin America, the pest causes important damage to Arabica coffee even when it is grown at elevations as low as 500 m asl. At these elevations however, the highest infestations are reported in shaded plantations, where temperature is in the range of 15-30°C and does not reach extreme values like those in full-sun coffee (Mariño et al., 2016). According to Mendesil et al. (2004), all immature life stages of *H. hampei* were found throughout the year on coffee grown on the Ethiopian highlands at elevations between 1,200 and 1,770 m asl, either in berries on the trees, or in the berries fallen on the ground. These reports suggest good adaptive capacities to various temperature conditions in *H. hampei* that may be considered in further studies of the pest thermal biology.

Conclusions

In conclusion, we developed an observation method that allowed the monitoring of development of the same individuals of *H. hampei* from egg to adult. The models presented here gave good predictions for immature stage development and survival according to temperature. Models also provided thermal requirements for immature stage development. With incorporation of oviposition model and validation under fluctuating

temperature, this will help understand the distribution of *H. hampei* in coffee production areas and predict the potential impact of climate change on the future distribution and abundance of this major pest of coffee.

Acknowledgements

We acknowledge the financial support for this research by the following organisations and agencies: The Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France; UK's Department for International Development (DFID); Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); and the Kenyan Government. The first author AGA Azrag was supported by a German Academic Exchange Service (DAAD) In-Region Postgraduate Scholarship as well as the National research foundation of South Africa to AA Yusuf & CWW Pirk. The views expressed herein do not necessarily reflect the official opinion of the donors.

References

Azrag, A. G., Murungi, L. K., Tonnang, H. E., Mwenda, D., & Babin, R. (2017). Temperature- dependent models of development and survival of an insect pest of African tropical highlands, the coffee antestia bug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae). *Journal of Thermal Biology*, *70*, 27–36.

Azrag, A. G., Pirk, C. W., Yusuf, A. A., Pinard, F., Niassy, S., Mosomtai, G., & Babin, R. (2018). Prediction of insect pest distribution as influenced by elevation: combining field observations and temperature-dependent development models for the coffee stink bug, *Antestiopsis thunbergii* (Gmelin). *PloS One*, *13*, e0199569.

Baker, P. S., Barrera, J. F., & Rivas, A. (1992). Life-history studies of the coffee berry borer (*Hypothenemus hampei*, Scolytidae) on coffee trees in Southern Mexico. *Journal of Applied Ecology*, *29*, 656–662.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., & Good, J. E. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, *8*, 1–16.

Barrera, J. F. (1994). Dynamique des populations du scolyte des fruits du caféier, *Hypothenemus hampei* (Coleoptera: Scolytidae), et lutte biologique avec le parasitoide *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae), au Chiapas, Mexique. PhD thesis, *Université Paul-Sabatier*. Toulouse III, France. p. 301.

Bergamin, J. (1943). Contribuição para o conhecimiento da biología da broca do café *Hypothenemus hampei* (Ferrari, 1867) (Col : Ipidae). *Archives of the Institute of Biology*, *14*, 31–72.

Bonato, O., Lurette, A., Vidal, C., & Fargues, J. (2007). Modelling temperature-dependent bionomics of *Bemisia tabaci* (Q-biotype). *Physiological Entomology*, *32*, 50–55.

Brun, L. O., Marcillaud, C., Gaudichon, V., & Suckling, D. M. (1989). Endosulfan resistance in *Hypothenemus hampei* (Coleoptera: Scolytidae) in New Caledonia. *Journal of Economic Entomology*, *82*, 1311–1316.

Brun, L. O., Marcillaud, C., Gaudichon, V., & Suckling, D. M. (1994). Cross resistance between insecticides in coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae) from New Caledonia. *Bulletin of Entomological Research*, *84*, 175–178.

Brun, L. O., Gaudichon, V., & Wigley, P. J. (1993). An artificial diet for continuous rearing of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). *International Journal of Tropical Insect Science*, *14*, 585–587.

Chami, A. (2003). Biología de la broca del café *Hypothenemus hampei :* Ferrari 1867. (Coleoptera: Scolytidae). Tesis. Decanato de Agronomía. *Universidad Centroccidental Lisandro Alvarado*. Venezuela. p. 129.

Costa, T. C. S., & Villacorta, A. (1989). Modelo acumulativo para *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Scolytidae) com base em suas exigencias termicas. *Anais da Sociedade Entomologica do Brasil 18*, 90–97.

Damon, A. (2000). A review of the biology and control of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Bulletin of Entomological Research*, *90*, 453–465.

Fand, B. B., Tonnang, H. E., Kumar, M., Kamble, A. L., & Bal, S. K. (2014). A temperature-based phenology model for predicting development, survival and population growth potential of the mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). *Crop Protection*, *55*, 98–108.

Fernández, S., & Cordero, J. (2007). Biología de la broca del café *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) en condiciones delaboratorio. *Bioagro*, *19*, 35–40.

Giraldo-Jaramillo, M., Garcia, A. & Parra, J. R. (2018). Biology, thermal requirements, and estimation of the number of generations of Hypothenemus hampei (Ferrari, 1867) (Coleoptera: Curculionidae) in the State of São Paulo, Brazil. *Journal of Economic Entomology*, 111, 2192–2200.

Hilbert, D. W., & Logan, J. A. (1983). Empirical model of nymphal development for the migratory grasshopper, *Melanoplus sanguinipes* (Orthoptera: Acrididae). *Environmental Entomology*, *12*, 1–5.

Jaramillo, J., Borgemeister, C., & Baker, P. (2006). Coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae): searching for sustainable control strategies. *Bulletin of Entomological Research*, *96*, 223–233.

Jaramillo, J., Chabi-Olaye, A., & Borgemeister, C. (2010). Temperature-dependent development and emergence pattern of *Hypothenemus hampei* (Coleoptera:

Curculionidae: Scolytinae) from coffee berries. *Journal of Economic Entomology*, *103*, 1159–1165.

Jaramillo, J., Chabi-Olaye, A., Kamonjo, C., Jaramillo, A., Vega, F. E., Poehling, H. M., & Borgemeister, C. (2009). Thermal tolerance of the coffee berry borer *Hypothenemus hampei*: predictions of climate change impact on a tropical insect pest. *PloS One*, *4*, e6487.

Jaramillo, J., Muchugu, E., Vega, F. E., Davis, A., Borgemeister, C., & Chabi-Olaye, A. (2011). Some like it hot: the influence and implications of climate change on coffee berry borer (*Hypothenemus hampei*) and coffee production in East Africa. *PloS One*, *6*, e24528.

Jonsson, M., Raphael, I. A., Ekbom, B., Kyamanywa, S., & Karungi, J. (2015). Contrasting effects of shade level and altitude on two important coffee pests. *Journal of Pest Science*, *88*, 281–287.

Kontodimas, D. C., Eliopoulos, P. A., Stathas, G. J., & Economou, L. P. (2004). Comparative temperature-dependent development of *Nephus includens* (Kirsch) and *Nephus bisignatus* (Boheman) (Coleoptera: Coccinellidae) preying on *Planococcus citri* (Risso) (Homoptera: Pseudococcidae): evaluation of a linear and various nonlinear models using specific criteria. *Environmental Entomology*, *33*, 1–11.

Le Pelley, R. H. (1968). Pests of coffee. *Longmans Green and Co*. London, UK. p.590.

Logan, J. A., Wollkind, D. J., Hoyt, S. C., & Tanigoshi, L. K. (1976). An analytic model for description of temperature dependent rate phenomena in arthropods. *Environmental Entomology*, *5*, 1133–1140.

Marchioro, C. A., Krechemer, F. S., Moraes, C. P., & Foerster, L. A. (2016). A stochastic model for predicting the stage emergence of *Plutella xylostella* under field conditions. *Annals of Applied Biology*, *169*, 190–199.

Mariño, Y. A., Pérez, M. E., Gallardo, F., Trifilio, M., Cruz, M., & Bayman, P. (2016). Sun vs. shade affects infestation, total population and sex ratio of the coffee berry borer (*Hypothenemus hampei*) in Puerto Rico. *Agriculture, Ecosystems and Environment*, *222*, 258–266.

Mendesil, E., Jembere, B., & Seyoum, E**.** (2004). Population dynamics and distribution of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) on *Coffea arabica* L. in Southwestern Ethiopia. *SINET: Ethiopian Journal of Science*, 27, 127–134.

Mujica, N., Sporleder, M., Carhuapoma, P., & Kroschel, J. (2017). A temperature-dependent phenology model for *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Journal of Economic Entomology*, *110*, 1333–1344.

Nielsen, A. L., Hamilton, G. C., & Matadha, D. (2008). Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). *Environmental Entomology*, *37*, 348–355.

Ngowi, B. V., Tonnang, H. E., Mwangi, E. M., Johansson, T., Ambale, J., Ndegwa, P. N., & Subramanian, S. (2017). Temperature-dependent phenology of *Plutella xylostella* (Lepidoptera: Plutellidae): simulation and visualization of current and future distributions along the Eastern Afromontane. *PloS One, 12*, e0173590.

O'Hara, R. B., & Kotze, D. J. (2010). Do not log-transform count data. *Methods in Ecology and Evolution, 1*, 118–122.

Ovuka, M., & Lindqvist, S. (2000). Rainfall variability in Murang'a District, Kenya: meteorological data and farmers' perception. *Physical Geography, 82*, 107–119.

R Development Core Team. (2016). R: a language and environment for statistical computing. Austria: R foundation for statistical computing. https://www.R-project.org/.

Régnière, J., Powell, J., Bentz, B., & Nealis, V. (2012). Effects of temperature on development, survival and reproduction of insects: experimental design, data analysis and modeling. *Journal of Insect Physiology*, *58*, 634–647.

Ruiz, L., Bustillo- Pardey, A. E., Flórez, F. J. P., & González, M.T. (1996). Ciclode vida de *Hypotbenemus hampei* en dos dikfas melúdicas. *Cenicafé*, *47*, 77–84.

Sharpe, P. J., & DeMichele, D.W. (1977). Reaction kinetics of poikilotherm development. *Journal of Theoretical Biology*, *64*, 649–670.

Sporleder, M., Kroschel, J., Quispe, M. R. G., & Lagnaoui, A. (2004). A temperature-based simulation model for the potato tuberworm, *Phthorimaea operculella* Zeller (Lepidoptera; Gelechiidae). *Environmental Entomology*, *33*, 477–486.

Tonnang, H. E., Juarez, H., Carhuapoma, P., Gonzales, J. C., Mendoza, D., Sporleder, M., Simon, R., & Kroschel, J. (2013). ILCYM – Insect Life Cycle Modeling. A software package for developing temperature-based insect phenology models with applications for local, regional and global analysis of insect population and mapping. *International Potato Center*. Lima, Peru. p. 193.

Vega, F. E., Franqui, R. A., & Benavides, P. (2002). Scientific note: The presence of the coffee berry borer, Hypothenemus hampei, in Puerto Rico: fact or fiction?. *Journal of Insect Science*, *2*, 1–3.

Vega, F. E., Blackburn, M. B., Kurtzman, C. P., & Dowd, P. F. (2003). Identification of a coffee berry borer associated yeast: does it break down caffeine?. *Entomologia Experimentalis et Applicata*, *107*, 19–24.

Vega, F. E., Infante, F., Castillo, A., & Jaramillo, J. (2009). The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae): a short review, with recent findings and future research directions. *Terrestrial Arthropod Reviews*, *2*, 129–147.

Wagner, T. L., Wu, H. I., Sharpe, P. J. H., & Coulson, R. N. (1984). Modeling distributions of insect development time: a literature review and application of the weibull function. *Annals of the Entomological Society of America*, *77*, 475–487.

Wagner, T. L., Olson, R. L., & Willers, J. L. (1991). Modeling arthropod development time. *Journal of Agricultural Entomology*, *8*, 251–270.

Wang, R. S., Lan, Z. X., & Ting, Y. C. (1982). Studies on mathematical models of the relationships between insect development and temperature. *Acta Ecologica Sinica*, *2*, 47– 57.

Worner, S. P. (1992). Performance of phenological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. *Environmental Entomology*, *21*, 689–699.

CHAPTER THREE

Modelling the impact of temperature on the demographic parameters of the African coffee white stem borer, *Monochamus leuconotus* (Pascoe) (Coleoptera: Cerambycidae)

Abstract

The African coffee white stem borer *Monochamus leuconotus* (Pascoe) (Coleoptera: Cerambycidae) is a destructive insect pest of Arabica coffee trees in African highlands. Our study aims to provide preliminary information on the pest biology as influenced by temperature, determine thermal thresholds, and provide life table parameters for *M. leuconotus* reared in the laboratory. The life cycle of *M. leuconotus* was studied at seven constant temperatures in the range 15-35°C, with 80 ± 5% RH and a photoperiod of L:D 12:12. Linear and nonlinear models were fitted to laboratory data to describe the impact of temperature on *M. leuconotus* development, mortality, fecundity and senescence. The complete life cycle was obtained between 18 and 30°C, with egg incubation period ranging 10.8-29.2 days. Larval development time was the longest with 194.2 days at 30°C and 543.1 days at 18°C. The minimum temperature threshold (T_{min}) was estimated at 10.7, 10.0 and 11.5°C, for egg, larva and pupa, respectively. The maximum temperature threshold (T_{max}) was estimated at 37.4, 40.6 and 40.0°C for egg, larva and pupa, respectively. The optimum temperature for immature stages' survival was estimated between 23.0 and 23.9°C. The highest fecundity was 97.8 eggs per female at 23°C. Simulated life table parameters showed the highest net reproductive rate (R_o) of 11.8 daughters per female at 26°C and maximal intrinsic rate of increase (r_m) between 26 and 28°C, with a value of 0.008. Our results will help understand *M. leuconotus* biology and may be used to predict the distribution and risk under climate change for this critical coffee pest.

Keywords: *Coffea arabica*, life cycle modelling, development time, life table parameters.

Introduction

The long-horned beetles under genus *Monochamus* contain approximately 150 species, most of them being important pests of agriculture and forestry, as well as vectors of diseases (Naves et al., 2008). The African coffee white stem borer, *Monochamus leuconotus* (Pascoe) (Coleoptera: Cerambycidae) is one of the most dangerous insect pests of Arabica coffee in Africa (Gichuhi et al., 2017; Liebig et al., 2018), with a distribution in western, eastern, central and southern parts of the continent (Schoeman et al., 1998; Rutherford and Phiri, 2006; Egonyu et al., 2015). It was first reported in the 1860s in south Africa, and then in the 1930s in eastern Africa (Kenya), attacking Arabica coffee at elevations below 1,600 m asl (Knight, 1939; Tapley, 1960; Schoeman et al., 1998). Currently, *M. leuconotus* is ranked as the most or the second most important pest of Arabica coffee after the coffee berry borer *Hypothenemus hampei* (Ferrari) in several countries including Tanzania, Uganda, Malawi and Zimbabwe, where infestation rates in farms are reported to be between 70 and 90% (Erbaugh et al., 2008; ICC, 2008; Egonyu et al., 2015).

Eggs of *M. leuconotus* are inserted by the reproductive females in the bark of coffee stems or main branches. The young larva feeds on the bark of the coffee trees damaging phloem, while older larvae bore galleries into the wood. Ring-barking and wood tunnelling disrupt sap flow leading to significant damages including yellowing of the leaves, stunted growth and dieback of coffee trees (Jonsson et al., 2015). Also, adult feeding causes damage to the buds, shoots, stem bark and skin of the green berries. Younger trees are more susceptible to *M. leuconotus* infestation, and trees less than three years old are often killed, while older ones can survive but become less productive (Jonsson et al., 2015). In

sub-Saharan Africa, global production loss due to *M. leuconotus* is estimated at 25% (Schoeman et al., 1995; Gichuhi et al., 2017).

Infested trees can be detected by ring-barking, the presence of frass on the stem due to larva feeding and exit holes about 1cm in diameter drilled by adult beetles as they exit the wood. Traditional control methods are still used by farmers to control the pest; they include cutting the infested stems, driving a wire into the galleries to kill the larvae and pupae, picking the adults and covering coffee tree stem with banana leaves to avoid oviposition by females (McNutt, 1975; Rutherford and Phiri, 2006). These methods are not effective enough and the dearth of knowledge on the biology and physiology of *M. leuconotus* makes it difficult to develop effective management strategies. Organochlorine insecticides such as endosulfan®, aldrin® and dieldrin® have been used in the past to control *M. leuconotus*. However, these chemicals today are banned entirely due to their toxicity, slow degradation in the environment and bioaccumulation (ICC, 2008; Jayaraj et al., 2016). A recent study in Uganda by Egonyu et al. (2015) showed that Arabica coffee variety KP423 was less susceptible to *M. leuconotus* infestation compared to other varieties such as KP162 and SL28.

Despite the importance of *M. leuconotus* in coffee production, little is known about its biology and physiology. This is mainly attributed to the fact that *M. leuconotus* spends the major part of its life (up to 2 years) inside the coffee wood, which makes it difficult to study its biology, especially under controlled conditions. Tapley (1960) reported a complete life cycle of 24 months under field conditions. Similarly, Schoeman et al. (1998) recorded a duration of 18 to 24 months in infested coffee logs kept in the laboratory. A recent study by Gichuhi et al. (2017) reported a complete life cycle of about 14 months

at 25°C, with a mean fecundity of 40 eggs per female, when the pest was reared on an artificial diet in the laboratory.

Nevertheless, the relationship between temperature and *M. leuconotus* life history has not been elucidated, as well as its thermal thresholds (minimum, optimum, and maximum temperatures) are still unknown. Besides, the demographic parameters such as net reproductive rate (R_o) and the intrinsic rate of increase (r_m), which indicate the population growth rate are still acking. Modeling the impact of temperature on *M. leuconotus* development and providing the pest thermal requirements will help predict the most suitable areas for the pest establishment in the context of global warming (Régnière et al., 2012). This will help coffee industry stakeholders adapt management strategies to changes in the pest distribution to prevent crop losses in the future. Therefore, in this chapter, we conducted a complete life table trial at seven constant temperatures (15–35°C) in order to provide the thermal requirements for the development, survival and fecundity of a rearing population of *M. leuconotus* using linear and nonlinear models. Furthermore, a compilation of the models was used to simulate the life table parameters of the rearing population under different temperature regimes.

Materials and methods

Colony initiation and egg production

Insects used for this study were obtained from a colony maintained by Gichuhi et al. (2017) in the coffee laboratory of the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. The authors initiated *M. leuconotus* colony with larvae

and pupae extracted from infested coffee stems obtained from coffee farms located on Mt. Kilimanjaro, Tanzania, and reared at 25°C ± 0.5°C, RH 80 ± 5% using an artificial diet (see Gichuhi et al., 2017 for the details). In the present study, eggs were obtained from the mated adults (20 males and 30 females) of the first laboratory generation reared by Gichuhi et al. (2017). These adults emerged in two groups; the first group (A) consisted of 12 males and 16 females, while adults of the second group (B) that emerged three months after adults of the first group in average consisted of 8 males and 14 females. Adults from group A were introduced into four Plexiglas cages (50 cm long, 40 cm large and 80 cm high), with in each cage 3 males and 4 females. Adults from group B were introduced in 4 cages, with in each cage 2 males and 3 or 4 females. Each cage had 6 freshly cut coffee sticks (50 cm long) as support for adults feeding and egg laying (eggs are inserted in the bark). The coffee sticks were slightly buried in a container with moist soil to avoid drying. Wet cotton bolls were provided in the container as a water source. The sticks were removed every 24 h from Plexiglas cages to extract the eggs and thereafter replaced with new ones.

Immature stages development and survival

Freshly laid eggs of ≤ 24 h old were carefully extracted from the sticks by cutting the bark around the eggs using a sharp scalpel blade, in such a way that eggs were still wrapped in a fine layer of phloem and wood. The extracted parts with eggs were introduced individually into small plastic containers (3.5 cm depth x 3.9 cm diameter), lined with wet cotton wool covered with a fine layer of sand and a paper towel at the top in order to maintain high humidity in the container. The extracted part was placed on the paper towel, and containers closed with lids containing windows made of fine muslin

cloth (0.1 µm) for ventilation. The containers were kept for five days in incubators (SANYO MIR-553 and MIR-554, Sanyo Electrical Ltd., Tokyo, Japan) set at the following seven constant temperatures:15, 18, 20, 23, 25, 30 and 35°C (± 0.5°C), with RH 80 ± 5%, and photoperiod 12:12 L:D. After this period, the phloem got dry and it was carefully separated from the wood, in order to facilitate egg observation. Eggs were observed daily for 60 days to monitor the incubation period. After egg hatching, the neonate larvae were carefully removed with a fine camel hair brush and transferred into plastic containers (3.5 cm depth x 3.9 cm diameter) containing an artificial diet, the recipe of which was given in Gichuhi et al. (2017). The eggs that did not hatch were observed for an additional two months, after which they were recorded as dead. We kept the neonate larvae individually to avoid cannibalism, and the diet was changed monthly. Three months after hatching, the larvae were transferred into bigger plastic containers (11.5 cm wide, 6.5 cm deep) with a ≈2 cm layer of artificial diet. The individuals were checked daily to monitor the moult to pupa stage as well as mortality. After pupation, the pupae were placed in a plastic container of the same size lined with a paper towel and monitored daily until adults emerged.

Reproduction and adult longevity

After emergence, adults were placed individually in plastic containers (11.5 cm wide, 6.5 cm deep) with coffee leaves and twigs as food, for the period of physiological and sexual maturation, as described by Gichuhi et al. (2017). Once the female began to feed, i.e. after four weeks in average, it was introduced into polyethylene terephthalate bottles (2 litters in volume) with a male for mating. The bottles were cut from the top and then covered with a fine muslin cloth (0.1 µm) fixed with a rubber band to keep the adults inside. The

bottles contained freshly cut coffee sticks (25 cm long) for feeding and egg laying that were slightly buried in moist soil. The sticks were checked daily to record the number of eggs laid by each female. The sticks were changed every three days, and survival of both males and females were monitored and recorded to assess longevity.

Model development

Modelling software

The Insect Life Cycle Modeling software (ILCYM version 3.0) (Tonnang et al., 2013) was used to model *M. leuconotus* development as influenced by temperature. ILCYM is an open source software built on two computer programs, Java and R (R Core Team, 2012). The software allows the modelling of the impact of temperature on immature stages' development and survival, and female longevity and fecundity, by fitting several non-linear functions. The best-fitted model is selected based on the coefficient of determination (R^2) and Akaike's Information Criterion (AIC) (Tonnang et al., 2013).

Development time for immature stages and adult longevity

Due to the skewness in the distribution of insects' development time, the development times of *M. leuconotus* immature stages and adult longevity at different constant temperatures were first normalised and then fitted to a probability frequency distribution. The cumulative frequency was plotted against ln-transformed development time in parallel lines, by fitting logit function for egg, larva, pupa and the adult male. The complementary log-log (CLL) function gave a better fit for the adult female longevity. The mathematical expressions of these functions are given below:

Logit distribution function: $f(x) = 1/(1 + \exp(-(a_i + b\ln x)))$

CLL distribution function: $f(x) = 1 - \exp(-\exp(a_i + b\ln x))$

where $f(x)$ is the probability density of the life stage to complete its development at normalised time x, $\ln x$ is the natural logarithm of the observed development times (in days), a_i is the intercept corresponding to temperature i and b is the common slope of the model (Tonnang et al., 2013).

Development rate for immature stages

Both linear and nonlinear models were used to predict the impact of temperature on the development rate for the immature stages of *M. leuconotus*. In the first step, the development rate of each immature stage was calculated by inverting the median development time (1/ median development time), and then plotted against temperature. Afterwards, a linear model was fitted to the development rate data to determine its relationship with temperature. Using parameters of the linear model, the minimum temperature threshold (T_{min}) and the thermal constant (k) which is represented in degree days (DD) were estimated as follow:

Linear model: $r(T) = a + bT$

Minimum temperature threshold: $T_{min} = -a/b$

Thermal constant: $k = 1/b$

where, $r(T)$ is the development rate at temperature T, a and b are the intercept and slope of the regression model, respectively, T_{min} is the minimum temperature threshold, and k is the thermal constant.

However, the linear model cannot correctly predict the impact of temperature on development rate at extreme temperatures due to the nonlinearity of the points. Hence, several nonlinear models were fitted to the data in order to elucidate the impact of extreme temperatures on immature development rate. The best fitted nonlinear model was selected based on the selection criteria (R^2 and AIC). Among the fifty-nine models that were fitted to the data, Logan model (Logan et al., 1976) was the best for all immature stages development rates. The mathematical equation for the model is given below:

$$\text{Logan model: } r(T) = \Upsilon\left\{\exp(\rho T) - \exp(\rho\, T_{max} - \frac{(T_{max} - T)}{v})\right\}$$

Where $r(T)$ is the development rate at temperature T; Υ, ρ, T_{max} and v are parameters from the Logan model (Logan et al., 1976).

Mortality rate for immature stages

The mortality rate was calculated for each immature stage at each constant temperature and fitted to forty-five nonlinear models that are embedded in ILCYM. Based on R^2 and AIC, Wang 1 model (Wang et al., 1982) gave the best prediction for the mortality rates for both egg and pupa. The polynomial function 4 gave the best fit to describe the impact of temperature on larval mortality. The mathematical equations of the models are given below:

$$\text{Wang 1 model: } m\,(T) = 1 - \frac{1}{\exp\left(\left(1 + \exp\left(-\frac{T - T_{opt}}{B}\right)\right) \times \left(1 + \exp\left(-\frac{T_{opt} - T}{B}\right)\right) \times H\right)}$$

$$\text{Polynomial function 4: } m(T) = \exp\,(b_1 + b_2 \times \mathrm{T} + b_3\sqrt{T})$$

Where, $m(T)$ is the mortality rate at temperature T; T_{opt}, B and H are Wang 1 model parameters; and b_1, b_2 and b_3 are polynomial function parameters (Tonnang et al., 2013).

Female fecundity and adult senescence

An exponential modified 1 model described age-specific fecundity, which refers to the cumulative proportion of egg production in relation to age of the females. The cumulative oviposition rate was plotted against normalised female age expressed as a ratio of age in days divided by median survival time. Age specific fecundity was computed using the formula:

$$f(x) = 1 - \exp\ -(aT + bT^2 + cT^3)$$

Where, $f(x)$ is the cumulative oviposition frequency at the normalised female age x; T is temperature; *a*, *b* and *c* are the model parameters (Tonnang et al., 2013).

The mean oviposition rate per female was calculated for each temperature regime as the total number of eggs laid by the females divided by the total number of females. Then, the below exponential polynomial function 12 was fitted to describe the relationship between temperature and oviposition:

$$f(T) = \exp\ (b_1 + b_2T + b_3 \log(T))$$

Where, $f(T)$ is the mean female fecundity at temperature T; b_1, b_2 and b_3 are the model parameters (Tonnang et al., 2013).

Senescence is a decline in organism's fitness due to ageing, and it is used to assess demographic parameters such as longevity and fecundity (Boggs, 2009). In ILCYM, the

terminology senescence is used for the adults rather than mortality to differentiate it from immature stage mortality (Tonnang et al., 2013). The adult senescence was calculated as the inverse of median longevity for both sexes (male and female) at each constant temperature, and then plotted against temperature. Afterwards, Stinner 4 model (Stinner et al., 1974) was fitted to describe the relationship between temperature and female senescence. On the other hand, Hilber and Logan 3 model gave the best fit for the male senescence. The following mathematical expressions were used:

$$\text{Stinner 4 model: } s(T) = \frac{C_1}{1 + \exp(k_1 + k_2 T)} + \frac{C_2}{1 + \exp(k_1 + k_2(2T_o - T))}$$

$$\text{Hilber and Logan 3: } s(T) = \Psi\left(\frac{(T - T_{min})^2}{(T - T_{min})^2 + D} - \exp - \frac{(T_{max} - (T - T_{min}))}{Dt}\right) + \theta$$

Where, $s(T)$ is the senescence rate at temperature T; C_1, C_2, k_1, k_2 and T_o are Stinner 4 model parameters; Ψ, T_{min}, T_{max}, D, Dt and θ are Hilbert and Logan 3 model parameters (Tonnang et al., 2013).

Simulation of life table parameters

All models used here to predict the impact of temperature on demographic parameters of *M. leuconotus* were compiled and used to simulate the life table parameters at different constant temperatures. Stochastic simulation (Curry et al., 1978) was used in ILCYM to estimate life table parameters, viz. 1) the intrinsic rate of natural increase (r_m) that determine the ability of a population to grow under specific ecological conditions, 2) the gross reproductive rate (*GRR*), which is known as the average number of daughters produced by a female throughout her lifespan, 3) the net reproductive rate (R_o), which is

similar to *GRR* but, takes into account the mortality rate of immature stages, 4) the mean generation time (*T*), which is the average time between the birth of parents and that of offspring, 5) doubling time (*Dt*), which is the time required for the population to double in size. Before the simulations, the sex ratio of 0.5 was determined in ILCYM for all the temperatures (Tapley, 1960; Schoeman et al., 1998; Gichuhi et al., 2017). The initial number of individuals used for the simulation was 150 at the egg stage, and the simulation was conducted for thirteen constant temperatures, from 20 to 32°C, with 1°C interval. The simulation was replicated three times and for each temperature bringing the number of runs to 450 individuals at egg stage. This was sought to determine the thermal limits of *M. leuconotus* population growth and to establish how an increase in temperature by 1°C can affect life table parameters of *M. leuconotus*.

Statistical analyses

The effect of temperature on *M. leuconotus* immature stages developmental time (in days) and adult longevity (in days) were separately submitted to the Generalised Linear Model (GLM) with a Poisson distribution in R (R Core Team, 2016), as recommended by O'Hara and Kotze (2010). Once significant differences were detected, the means were separated using Tukey test at $\alpha = 0.05$. On the other hand, the life table parameters (intrinsic rate of increase, gross reproduction rate, net reproduction rate, mean generation time, doubling time, and the finite rate of increase) obtained from the simulations were also separately subjected to an analysis of variance (ANOVA) in R (R Core Team, 2016). Each life table parameter was set as the dependent variable, with the temperature being the independent variable.

Results

Development time of immature stages and adult longevity

Mean development times of immature stages and adult longevity at each constant temperature are shown in Table 3.1. Temperature had a significant effect on development times and adult longevity of *M. leuconotus* ($P < 0.0001$) (Table 3.1). However, there was no significant difference between development times at 23 and 25°C, for all immature stages. Eggs successfully developed to the larval stage at all tested temperatures (in the range 15-35°C), with an incubation period ranging from 10.8 ± 2.4 to 44.8 ± 6.3 days (mean ± SD) and significantly impacted by temperature ($\chi^2 = 378.05$, $df = 371$, $P < 0.0001$) (Table 3.1). Among immature stages, larvae had the longest development time with a range of 194.2 ± 25.7 to 543.1 ± 113.1 days in the temperature range 18-30°C. The longest mean development time for the pupal stage was 48.6 ± 8.1 days at 18°C, while the shortest was 17.7 ± 2.7 days at 30°C. Average female longevity was the longest at 20°C with 168.3 ± 58.2 days, compared to 116.0 ± 49.9 days for the male at 25°C. The frequency distribution of development times for egg, larva, pupa and the adult male was well described by a logit model ($R^2 = 0.94$-0.98, AIC = 455.3-1189.2) (Table 3.2). However, the frequency distribution of female longevity fitted a CLL distribution (larva: $R^2 = 0.91$, AIC = 643.3) (Table 3.2).

Table 3.1 Observed mean development times (in days ± SD) of immature stages and adult longevity of *Monochamus leuconotus* reared at different constant temperatures in the laboratory. N is the total number of eggs used for the experiment at each constant temperature, and *n* is the number of each life stage used to calculate the mean at each constant temperature.

Temp (°C)	N	Immature stage development time (days)						Adult longevity (days)			
		n	Egg	*n*	Larva	*n*	Pupa	*n*	Female	*n*	Male
15	50	30	44.80 ± 6.31a	30	-		-		-		-
18	76	63	29.17 ± 6.50b	22	543.05 ± 113.10a	15	48.60 ± 8.08a	8	154.00 ± 31.01a	7	38.57 ± 15.12a
20	100	71	22.37 ± 6.52c	42	408.48 ± 108.45b	35	41.50 ± 8.23b	18	168.28 ± 58.15a	17	94.31 ± 64.96b
23	85	72	15.88 ± 1.77d	39	292.89 ± 49.67c	37	29.08 ± 4.39c	16	135.63 ± 27.34b	21	99.23 ± 60.00c
25	81	67	14.28 ± 4.11d	42	285.86 ± 46.96c	35	26.03 ± 4.21cd	17	113.53 ± 31.49c	18	116.00 ± 49.99d
30	92	61	10.77 ± 2.43e	27	194.15 ± 25.66e	19	17.68 ± 2.67e	10	79.00 ± 18.44d	9	85.00 ± 31.42e
35	53	14	12.71 ± 1.81de	14	-		-		-		-
χ^2			378.05		172.15		125.17		68.11		64.56
df			371		167		136		64		66
P-value			< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001

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

Table 3.2 Statitics of goodness of fit and parameters estimated for logit and complementary log-log (CLL) functions (*a* = y-intercept, *b* = common slope), fitted to cumulated frequency distributions of development times of immature stages and adult longevity of *Monochamus leuconotus* reared at different constant temperatures in the laboratory.

Model parameters (mean ± SE)	Immature stage development time				Adult longevity	
	Temperature (°C)	Egg	Larva	Pupa	Female	Male
Intercept (*a*) at:	15	-26.15 ± 0.59	-	-	-	-
	18	-23.12 ± 0.51	-52.86 ± 1.04	-39.04 ± 0.98	-22.54 ± 0.66	-10.42 ± 0.32
	20	-21.24 ± 0.48	-50.56 ± 0.99	-37.47 ± 0.93	-23.48 ± 0.69	-12.28 ± 0.37
	23	-18.82 ± 0.43	-47.62 ± 0.94	-33.89 ± 0.85	-22.07 ± 0.65	-13.13 ± 0.40
	25	-18.04 ± 0.41	-47.59 ± 0.94	-32.73 ± 0.82	-21.20 ± 0.62	-13.67 ± 0.41
	30	-16.07 ± 0.37	-44.23 ± 0.87	-28.90 ± 0.73	-19.47 ± 0.57	-12.81 ± 0.39
	35	-18.05 ± 0.42	-	-	-	-
Slope (*b*)		6.95 ± 0.15	8.43 ± 0.17	10.17 ± 0.25	4.45 ± 0.13	2.96 ± 0.09
R^2		0.94	0.95	0.98	0.91	94
AIC		949.06	1189.20	455.26	643.32	548.99

Development rate of immature stages

Minimum temperature thresholds for development were estimated from the linear models fitted to the relationship between development rate and temperature (Figure 3.1) at 10.7, 10.0 and 11.5°C, for egg, larva and pupa, respectively, with thermal constants of 192.3, 3333.3, and 322.6 degree days, respectively. The model gave a good fit with R^2 ranging between 0.97 and 0.99, and AIC between −66.39 and −46.85. The Logan model gave the best fit for the relationship between development rate and temperature for all immature stages of *M. leuconotus* (R^2 = 0.92-0.99; AIC between −56.06 and −29.70) (Figure 3.1; Table 3.3). The maximum temperature threshold for egg, larva and pupa development was estimated from the model at 37.4, 40.6 and 41.0°C, respectively. (Figure 3.1; Table 3.3).

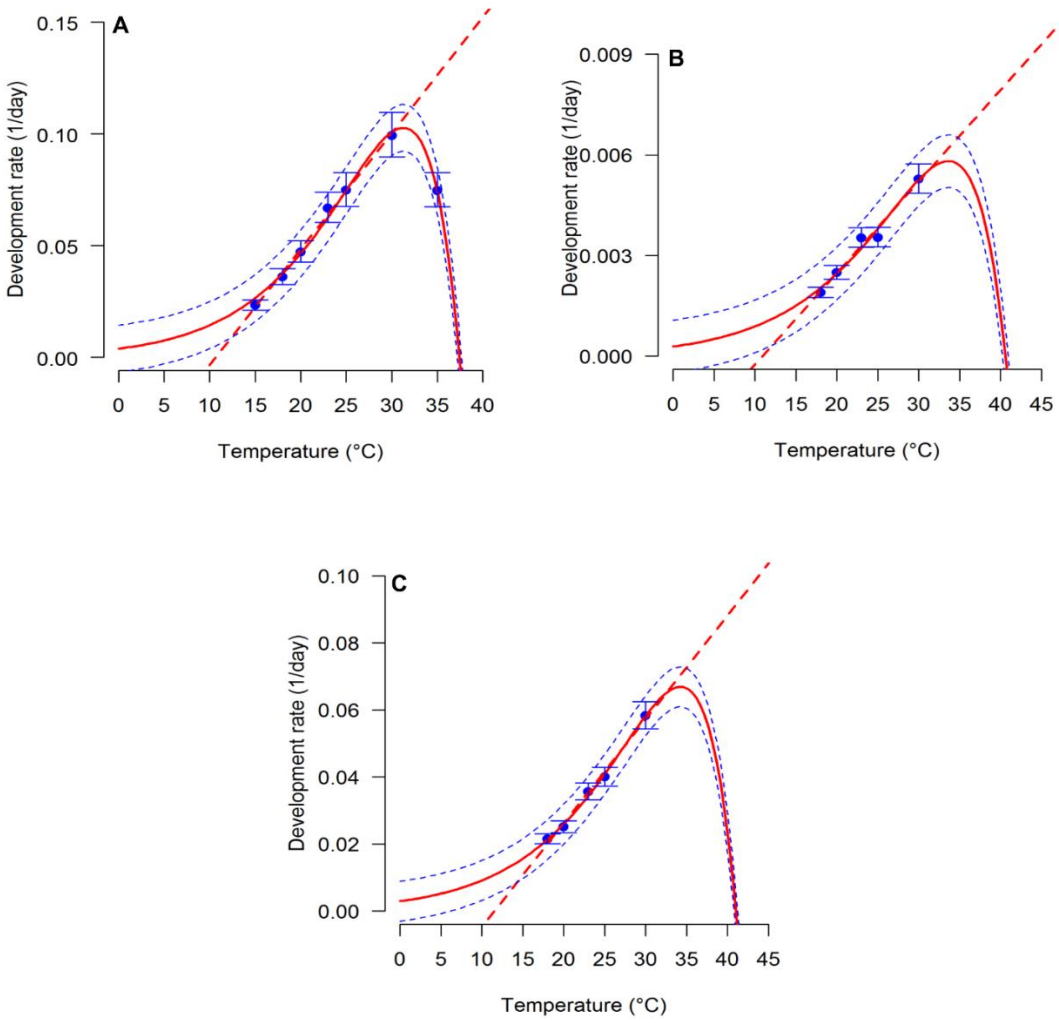


Figure 3.1 Model fitting to the relationship between development rate of *Monochamus leuconotus* immature stages and temperature, with: A) egg; B) larva and C) pupa. The blue points are the observed values with bars representing the standard deviation. Fitted models are the dashed straight lines for linear model and solid lines for the Logan model (egg) and Sharpe and DeMichele 3 and 2 models (larva and pupa). Dashed lines in blue above and below represent the upper and lower 95% confidence interval.

Table 3.3 Statistics of goodness of fit and parameters of models fitted to the relationship between development rate of *Monochamus leuconotus* immature stages and temperature, *F*: F-test statistic, *df*.: degree of freedom, *p*: probability value, R^2: coefficient of determination, and AIC: Akaike's Information Criterion.

Life stage	Model mane	Model parameters (± SE)		Statistics				
				F	*df.*	*P*	R^2	AIC
Egg	Logan	Υ	0.012 ± 0.002	124.62	3,3	0.001	0.99	−56.06
		T_{max}	37.428 ± 0.001					
		ρ	0.158 ± 2.440					
		v	5.961 ± 0.020					
Larva		Υ	0.0004 ± 0.000	42.19	3, 11	< 0.001	0.92	−50.31
		T_{max}	40.643 ±0.026					
		ρ	0.129 ± 0.001					
		v	6.356 ± 0.144					
Pupa		Υ	0.003 ± 0.001	88.85	3, 11	< 0.001	0.96	−29.70
		T_{max}	41.01 ± 0.009					
		ρ	0.122 ± 0.000					
		v	5.672 ± 0.207					

Mortality rate of immature stages

Temperature had a significant effect on the mortality rate of *M. leuconotus* immature stages ($P \leq 0.05$) (Figure 3.2; Table 3.4). The temperature-dependent mortality of egg and pupa stage were well described by the Wang 1 model (R^2 = 0.91-0.93 and AIC between −13.04 and −13.76). A polynomial function 4 gave the best fit for the larva stage (R^2 = 0.97 and AIC = −16.92) (Figure 3.2; Table 3.4). Optimum temperature for the survival of *M. leuconotus* immature stages was estimated between 23.0 and 23.9°C from the models. The larva stage did not survive at 15 and 35°C (Figure 3.2B). The larva stage showed the highest mortality at all temperatures with 100, 65, 40, 45, 39, 54 and 100% at constant temperatures of 15, 18, 20, 23, 25, 30 and 35°C, respectively.

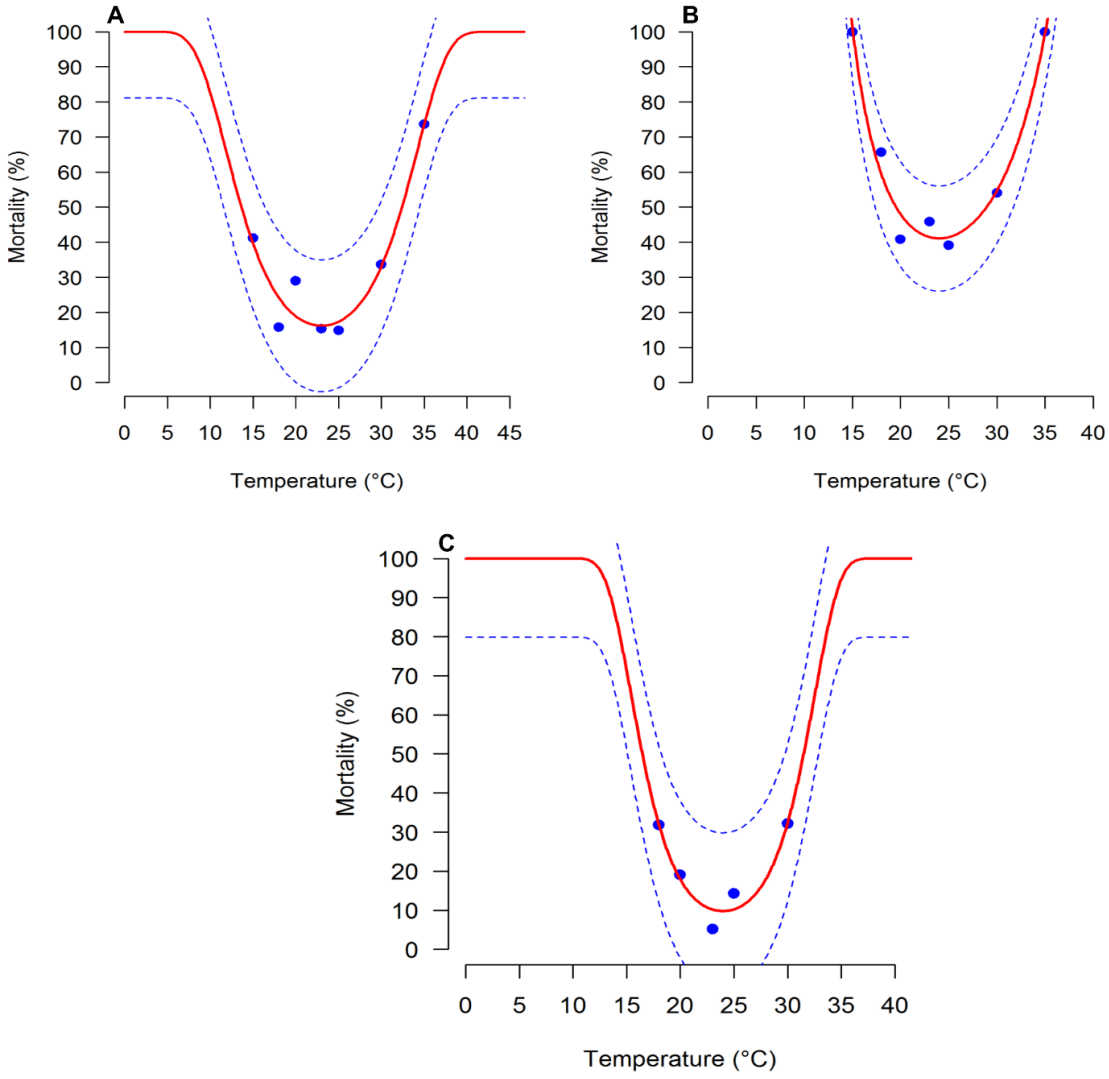


Figure 3.2 Model fitting to the relationship between the mortality rate of *Monochamus leuconotus* immature stages and temperature, with: A) egg; B) larva and C) pupa. The blue points are the observed values. The solid red lines are the fitted models, Wang 1 model for egg and pupa, and exponential polynomial function 4 for larva. Dashed lines in blue above and below represent the upper and lower 95% confidence interval.

Table 3.4 Statistics of goodness of fit and parameters of models fitted to the relationship between the mortality rate of *Monochamus leuconotus* immature stages and temperature. *F*: F-test statistic, *df*: degree of freedom, *p*: probability value, R^2: coefficient of determination, and AIC: Akaike's Information Criterion.

Life stage	Model mane	Model parameters (mean ± SE)		Statistics				
				F	*df*	*P*	R^2	AIC
Egg	Wang 1	T_{opt}	23.019 ± 0.607	27.072	2,4	0.005	0.93	−13.76
		B	3.588 ± 0.421					
		H	0.044 ± 0.009					
Larva	Exponential polynomial function 4	b_1	19.668 ± 1.748	69.710	2,4	< 0.001	0.97	−16.92
		b_2	0.857 ± 0.075					
		b_3	-8.393 ± 0.733					
Pupa	Wang 1	T_{opt}	23.972 ± 0.326	11.433	2,2	0.050	0.91	−13.04
		B	2.337 ± 0.374					
		H	0.026 ± 0.008					

Female fecundity and adult senescence

Temperature significantly influenced female fecundity in *M. leuconotus* (Figure 3.3; Table 3.5) with the exponential modified 1 model giving the best fit to the age-specific cumulative oviposition rate, with $R^2 = 0.97$ and ACI= −2752.9 (Figure 3.3A; Table 3.5). The lowest mean fecundity was 38.7 eggs per female at 30°C, while the highest was 97.81 eggs per female at 23°C (Figure 3.3B). The exponential polynomial function 12 gave the best fit to the relationship between *M. leuconotus* female fecundity and temperature ($R^2 = 0.96$ and ACI= 36.68) (Figure 3.3B; Table 3.5). The model showed that females of *M. leuconotus* might be able to lay eggs under temperatures between 11 and 40°C, with a maximum fecundity at around 23°C (Figure 3.3B; Table 3.5). The adult senescence of both male and female was significantly affected by temperature ($P < 0.05$). The Stinner 4 model described the impact of temperature on *M. leuconotus* female senescence with $R^2 = 0.78$ and AIC= −33.53 (Figure 3.3C; Table 3.5), while Hilber and Logan 3 model was best fit to the male senescence $R^2 = 0.71$ and AIC= −20.42 (Figure 3.3D; Table 3.5).

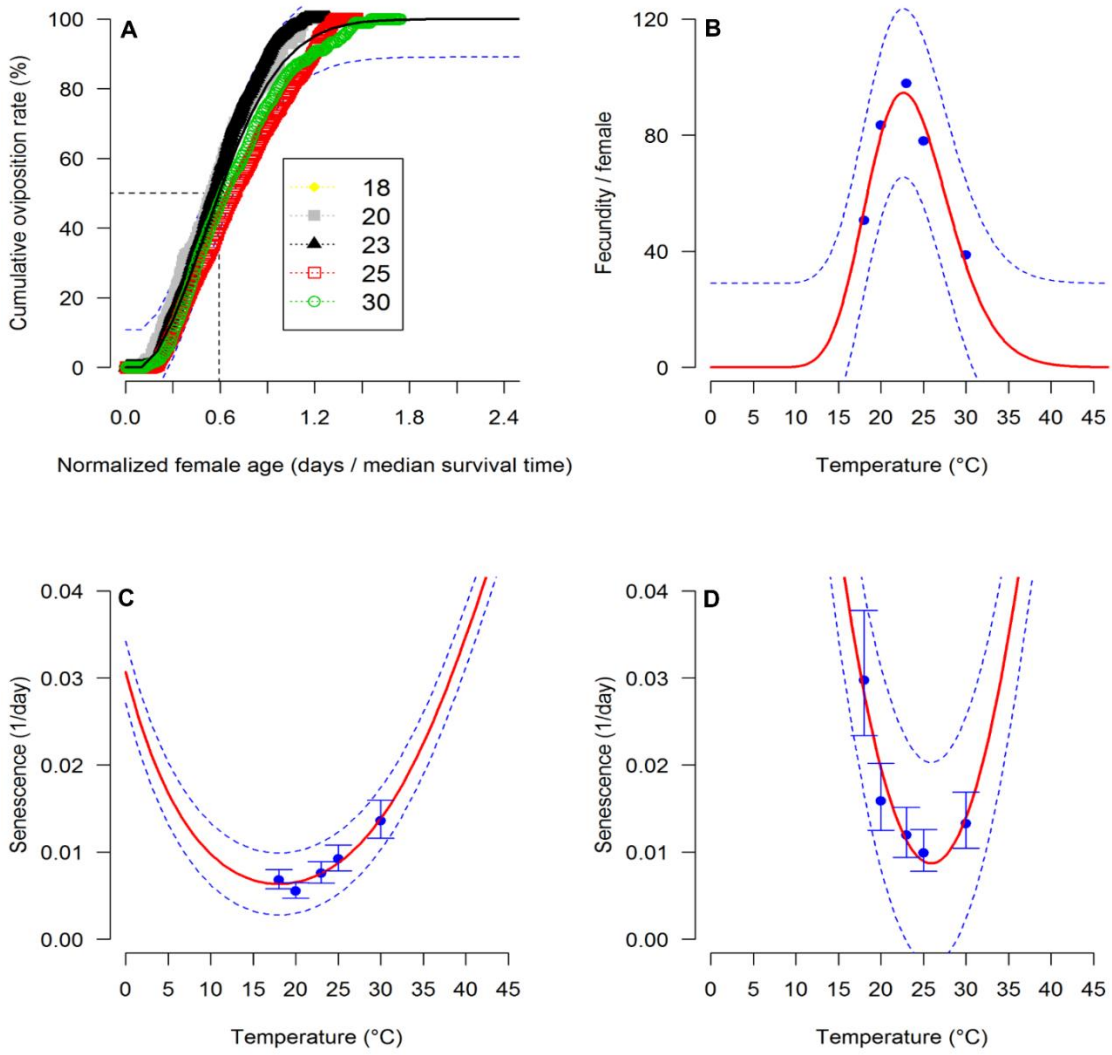


Figure 3.3 Model fitting to the relationship between fecundity of *Monochamus leuconotus* female and temperature, and adult senescence with temperature with: A) cumulative oviposition fitted to exponential modified 1 model; B) average fecundity of the females fitted to exponential polynomial function 12; C) female senescence rates fitted to Stinner 4 model and D) male senescence rates fitted to Hilbert and Logan 3 model. The blue points are the observed values with bars representing the standard deviation. The solid red lines are the fitted models with dashed lines in blue above and below representing the upper and lower 95% confidence interval.

Table 3.5 Statistics of goodness of fit and parameters of models fitted to describe the relationship between *Monochamus leuconotus* cumulative oviposition, total oviposition and adult senescence and temperature *F*: F-test statistic, *df*: degree of freedom, *p*: probability value, R^2: coefficient of determination, and AIC: Akaike's Information Criterion.

Demographic parameters	Model name	Model parameters		Statistics				
				F	*df.*	*P*	R^2	AIC
Relative oviposition	Exponential modified 1	a	-0.243 ± 0.050	17360.0	2,818	< 0.001	0.97	−2410.9
		b	2.451 ± 0.177					
		c	-0.120 ± 0.143					
Total oviposition	Exponential polynomial function 12	b_1	-43.639 ± 8.285	25.13	2,2	0.038	0.96	36.68
		b_2	-1.004 ± 0.173					
		b_3	22.733 ± 3.913					
Female senescence	Stinner 4	C_1	4.036 ± 0.628	8.86	4,10	0.002	0.78	−33.53
		C_2	0.084 ± 0.052					
		k_1	4.826 ± 0.292					
		k_2	0.129 ± 3.065					
		T_o	2.792 ± 0.02					
Male senescence	Hilbert and Logan 3	Ψ	994025.5 ± 0.000	4.39	5,9	0.026	0.71	−20.42
		T_{min}	25.899 ± 0.564					
		T_{max}	38.567 ± 0.000					
		D	3134208079 ± 0.00					
		Dt	0.017 ± 0.000					
		θ	0.008 ± 0.002					

Life table parameters

Temperature had a significant effect on the simulated life table parameters of *M. leuconotus* ($P < 0.0001$) (Table 3.6). The intrinsic rate of natural increase r_m was maximal between 26 and 28°C, with a value of 0.008, and negative at 20, 21 and 32°C. The highest *GRR* was at 27°C, with 40.9 daughters per female, while the lowest was at 20°C, with 0.58 daughters per female. The net reproductive rate R_o ranged from 0.05 daughters per female per generation at 20°C, to 11.8 daughters per female per generation at 26°C. The mean generation time *T* decreases with an increase in temperature with the longest time (363 days) at 20°C, and shortest (223.9 days) at 32°C. The time required for the population to double (D_t) was maximal at 22°C, with 245.4 days and minimal at 27°C, with 82.8 days. The highest finite rate of increase λ was at temperatures between 26 and 28°C, with a value of 1.008, while the shortest was 22°C, with a value of 1.003 (Table 3.6).

Table 3.6 Simulated life table parameters (± SD) of *Monochamus leuconotus* at different constant temperatures (number of eggs used for the simulation = 150). r_m: intrinsic rate of natural increase *GRR*: gross reproduction rate, R_o: net reproduction rate, *T*: mean generation time, D_t: doubling time, and λ: finite rate of increase.

Temperature (°C)	r_m	*GRR*	R_o	*T*	D_t	λ
20	-0.008 ± 0.001	0.58 ± 0.44	0.05 ± 0.02	363.01 ± 0.99	-	-
21	-0.001 ± 0.002	2.30 ± 0.97	0.86 ± 0.42	352.51 ± 1.06	-	-
22	0.002 ± 0.001	5.07 ± 3.05	2.31 ± 1.39	342.54 ± 9.70	245.36 ± 98.48	1.003 ± 0.001
23	0.005 ± 0.000	9.95 ± 0.98	4.96 ± 0.32	333.85 ± 8.92	144.86 ± 9.26	1.005 ± 0.000
24	0.007 ± 0.001	20.26 ± 2.14	9.41 ± 2.20	321.57 ± 4.54	100.79 ± 8.64	1.007 ± 0.001
25	0.007 ± 0.001	26.32 ± 4.71	10.59 ± 1.75	315.36 ± 2.69	93.41 ± 7.87	1.007 ± 0.001
26	0.008 ± 0.001	34.60 ± 8.59	11.81 ± 2.50	308.64 ±11.34	87.95 ± 11.68	1.008 ± 0.001
27	0.008 ± 0.000	40.91 ± 3.96	11.16 ± 2.35	285.19 ± 11.89	82.81 ± 4.96	1.008 ± 0.000
28	0.008 ± 0.001	35.79 ± 12.89	8.23 ± 3.16	256.33 ± 32.89	87.21 ± 11.67	1.008 ± 0.001
29	0.007 ± 0.000	40.57 ± 14.57	6.69 ± 1.04	264.21 ± 4.42	97.13 ± 6.60	1.007 ± 0.000
30	0.004 ± 0.002	25.38 ± 9.04	3.15 ± 1.36	254.15 ± 2.79	197.24 ± 112.61	1.004 ± 0.002
31	0.001 ± 0.000	15.92 ± 4.38	1.15 ± 0.01	237.95 ± 1.74	-	-
32	-0.004 ± 0.004	8.67 ± 8.90	0.56 ± 0.42	223.90 ± 18.71	-	-
F	41.01	12.09	20.06	42.01	59.24	12.96
df	12, 25	12, 25	12, 25	12, 25	9, 17	9, 17
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Discussion

Colony initiation and rearing method

In this study, we provided the first description of the impact of different constant temperatures on the development, survival and reproduction in *M. leuconotus*. Previous studies on the basic biology of this important pest were either carried out on coffee trees in the field (Knight, 1939; Tapley, 1960) or in the laboratory with observing immature development in coffee wood (Schoeman et al., 1998). However, Gichuhi et al. (2017) provided an innovative method for studying the biology of *M. leuconotus* by developing an artificial diet for the larva and provided necessary information on its biology when reared at 25°C. In our study, we used the first generation of *M. leuconotus* obtained by Gichuhi et al. (2017) as a colony. Using the rearing method of the same authors, we obtained a complete life cycle and fecundity for the second generation of *M. leuconotus* at five constant temperatures. Then, we provided the thermal requirements by calculating the minimum and maximum temperature thresholds and computed the life table parameters that describe the population growth rate of *M. leuconotus* for the first time.

Here, we improved the method used to observe *M. leuconotus* immature stages, starting from the incubation of eggs. Our method allowed us to easily extract eggs from the bark and visually observe them throughout the incubation period. In addition, we increased the overall egg hatchability by 47% which is more than 1-fold, compared to that obtained by Gichuhi et al. (2017). We also used polyethylene bottles (volume 2 L) and coffee sticks for the adults feeding and fecundity assessment. The advantages of this method are: the bottles are not expensive and small in size, which makes it easy to store many in

incubators. It also allows following daily fecundity of each female separately by coupling the male and female in one bottle.

Development of immature stages

Egg incubation period ranged from 10.8 to 44.8 days under temperatures ranging 15-30°C. Our result at 25°C (14.3 days) was in line with that of Schoeman et al. (1998), who obtained an incubation period of 15 days at a temperature of 26 ± 2°C. However, it was different from those of Tapley (1960) and Gichuhi et al. (2017), who obtained 21-23 days under field conditions, and 26.6 days at 25°C, respectively. This variation may be due to our observation method, where we used moist sand to maintain egg with high moisture. It has been reported that the moisture level in plant tissues is a crucial factor that determines Cerambycid egg hatchability (Hanks, 1999). This was confirmed by our study that showed egg hatchability of *M. leuconotus* increased to 86%, compared to that of Gichuhi et al. (2017) who obtained 39%. The development time of the larval stage was highly variable in our study, even for the individuals reared in the same conditions. For example, the duration of the larval stage ranged 330-751 days at 18°C, 209-609 days at 23°C and 169-346 days at 25°C. Similar variation was observed by Gichuhi et al. (2017) for the first generation of *M. leuconotus* reared on artificial diet. The variation can be explained by the fact that *M. leuconotus* adults have two emerging patterns in the year (March to May, and September to November), which are linked to the two rainfall seasons in East Africa (Tapley, 1960; Schoeman et al., 1998; Liebig et al., 2018). Therefore, the variation in the larval development may be an adaptation to avoid the emergence of all

the adult population at the same time in the year in order to limit the risk of failing reproduction due to sub-optimal environmental conditions.

The average development time for the larva stages ranged from 194.2 to 543.1 days for all temperature regimes we tested. This was shorter than the report of Tapley (1960), who reported 600 days when the larva was reared on coffee wood. The difference between the two studies may be explained by the use of an artificial diet that might have accelerated larval development as a result of better nutritional properties in comparison to coffee wood which contain less nutrients (Gichuhi et al., 2017). However, the development time of the pupa we obtained at 25°C was similar to those of Schoeman et al. (1998) and Gichuhi et al. (2017).

Thermal thresholds

Here, we provide a comprehensive overview of the relationship between temperature, development and survival of *M. leuconotus*. The minimum temperature thresholds (T_{min}) for the immature development rate ranged between 10.0 and 11.5°C. At 35°C, we had a few larvae (14), as most of them had died at the earlier stages due to the quick dryness of the diet. Therefore, 35°C might not be the maximum temperature threshold for the larval development. Logan model predicted the maximum temperature thresholds at 37.7, 40.6, and 41.0°C, for *M. leuconotus* egg, larva and pupa development rates, respectively. The same model has estimated the maximum threshold for another tropical species, the diamondback moth, *Plutella xylostella* L. in Kenya at 40.6, 40.7 and 38°C, for egg, larva, and pupa, respectively, with a larger thermal window similar to *M. leuconotus* immature stages (Ngowi et al., 2017). We found larvae to be more susceptible to the temperature in

comparison to other life stages with a mortality rate between 39 and 100% at all tested temperatures. Also, we observed that some larvae were deformed when they stopped feeding for the pupation, as well as pupae when they were emerging to adults at all temperatures. Although this was in contrast to early reports by Gichuhi et al. (2017), who obtained 10% mortality of *M. leuconotus* larvae at 25°C. We think that these observations might be attributed to the inbreeding in the colony since there were no additional individuals added from the field.

Oviposition

Gichuhi et al. (2017) reported that *M. leuconotus* adults, especially females, need approximately one month post eclosion to be physiologically and sexually mature, which was confirmed by our study. However, one of the behaviours we observed is that, when we coupled sexually immature females with mature males, they show aggression by fighting each other inside the container and ended up with cuts off female's antennae and/or legs. This behaviour may be explained by the fact that males were mainly focused on mating, while females were not ready, confusing the males that might have taken immature females as competitor males. The mean number of eggs laid by a female was variable according to temperature, with an average of 97.8 eggs at 23°C, and 38.7 eggs at 30°C. However, at 25°C (77.9 eggs) oviposition was twice higher than that of Knight (1939) and Gichuhi et al. (2017), who reported an average of 40 eggs per female. Our results are more in line with the report of Schoeman et al. (1998), who obtained an average of 80.5 eggs per female at 26 ± 2°C. According to the oviposition models, *M. leuconotus* females can lay eggs under a wide range of temperatures (11-40°C). This makes it unique

from the other major coffee pests in east Africa such as coffee berry borer *Hypothenemus hampei* (Ferrari) and antestia bug *Antestiopsis thunbergii* (Gmelin) that require a temperature range of 15-32°C and 15-30°C, respectively, for oviposition (Jaramillo et al., 2009; Azrag et al., 2017).

Life table parameters

This study is the first report on the demography of *M. leuconotus*. The results we obtained here will help in predicting the pest distribution and population dynamics in coffee plantations. We found that the net reproductive rate (R_o) was highest at 26°C, with 11.8 daughters per female. This parameter takes into account the mortality rate of immature stages during the life cycle, and it indicates the average of the population growth rate from one generation to the other (Ahmed et al., 2016). However, the intrinsic rate of natural increase (r_m), which indicates the capacity of a population to grow in given conditions was maximal between 26 and 28°C, with a value of 0.008. This value is low when compared with an intrinsic rate of increase for *Monochamus galloprovincialis* (Olivier) (r_m = 0.05) at the temperature of 24-26°C (Akbulut et al., 2007). The difference could be explained by our rearing conditions, especially the artificial diet that speeded up larvae development and resulted in adults of small size, compared to the field population, thus, lower r_m. Nevertheless, the populations of *M. leuconotus* can grow at temperatures ranging from 22-31°C. Our results showed that the rearing population of *M. leuconotus* could complete only one generation per year at all tested temperatures. However, previous reports showed that *M. leuconotus* need one year and half to two years to complete one generation under field conditions (Knight, 1939; Tapley, 1960; Schoeman

et al., 1998). Therefore, the life table parameters we obtained might not reflect what happens in the field and thus should be considered with caution.

Relationship between the thermal window and ecological traits

Contrary to other coffee pests such as *Antestiopsis thunbergii* (Gmelin), *M. leuconotus* has a broad thermal window (11-40°C). More generally, when compared to other insects, this thermal window of 29°C is very large and may be an adaptation due to the fact that immature stages cannot escape sub-optimal environmental conditions, because they are attached to the coffee wood. The air temperature in the field could vary from the under-bark microclimate due to the tree shade and humidity level in the plant tissues. This makes it difficult to generalise laboratory findings to the field populations. Therefore, this variation of the microclimate needs to be considered when exploiting such information to make a geographic prediction. However, under field conditions, it has been proven that *M. leuconotus* infestation is as high as 70% at elevations between 1,100-1,600 m asl, and there was no relationship between its distribution and elevation, confirming the large thermal window (Babin et al., 2017).

On the one hand, daily extreme temperatures could reach 40°C at low elevations ≈1,000-1,100 m asl, especially in summer (Azrag et al., 2018). On the other hand, the extreme minimum temperatures at high elevations ≈1,600-1,800 can reach 13°C (Azrag et al., 2018), which further explains the ability of *M. leuconotus* to adapt to a wide range of temperatures, reflected by the large thermal window of the pest. In Uganda, reported infestation levels by this pest was higher on highly shaded coffee plantations at elevations between 1,511 and 1,840 m asl (Jonsson et al., 2015). However, in Kilimanjaro region, Tanzania, infestation by *M. leuconotus* was low in highly shaded plantations at elevations

between 1,100 and 1,600 m asl (Babin et al., 2017). These results seem to indicate that temperature is not playing a crucial role on the infestation level of *M. leuconotus*, as it was demonstrated for *A. thunbergii* (Azrag et al, 2017; 2018 chapter five). Thus, other factors such as rainfall and natural enemies need to be investigated in future studies.

Conclusions

Our study provided hitherto data on temperature-dependent development models for *M. leuconotus* as well as its thermal thresholds. The models fitted to immature development time and rate, mortalities, adult senescence and female fecundity were used to simulate the life table parameters. This information will be useful for further studies on the thermal biology of this important pest. Besides, our results will help understand distribution and population dynamics of *M. leuconotus* and predict associated risk under global climate warming.

Acknowledgements

We acknowledge the financial support for this research by the following organisations and agencies: The Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France; UK's Department for International Development (DFID); Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); and the Kenyan Government. The first author AGA Azrag was supported by a German Academic Exchange Service (DAAD) In-Region Postgraduate Scholarship as well as the National

research foundation of South Africa to AA Yusuf & CWW Pirk. The views expressed herein do not necessarily reflect the official opinion of the donors.

References

Ahmed, A. G., Murungi, L. K., & Babin, R. (2016). Developmental biology and demographic parameters of antestia bug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae), on *Coffea arabica* (Rubiaceae) at different constant temperatures. *International Journal of Tropical Insect Science*, *36*, 119–127.

Akbulut, S., Keten, A., Baysal, I., & Yüksel, B. (2007). The effect of log seasonality on the reproductive potential of *Monochamus galloprovincialis* Olivier (Coleoptera: Cerambycidae) reared in black pine logs under laboratory conditions. *Turkish Journal of Agriculture and Forestry*, *31*, 413–422.

Azrag, A. G., Murungi, L. K., Tonnang, H. E., Mwenda, D., & Babin, R. (2017). Temperature-dependent models of development and survival of an insect pest of African tropical highlands, the coffee antestia bug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae). *Journal of Thermal Biology*, *70*, 27–36.

Azrag, A. G., Pirk, C. W., Yusuf, A. A., Pinard, F., Niassy, S., Mosomtai, G., & Babin, R. (2018). Prediction of insect pest distribution as influenced by elevation: combining field observations and temperature-dependent development models for the coffee stink bug, *Antestiopsis thunbergii* (Gmelin). *PloS One*, *13*, e0199569.

Babin, R., Azrag, A. G., Gichuhi, J. M., & Guandaru, E. K. (2017). Bioecology of a major pest of Arabica coffee in Eastern Africa highlands, the African coffee white stem borer, *Monochamus leuconotus* (Coleoptera: Cerambycidae). *22nd Meeting and Conference of the African Association of Insect Scientists*. ARC, Wad Medani, Sudan.

Boggs, C. L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology*, *23*, 27–37.

Curry, G. L., Feldman, R. M., & Smith, K. C. (1978). A stochastic model of a temperature-dependent population. *Theoretical Population Biology*, *13*, 197–213.

Egonyu, J. P., Kucel, P., Kagezi, G., Kovach, J., Rwomushana, I., Erbaugh, M., Wekono, R., Salifu, D., & Kyamanywa, S. (2015). *Coffea arabica* variety KP423 may be resistant to the cerambycid coffee stemborer *Monochamus leuconotus*, but common stem treatments seem ineffective against the pest. *African Entomology*, *23*, 68–74.

Erbaugh, M. J., Donnermeyer, J., Kyamanywa, S., & Kucel, P. (2008). The role of extension in the assessment process: identifying production constraints among arabica coffee producers in Eastern Uganda. In: *Proceedings of the 24th Annual Conference of the Association for International Agricultural and Extension Education*. Earth University, Costa, Rica.

Gichuhi, J. M., Ndegwa, P. N., Mugo, H. M., Guandaru, E. K., & Babin, R. (2017). Rearing method and developmental biology of the African coffee white stem borer, *Monochamus leuconotus* (Coleoptera: Cerambycidae). *Journal of Economic Entomology*, *110*, 1120–1126.

Hanks, L. M. (1999). Influence of the larval host plant on reproductive strategies of cerambycid beetles. *Annual review of entomology*, *44*, 483–505.

ICC. (2008). Integrated white stem borer management in smallholder coffee farms in India, Malawi and Zimbabwe: final project report. *International Coffee Council*. London, UK.

Jaramillo, J., Chabi-Olaye, A., Kamonjo, C., Jaramillo, A., Vega, F. E., Poehling, H. M., & Borgemeister, C. (2009). Thermal tolerance of the coffee berry borer *Hypothenemus hampei*: predictions of climate change impact on a tropical insect pest. *PloS One*, *4*, e6487.

Jayaraj, R., Megha, P., & Sreedev, P. (2016). Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdisciplinary Toxicology*, *9*, 90–100.

Jonsson, M., Raphael, I. A., Ekbom, B., Kyamanywa, S., & Karungi, J. (2015). Contrasting effects of shade level and altitude on two important coffee pests. *Journal of Pest Science*, *88*, 281–287.

Knight, C. D. 1939. Observations on the life-history and control of the white borer of coffee in Kenya. *The East African Agricultural Journal*, *5*, 61–67.

Liebig, T., Babin, R., Ribeyre, F., Läderach, P., van Asten, P., Poehling, H. M., Jassogne, L., Cilas, C., & Avelino, J. (2018). Local and regional drivers of the African coffee white stem borer (*Monochamus leuconotus*) in Uganda. *Agricultural and Forest Entomology*, *20*, 514–522.

Logan, J. A., Wollkind, D. J., Hoyt, S. C., & Tanigoshi, L. K. (1976). An analytic model for description of temperature-dependent rate phenomena in arthropods. *Environmental Entomology*, *5*, 1133–1140.

McNutt, D. N. (1975). Pests of coffee in Uganda, their status and control. *Proceedings of the National Academy of Sciences of the United States of America*, *21*, 9–18.

Naves, P. M., Sousa, E., & Rodrigues, J. M. (2008). Biology of *Monochamus galloprovincialis* (Coleoptera, Cerambycidae) in the pine wilt disease affected zone, Southern Portugal. *Silva Lusitana*, *16*,133–148.

Ngowi, B. V., Tonnang, H. E., Mwangi, E. M., Johansson, T., Ambale, J., Ndegwa, P. N., & Subramanian, S. (2017). Temperature-dependent phenology of *Plutella xylostella* (Lepidoptera: Plutellidae): simulation and visualization of current and future distributions along the Eastern Afromontane. *PloS One*, *12*, e0173590.

O'Hara, R. B., & Kotze, D. J. (2010). Do not log-transform count data. *Methods in Ecology and Evolution*, *1*, 118–122.

R Development Core Team. (2012). R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. http://www.R-project.org/.

R Development Core Team. (2016). R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. http://www.R- project.org/.

Régnière, J., Powell, J., Bentz, B., & Nealis, V. (2012). Effects of temperature on development, survival and reproduction of insects: experimental design, data analysis and modeling. *Journal of Insect Physiology*, *58*, 634–647.

Rutherford, A., & Phiri, N. 2006. Pests and diseases of coffee in Eastern Africa: A technical and advisory manual. *CAB International*. Wallingford, UK.

Schoeman, P. S., Hamburg, H., & Pasques, B. P. (1998). The morphology and phenology of the coffee white stem borer, *Monochamus leuconotus* (Pascoe) (Coleoptera: Cerambycidae), a pest of arabica coffee. *African Entomology*, *6*, 83–89.

Stinner, R. E., Gutierrez, A. P., & Butler, G. D. (1974). An algorithm for temperature-dependent growth rate simulation. *The Canadian Entomologist*, *106*, 519–524.

Tapley, R. G. (1960). The white coffee borer, *Anthores leuconotus* Pasc. and its control. *Bulletin of Entomological Research*, *51*, 279–301.

Tonnang, H. E., Juarez, H., Carhuapoma, P., Gonzales, J. C., Mendoza, D., Sporleder, M., Simon, R., & Kroschel, J. (2013). ILCYM – Insect Life Cycle Modeling. A software package for developing temperature-based insect phenology models with applications for local, regional and global analysis of insect population and mapping. *InternationalPotato Center*. Lima, Peru, p. 193.

Wang, R., Lan, Z., & Ding, Y. (1982). Studies on mathematical models of the relationship between insect development and temperature. *Acta Ecologica Sinica*, *2*, 47–57.

CHAPTER FOUR

Factors affecting the distribution and seasonal variation of two stink bugs on Arabica coffee, *Antestiopsis thunbergii* and *Antestiopsis facetoides*

Abstract

The stink bugs *Antestiopsis* spp. (Hemiptera: Pentatomidae) consist of a species complex with some of them being major coffee pests in Africa. Three species *A. thunbergii*, *A. intricata* and *A. facetoides* have been identified in coffee plantations of East Africa, with *A. thunbergii* being the most important economically. This study aimed at characterising the seasonal variation of *A. thunbergii* and *A. facetoides* at different elevations and identifying the factors that affected their distribution and seasonal variations. The populations of both species were assessed monthly for two years in coffee farms, with different shade levels, located on the Aberdare range, in Kenya, at elevations between 1,300 and 1,900 m asl. Climatic data were measured over the elevation gradient using data loggers. The number of berries at different maturation stages were counted and recorded on each visit. The result showed that *A. facetoides* was the most dominant species, accounting for 70% of the total bug population. The two species had a similar trend in seasonal variation, with a peak between June and August. However, elevation had an effect on their distribution, with *A. facetoides* being the most abundant at the lowest elevations, while *A. thunbergii* were the most abundant in plantations at high elevation. The same was true for the shade level, with *A. thunbergii* being the most abundant in highly shaded plantations, while *A. facetoides* was mostly found in full-sun plantations. The number of both species was positively correlated with the number of mature green berries. However, the number of the bugs was negatively correlated with the number of ripe berries that contributed for 57.7% and 35.9% in the variation of *A. thunbergii* and *A. facetoides* populations, respectively. Our results will help understand the distribution and population dynamics of both species and develop appropriate control measures.

Key words: *Coffea arabica*, Antestia bugs, elevation, seasonal variation, potato test

Introduction

Arabica coffee, *Coffea arabica* L. is one of the most important cash crops for many developing countries (DaMatta, 2004; DaMatta and Ramalho, 2006). It is grown in tropical and sub-tropical highlands where annual temperature ranges between 18 and 23°C, with a little seasonal fluctuation, and rainfall between 1,600 and 2,000 mm (DaMatta and Ramalho, 2006). Although it is an important crop worldwide, climate variability, especially temperature increases, droughts, and insect pests and diseases pose major threats to the production of *C. arabica* (DaMatta and Ramalho, 2006; Ovalle-Rivera et al., 2015). To mitigate the effect of temperature rise, growing coffee under shade trees has become a widespread practice by the farmers (Beer et al., 1998; Albertin and Nair, 2004). Shade trees provide a suitable microclimate for coffee production, even when the temperatures are extremely high. It also plays a vital role in water conservation and biodiversity in coffee plantations (Teodoro et al., 2009; Railsback and Johnson, 2014). However, the density of shade on coffee farms differs depending on tree diversity and density (Teodoro et al., 2009). This usually leads to variations in abiotic factors such as temperature and relative humidity, which have potential effects on the population densities and dynamics of coffee insect pests (Azrag et al., 2018).

The antestia bug *Antestiopsis* spp. (Hemiptera: Pentatomidae) consist of a dozen species complex (Greathead, 1966). Three of these species, *Antestiopsis thunbergii* (Gmelin), *A. intricata* (Ghesquière and Carayon) and *A. facetoides* (Greathead) (Greathead, 1966; Babin et al., 2018) are known to damage coffee. These are distinct in their morphologies with striking patterns and markings in black, white and orange on their body (Greathead, 1966; Babin et al., 2018). The similarity in colour makes it difficult to distinguish between

the species (Figure 1.1). However, the geographical distribution of these species differs, with *A. thunbergii* being found in east and southern parts of Africa, *A. intricata* in West Africa and some parts of East Africa (Ethiopia and Uganda). While the distribution of *A. facetoides* is limited to Kenya and Tanzania and it was found together with *A. thunbergii* in coffee plantations.

Antestia bugs feed on all vegetative parts of the coffee plant and its fruits, resulting in severe crop losses (Ahmed et al., 2016). The mature green (unripe) coffee berries are the most preferred food source, and they highly contribute to the bug's development and reproduction (Le Pelley 1942; Ahmed et al., 2016). Two types of feeding damage have been described, a direct damage which results in flower and fruit fall, necrosis on the coffee beans, and beans weight loss (Cilas et al., 1998) and an indirect damage due to the fungi *Nematospora* spp. that colonise the beans through the feeding lesions, leading to endosperm putrefaction and damage known as 'zebra beans' (Le Pelley, 1942; Ribeyre and Avelino, 2012). The extent of the yield loss due to these damages are estimated to be about 45%, with one or two bugs per tree as the economic threshold for implementation of pest control (McNutt, 1979; Cilas et al., 1998). In the recent past, some studies showed that *A. thunbergii* transmits a bacteria *Pantoea coffeiphila* (Enterobacteriaceae) that causes the 'potato taste' defect (PTD), especially in the African Great Lakes region (Matsuura et al., 2014; Gueule et al., 2015). In this region, the severity of PTD on coffee beans increased from 18% in 2012 to 51% in 2013 (MOAAR, 2014 cited in Ahmed et al., 2016). It reduces the quality and competitiveness of Arabica coffee beans in foreign markets and hence lowers profit margins.

Although antestia bugs threaten coffee production in Africa, there is still little knowledge on the bioecology of these pests, especially the seasonal variations and factors affecting their distribution and population densities. Solid knowledge on the spatial and temporal distribution patterns of an insect pest is crucial for effective control measures (Rao et al., 2000). This knowledge should be based on the response of the insects' population to its habitat and the surrounding environment, in order to identify key factors that affect population dynamics. The population density of *A. thunbergii* in Rwanda has been reported to peak in February with a density of 45 bugs per tree (Foucart and Brion, 1959). Thereafter, declining to its minimal level in March, after which it increases again between June and September with a second peak in August (Foucart and Brion, 1959). On the other hand, Azrag et al. (2018) found higher populations in June, compared to the period of October and January in the Kilimanjaro region at elevations between 1,000 and1,600 m asl. Yet, factors involved in the seasonal variations are not well understood. Therefore, the objectives of this study were to describe the seasonal variation of the two antestia bugs species *A. thunbergii* and *A. facetoides* at different elevations and to identify the factors involved in the infestation level and distribution for the two species.

Materials and methods

Study site and experimental design

This study was conducted in 28 small holding coffee farms located on the Aberdare range (0.7096°S – 37.0835°E and 0.6948°S – 36.9226°E), with elevations between 1,300 and 1,900 m asl. The selected farms were small with approximately 100 coffee trees each and the most common coffee variety on these holdings was SL28. This variety is old in Africa

and it was introduced into Kenya since the 1930s (World Coffee Research, 2018). The variety SL28 is suited for medium to high altitudes and shows resistance to drought but is susceptible to the major diseases of coffee (World Coffee Research, 2018). This agricultural area is characterised by a binomial rainfall regime: a long rainy season occurring from mid-March to end of May (being the most important for coffee growing), and a short one from mid-October to December, (Ovuka and Lindqvist, 2000). The coolest and warmest seasons are from June to August and January to March, respectively. Variation in elevation and climate leads to different agro-ecological zones in the area that harbours diverse types of crops. Coffee plantations mixed with food crops such as maize, *Zea mays* L., beans (*Vigna* spp.), banana *Musa* spp., and trees such as *Grevillea robusta* (A. Cunn. ex R. Br.) and *Macadamia* spp. which provide coffee trees with shade are usually found at elevations between 1,000 and 1,900 m asl. In our study, we divided the elevation gradient into three zones: the low zone (1,300-1,450 m asl), the mid zone (1,500- 1,700 m asl), and the high zone (1,750-1,900 m asl). Each zone had nine coffee farms with the exception of the lower zone, which had 10 farms. Coffee farms in each zone were visually categorised as having either full sun, low shade, moderate shade, or high shade (Figure 4.1).

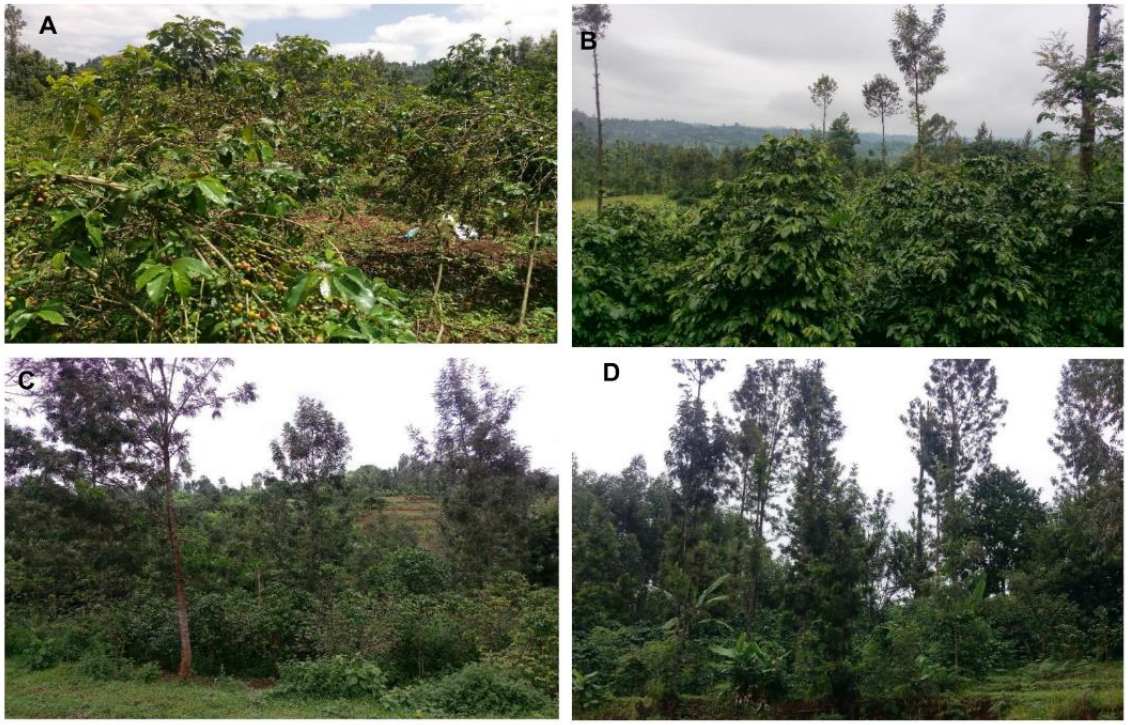


Figure 4.1 Shade classification on the coffee plantations, A: full sun, B: low shade, C: moderate shade and D: high shade.

Climatic data

A total of 11 data loggers (iButtons Hygrochron, Maxim Integrated, San Jose, USA) were deployed on coffee farms across the elevation gradient (1,300-1,900 m asl) to record daily temperature and relative humidity. These were hung on coffee tree branches at approximately 2 m above the ground. Temperature and humidity were recorded hourly for the two years duration of the project. Average monthly temperature and humidity across the study site were calculated and used for the determination of climatic variation in the study sites.

Monitoring of antestia bug populations and coffee berry development

Monthly counts of antestia bugs were carried out on the selected coffee farms. The antestia bugs found in the study area were identified as *A. thunbergii* and *A. facetoides* using the morphological key developed by Greathead (1966). On each farm and during each observation round, 15 trees were randomly selected on the two sides of a diagonal line across the farm (Azrag et al., 2018). On each tree, numbers of *A. thunbergii* and *A. facetoides* were counted and recorded irrespective of their life stages. We were not able to distinguish between egg batches of the two species hence, did not record them in our study. To assess the availability of the coffee berries at different maturation stages (developing, mature green and ripe berries), berries were counted monthly on the same trees throughout the year. In each tree, two branches were selected, one at the top and one at the bottom of the tree. One fruit node was randomly selected in each branch and the number of developing, green mature and ripe berries were recorded.

Statistical analyses

A descriptive analysis was used to describe the variation in the mean monthly temperature and relative humidity within the study area, as well as the number of antestia bugs per month (monthly average for the two years) at different elevation zones. Repeated measures ANOVA were performed using an ezANOVA function in 'ez' package (Lawrence, 2016) in R 3.3.0 (R Core Team, 2016). The monthly average of antestia bugs per farm (average for the two years) was computed for each species and used as the dependent variable. Shade class (four levels) and elevation (three zones) were factors for each coffee farm, and the observation date (month) was included as a random effect to

account for the repeated measures. Once significant difference was detected, data were subjected to pairwise comparisons of the means using Bonferroni test at $\alpha = 0.05$.

To understand factors that influenced monthly variation in the number of antestia bug, we carried out Spearman correlation analyses with environmental variables (monthly mean temperature and humidity) and coffee berry maturation stage (monthly number of developing, mature and red berries per node) as independent variables. Since there is a correlation between the independent variables (i.e. temperature, humidity and berry maturation stage), we could not use the ordinary regression methods to separate the effects of a single independent variable on the dependent variable. To solve this problem, we used a hierarchical partitioning analysis, which is a technique that deals with the problem of collinearity that exists between the independent variables. This method considers all possible models in a multivariate regression and it estimates the percentage of explained variance for each independent variable into independent and joint contribution with all other variables (Mac Nally, 1996; Mac Nally and Walsh, 2004). Specifically, we used hierarchical partitioning to quantify the independent contribution of each independent variable to the monthly number of antestia bug using R packages 'hier.part' (Walsh and Mac Nally, 2015).

Results

Variation in climate and coffee berry maturation stage

The climatic conditions (monthly mean temperature and relative humidity) in the study area and berry maturation on coffee trees throughout the year are shown on Figure 4.2.

The monthly mean temperature ranged between 17.7 and 23.2°C, with March and July being the hottest and coolest months, respectively (Figure 4.2A). The highest mean relative humidity was obtained in November with a value of 85%, while the lowest was 61% recorded in March. By contrast, the monitoring of coffee berry maturation showed that the mean number of developing berries was the highest between January and June, with a peak at 4 berries per node in April (Figure 4.2B). The mean number of mature green berries peaked between July and September, with 4-5 berries per node. The mean number of ripe berries had two peaks, the first one between April and July, which corresponds to the small harvesting season in the region, and the second between August and January with a peak in November, which corresponds to the main harvesting season in the region (Figure 4.2B).

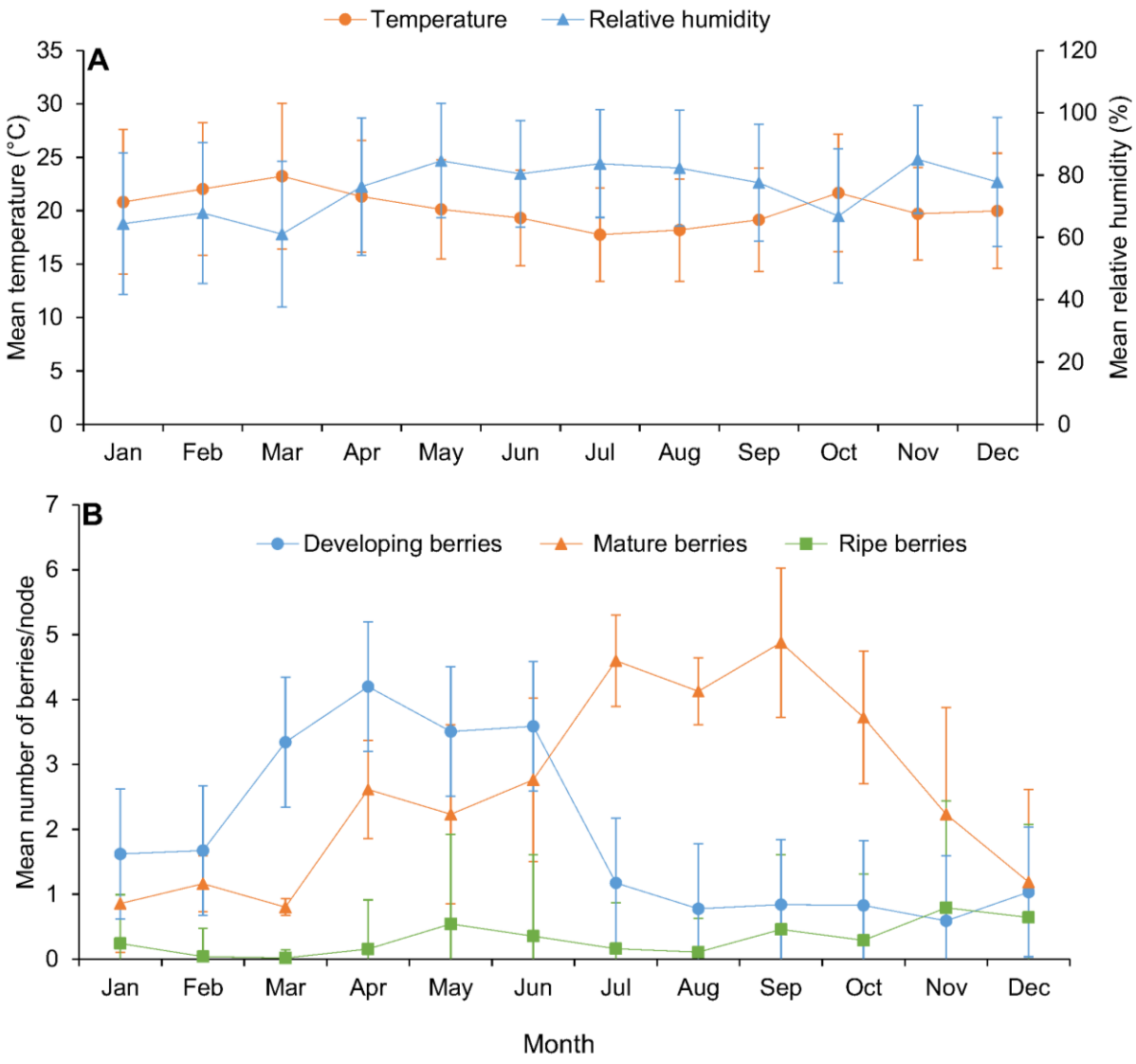


Figure 4.2 Monthly climatic data (average temperature and relative humidity for years 2016 and 2017 ± SD) (A), and B: coffee berry maturation stage (mean number of developing, mature and ripe berries per node ± SD), recorded in coffee farms located on elevation ranged between 1,300 and 1,900 m asl, on the Aberdare range in Murang'acounty, Kenya.

Seasonal variation of antestia bug populations

In total, 2041 antestia bugs (both nymphs and aduts) belonging to the two species *Antestiopsis thunbergii* and *A. facetoides* were observed in this study. *Antestiopsis facetoides* was dominant across the study area, accounting for 70% of the total bug population. Both species were found throughout the year in coffee plantations with two peaks across all elevation gradients (Figure 4.3). The number of *A. thunbergii* (both nymphs and adults) per month (monthly average for the two years) started to build up from January and reached its peak in April with 13 and 23 bugs at high (1,750-1,900 m asl) and medium (1,500- 1,700 m asl) elevations, respectively (Figure 4.3A). However, no *A. thunbergii* were observed on all the ten farms at the lower elevations (1,300-1,450 m asl) during this period. The number of *A. thunbergii* per month started increasing again between June and September with a peak between July and August at all elevations (Figure 4.3A). On the other hand, variation in the number of *A. facetoides* followed the same trend as those of *A. thunbergii* being higher in April at low and medium elevations (Figure 4.3B). Afterwards, it decreased from May to October at medium and high elevations. Globally, the number of antesia bug per month (the two species) in the study area started increasing from April and reached the highest peak in July (Figure 4.3C). Afterwards, it decreased and reached it minimal levels in November (Figure 4.3C).

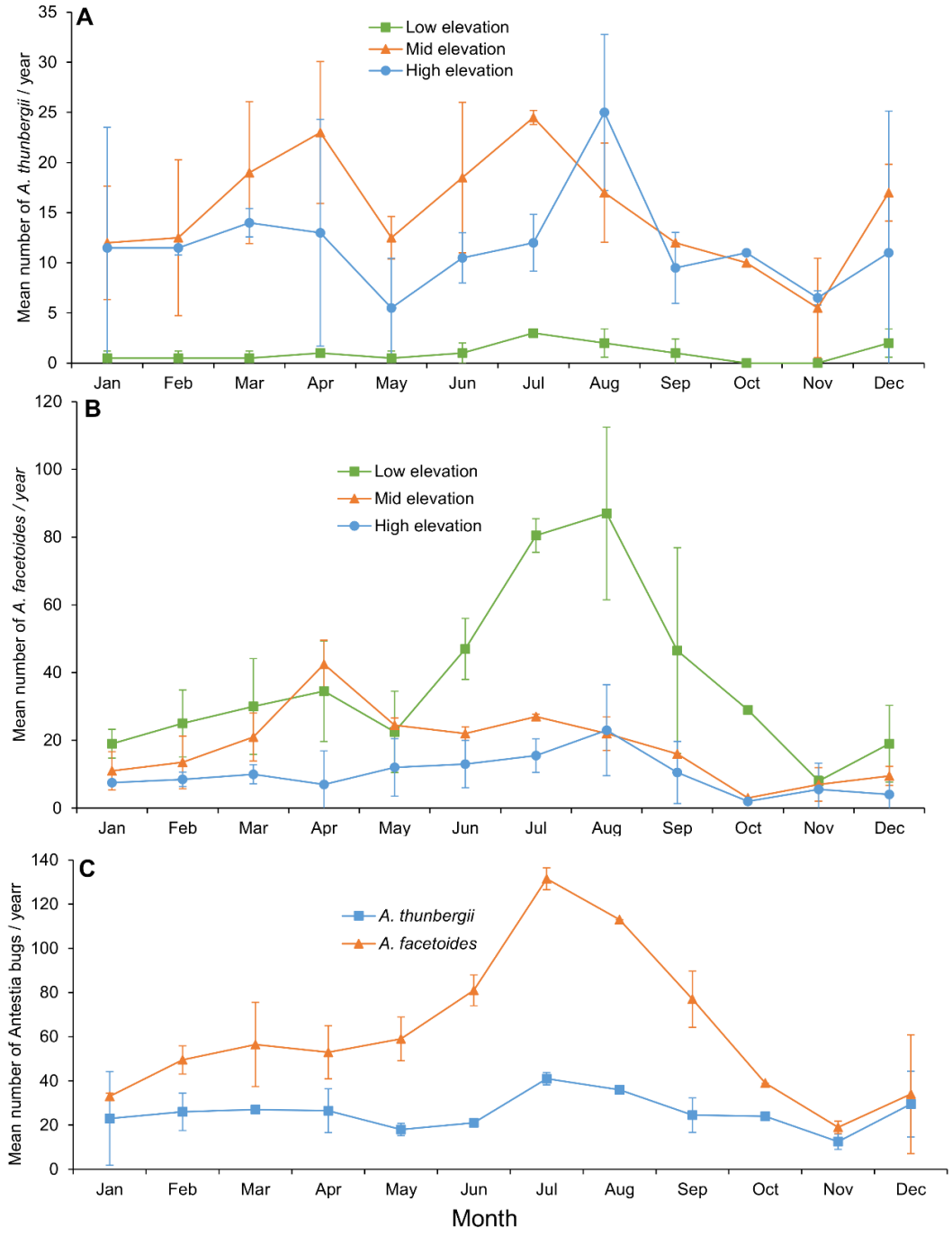


Figure 4.3 Seasonal variation (mean number of antestia bugs (both nymphs and adults) per month monitored for two years 2016 and 2017 ± SD) of A: *Antestiopsis thunbergii* and B: *Antestiopsis facetoides* over different elevation zones (low:1,300-1,450 m asl; mid: 1,500-1,700 m asl; and high: 1,750-1,900 m asl), and C: the total number of the bugs for all elevations (1,300-1,900 m asl), on the Aberdare range in Murang'a county, Kenya.

Factors influencing the seasonal variation of antestia bug populations

Table 4.1 shows the results of an analysis of the correlation between the number of *A. thunbergii* and *A. facetoides* per month as dependent variables, and climatic data (average monthly temperature and humidity) and coffee berry maturation stage (monthly number of berries per node) as independent variables. A significant negative correlation was detected between the number of *A. thunbergii* per month and the monthly number of ripe berries per node ($r = -0.56$; $P < 0.05$). The correlation between the number of mature green berries per node on coffee trees and the number of *A. thunbergii* was weak and not significant ($r = 0.189$; $P > 0.05$). On the other hand, the number of *A. facetoides* per month was positively correlated with the number of mature berries per node ($r = 0.64$; $P < 0.05$), and negatively with temperature ($r = -0.58$; $P < 0.05$). The hierarchical partitioning analysis showed that the majority (57.7%) of the explained variance on the monthly number of *A. thunbergii* was related to the independent effects of the ripe berries (Figure 4.4A). The second most influenced variable on this species was temperature, with an independent contribution of 18.2%. In contrast, the variation in the number of *A. facetoides* per month was mostly explained by ripe berries (35.9%), followed by temperature (29.4%) and mature berries (18.7%) (Figure 4.4B).

Table 4.1 Spearman correlations between the number *Antestiopsis thunbergii* and *Antestiopsis facetoides* per month and explanatory variables (climatic variables and berry maturation stage on coffee trees)

	A. thunbergii	*A. facetoides*	Developing berries	Mature berries	Ripe berries	Temperature
A. thunbergii						
A. facetoides	0.441 ns					
Developing berries	−0.063 ns	0.209 ns				
Mature berries	0.189 ns	0.643*	0.294 ns			
Ripe berries	−0.559*	−0.329 ns	−0.308 ns	0.168 ns		
Temperature	−0.119 ns	−0.580*	0.343 ns	−0.734**	−0.364 ns	
Humidity	−0.196 ns	0.322 ns	−0.259 ns	0.489 ns	0.552*	−0.776**

Significant codes for the correlation: * $p \leq 0.05$ and ** $p \leq 0.01$; ns = correlation not significant

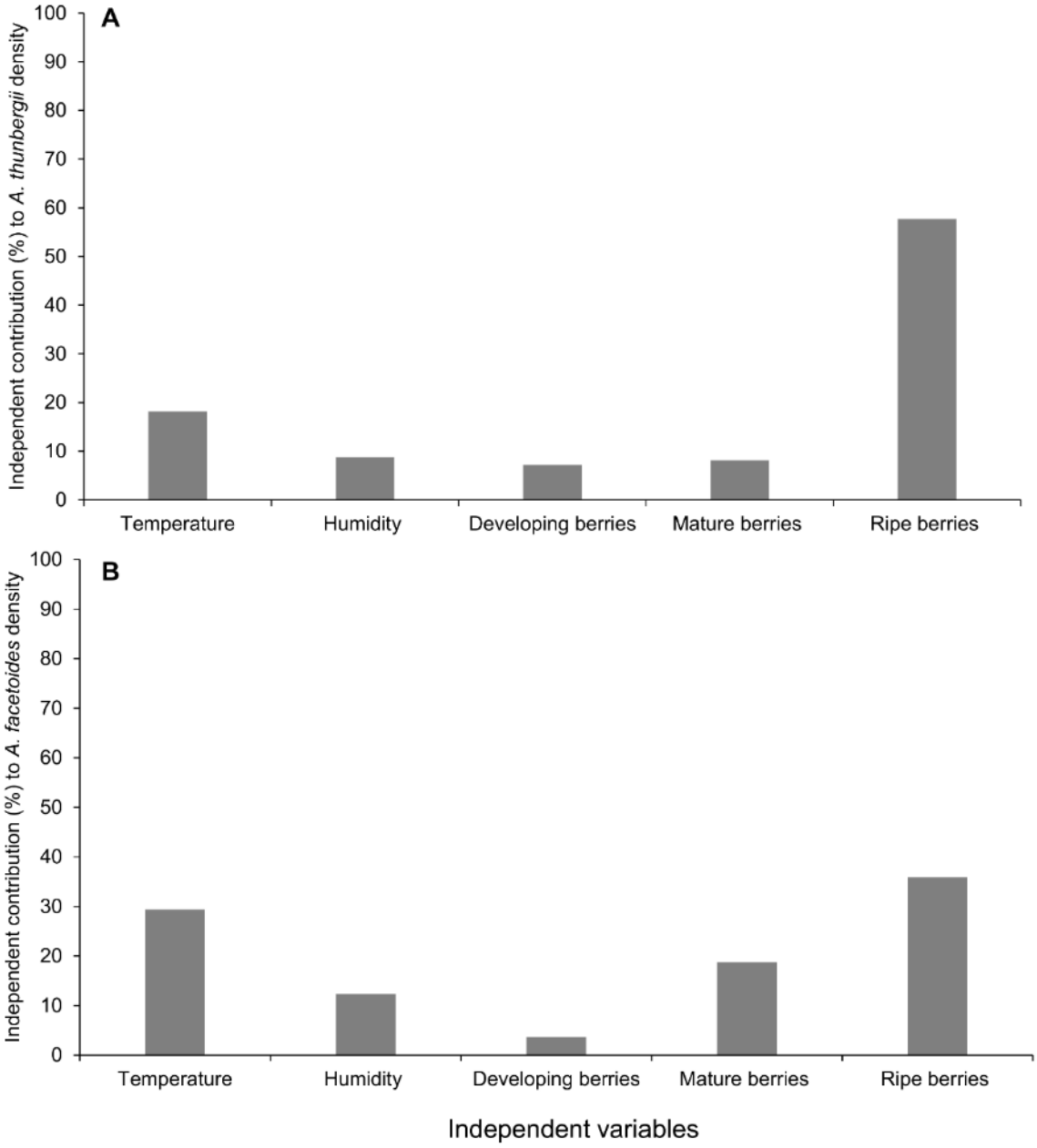
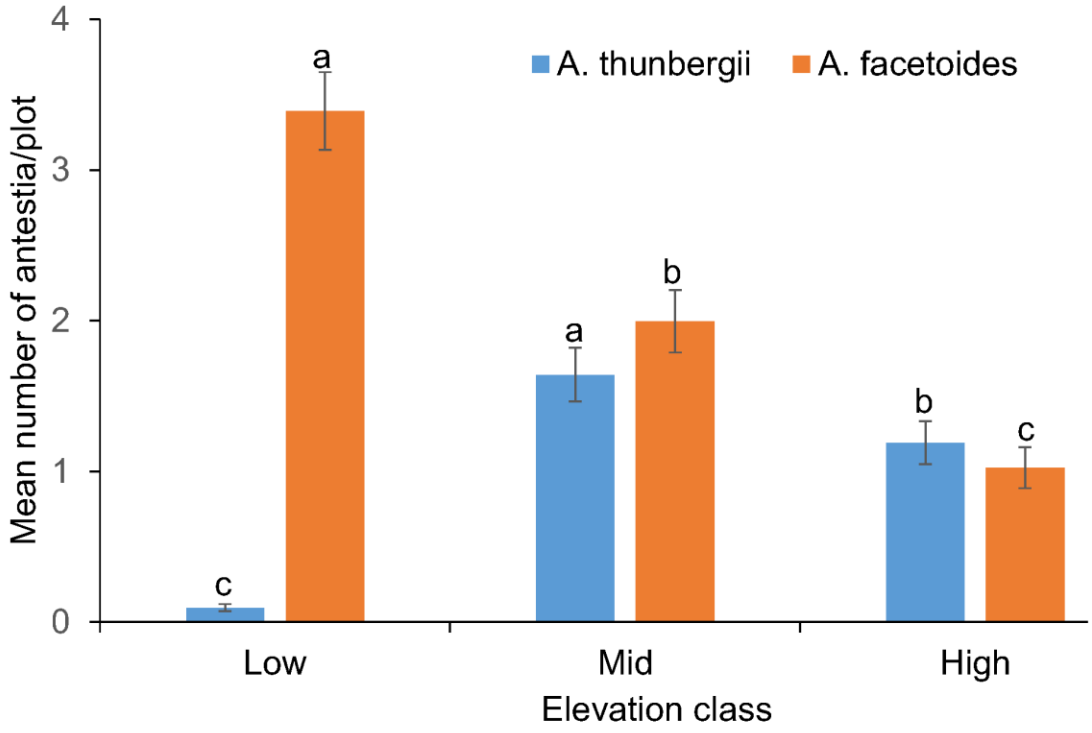


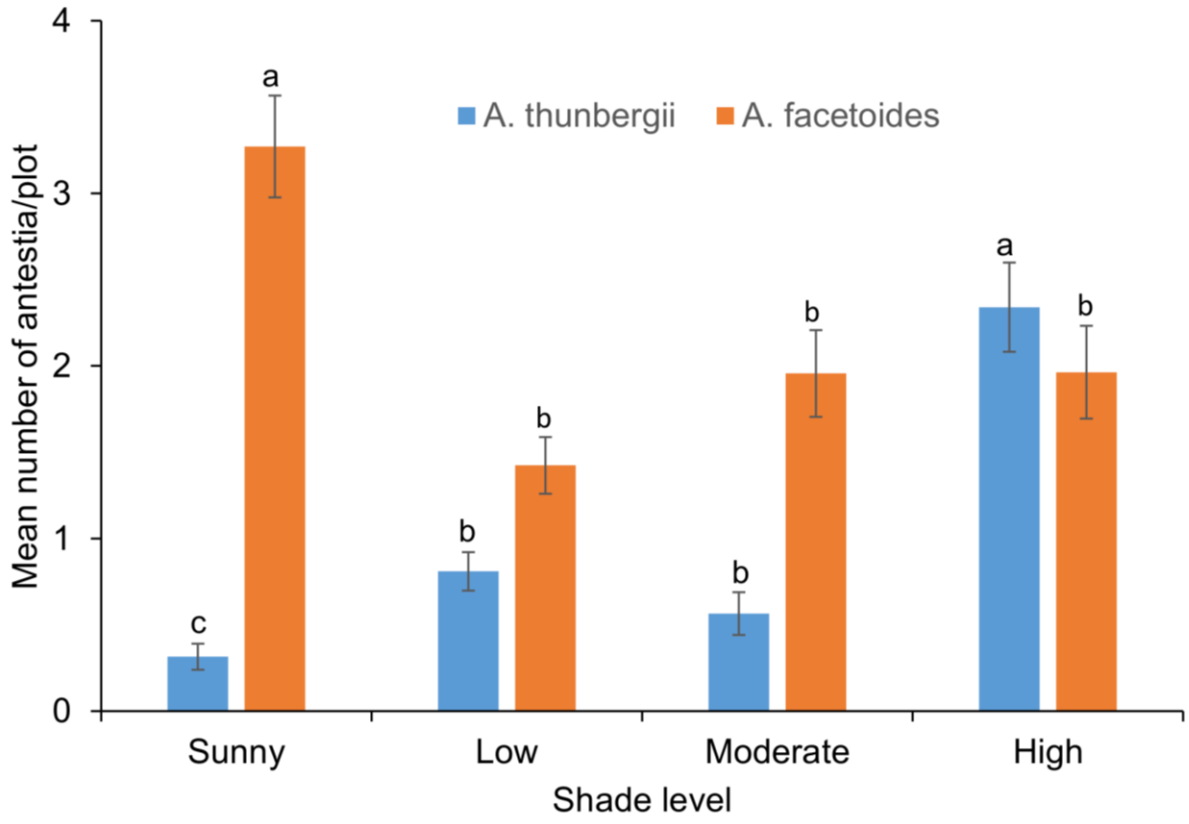
Figure 4.4 Hierarchical partitioning analyses showing the independent effects (percentage of the explained variance) of the environmental variables (monthly average temperature and humidity) and coffee berry maturation stage (monthly mean number of developing, mature and ripe berries per node) on the number of antestia bugs per month, with, A: *Antestiopsis thunbergii* and B: *Antestiopsis facetoides*.

Effect of elevation and shade on the population of antestia bug

Elevation had a significant effect on the number of antestia bug (Figure 4.5) (average per farm per year). The highest number of *A. thunbergii* was found on farms at medium elevations (1,500- 1,700 m as) (1.6 bugs), followed by the farms at the highest elevation (1,750-1,900 m asl) (1.2 bugs) ($F_{2,633} = 14.43$, $P < 0.001$) (Figure 4.5). On the other hand, the number of *A. facetoides* was significantly higher on farms at the lower elevation (3.4 bugs) and it decreased with an increase in elevation ($F_{2,633} = 22.18$, $P < 0.001$) (Figure 4.5). The number of antestia bugs per farm was also affected by shade in coffee plantations (Figure 4.6). The number of *A. thunbergii* individuals per farm was higher in plantations with high shade, compared to ones with low and moderate shade and full sun ($F_{3,633} = 12.24$, $P < 0.001$). By contrast, the number of *A. facetoides* was higher in sunny plantations compared to low and moderate shade ($F_{3,633} = 3.16$, $P < 0.05$) (Figure 4.6).



Figurer 4.5 Number of antestia bugs (mean number/farm/ year ± SE, for the population monitored for two years 2016 and 2017), *Antestiopsis thunbergii*, and *Antestiopsis facetoides* for the different elevation zones (Low: 1,300-1,450 m asl, mid: 1,500-1,700 m asl, and high: 1,750-1,900 m asl) located on the Aberdare range in Murang'a county, Kenya.



Figurer 4.6 Number of antestia bugs (mean number/farm/year ± SE, for the population monitored for two years 2016 and 2017), *Antestiopsis thunbergii*, and *Antestiopsis facetoides* for different shade levels in coffee plantations located on the Aberdare range in Murang'a county, Kenya.

Discussion

Our results showed that the seasonal variations of *A. thunbergii* and *A. facetoides* throughout the year had a similar trend in coffee plantations, with two peaks at all elevations (low, 1,300-1,450 m asl; mid, 1,500-1,700 m asl; and high, 1,750-1,900 m asl). The highest population densities for antestia bugs in the in Murang'a were observed in August with a mean of 0.4 and 0.6 bugs per tree at high and low elevations, respectively, and in July (0.4 bugs per tree) at medium elevation. However, the lowest and highest densities we obtained in different farms ranged between 0 and 1.7 bugs per tree. A density of 1 bug per tree is considered as economic threshold level that requires the implementation of the control measures. A recent study in Rwanda by Bigirimana et al. (2019) obtained a density of 0 to 6 bugs per tree for the different farms with a mean density of 0.5 bugs per tree for the study site (elevation between 1,381 and 2,135 m asl), which is consistent with our findings. However, the mean density we obtained here was lower compared to the findings of Azrag et al. (2018) who obtained 1.5 bugs per tree in Tanzania. These low densities could be linked to good cultural farming practices like pruning that was adopted by the farmers in our study area. Good pruning practices expose antestia bugs to extreme temperatures, which can be lethal, especially for the immature stages. Also, it provides a suitable environment for the natural enemies to easily target the pest.

Overall, the highest number of antestia bug was observed between June and August at all elevations. This period corresponds to the cold season in Murang'a with lots of mature green berries that are available on coffee trees. Similar results were obtained by Azrag et al. (2018) for *A. thunbergii* in Mt. Kilimanjaro, Tanzania, where the population of the

bug was higher in cold season compared to short rainy and warm dry seasons. The same was true for the report of van der Meulen and Schoeman (1990), who obtained high populations of immature stages for *A. thunbergii* when lots of green berries were available on the trees in South Africa. Azrag et al. (2017) suggested that the availability and quality of food could play a vital role in the seasonal variation of *A. thunbergii*, compared to some abiotic factors such as temperature. This was true due the positive correlation we found between the number of antesia bug and the presence of mature green berries. Furthermore, Le Pelley (1942) reported that the mature green berries are the most preferable food for antestia bugs and the most important for their development, survival and reproduction. Indeed Njihia et al. (2017) have shown that the second nymph instar for *A. thunbergii* were strongly attracted to odours from mature green berries supporting our observations. Therefore, the availability of food, especially mature green berries is one of the key factors explaining the high populations of both species between June and August.

The lowest number of *A. thunbergii* and *A. facetoides* were observed in May and November at all elevations which coincides with the period when most of the berries on the trees are ripe. Le Pelley (1942) showed that when antestia bugs feed on the ripe berries only, they cannot produce offspring. Thus, it could be possible that antestia bug adults in the field look for an alternative food source for survival when the berries become ripe. Our results strengthened the findings of Njihia et al. (2018), that showed the volatiles of the ripe berries significantly repels the second nymph instar of *A. thunbergii*. Therefore, the presence of the ripe berries was one of the key factors that explained the low populations for both species in May and November (small and main harvesting seasons).

In our study, *A. thunbergii* and *A. facetoides* had a different distribution over the elevation gradient, which suggests that they have different thermal requirements. The highest number of *A. thunbergii* was found at medium elevations (1,500-1,700 m asl), where the mean annual temperature ranged between 19.9 and 21.7°C, followed by the high elevations (1,750-1,900 m asl) where the mean temperature is between 18.9 and 19.7°C. Our results showed that temperature is the second most crucial factor that negatively affected the number of *A. thunbergii* after ripe berries. It has been established that the extreme temperatures (below 19°C and above 26°C) negatively affect the reproduction and intrinsic rate of increase that determine the population growth rate for *A. thunbergii* (Ahmed et al., 2016; Azrag et al., 2017). Therefore, the high number of *A. thunbergii* at mid-elevations could be explained by the daily minimum and maximum temperatures that are appropriate for *A. thunbergii* development, survival and reproduction, compared to the low and high elevations where daily temperatures can be lethal for the immature stages (Azrag et al., 2017). Azrag et al. (2018) showed a high risk of *A. thunbergii* at elevations between 1400-1800 m asl on Kilimanjaro slope, under current and future (the year 2055) climatic conditions.

On the other hand, the number of *A. facetoides* was the highest in the study area and mostly found at the lower elevations (1,300-1,450 m asl), where the annual mean temperature ranged between 20.6 and 22°C, followed by the mid elevations (1,500-1,700 m asl). To the best of our knowledge, the thermal biology of *A. facetoides* at different constant temperatures has not been studied and literature on this species is scarce. This is mainly due to the lower economic importance of *A. facetoides* in coffee plantations in Africa, compared to the two other species *A. thunbergii* and *A. intricata* that cause high

damages to Arabica coffee (Greathead, 1966; Babin et al., 2018). Therefore, it is difficult to explain the effect of temperature and elevation on the distribution of *A. facetoides* at the moment. However, our results suggest that *A. facetoides* has a wider thermal window with a preference for a warmer climate, compared to *A. thunbergii*, which could explain its high number at the lower elevations.

Shade level on coffee plantations had an opposite effect on the populations of *A. thunbergii* and *A. facetoides*, which confirms the different thermal requirements of both species. The number of *A. thunbergii* were higher in high shade coffee plantations, compared to sunny, low and moderate shade. Earlier on, Mugo et al. (2013) reported high infestation level of *A. thunbergii* in shaded coffee compared to full sun plantations in Kenya. In addition, a study by Kirkpatrick (1937) showed that *A. thunbergii* prefers cool climate and populations are usually higher in bushy coffee trees which they use as shade to avoid high temperatures during the day. This behaviour was explained by Azrag et al. (2017) who reported that the susceptibility of immature stages to extreme temperatures as a key factor for *A. thunbergii* to seek cool habitat for the development and population growth. By contrast, the number of *A. facetoides* was higher on sunny coffee plantations, and low in low, moderate and high shaded coffee. Presently, we do not know the mechanism behind the positive effect of sunny plantations on *A. facetoides* populations. However, one explanation could be that *A. facetoides* requires high temperature for the development and population growth as we mentioned earlier, and this might be the reason for the preference of a warmer climate. Also, it could be possible that shaded plots offer higher diversity and abundance of natural enemies of *A. facetoides* (i.e. parasitoids, and birds) which lower its population in shaded plantations.

Conclusions

The antestia bug species *A. thunbergii* and *A. facetoides* have a different distribution over elevation gradients, as well as under different shade covers which suggest different thermal requirements. However, the seasonal variations of *A. thunbergii* and *A. facetoides* display similar trends over two years and the population was higher when lots of mature green berries were available. In addition, when the berries are ripened, number of both species decreases. These results suggest that the presences of food, especially mature green berries on the trees is the most important factor resulting in seasonal population variation of antestia bugs.

Acknowledgements

We acknowledge the financial support for this research by the following organisations and agencies: The Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France; UK's Department for International Development (DFID); Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); and the Kenyan Government. The first author AGA Azrag was supported by a German Academic Exchange Service (DAAD) In-Region Postgraduate Scholarship as well as the National research foundation of South Africa to AA Yusuf & CWW Pirk. The views expressed herein do not necessarily reflect the official opinion of the donors.

References

Ahmed, A. G., Murungi, L. K., & Babin, R. (2016). Developmental biology and demographic parameters of antestia bug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae), on *Coffea arabica* (Rubiaceae) at different constant temperatures. *International Journal of Tropical Insect Science*, *36*, 119–127.

Albertin, A., & Nair, P. K. R. (2004). Farmers' perspectives on the role of shade trees in coffee production systems: An assessment from the Nicoya Peninsula, Costa Rica. *Human Ecology*, *32*, 443–463.

Azrag, A. G., Pirk, C. W., Yusuf, A. A., Pinard, F., Niassy, S., Mosomtai, G., & Babin, R. (2018). Prediction of insect pest distribution as influenced by elevation: combining field observations and temperature-dependent development models for the coffee stink bug, *Antestiopsis thunbergii* (Gmelin). *PloS One*, *13*, e0199569.

Azrag, A. G., Murungi, L. K., Tonnang, H. E., Mwenda, D., & Babin, R. (2017). Temperature-dependent models of development and survival of an insect pest of African tropical highlands, the coffee antestia bug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae). *Journal of Thermal Biology*, *70*, 27–36.

Babin R., Mbondji Mbondji, P., Mendesil, E., Mugo, H. M., Lee, J-H., Serracin, M., Rukazambuga, N. D. T. M., & Miller, T. A. (2018). The antestia bug complex in Africa and Asia. In: McPherson, J. E, editor. Invasive stink bugs and related species (Pentatomoidea): Biology, higher systematics, semiochemistry, and management. *CRC Press*. USA. pp. 465–493.

Beer, J., Muschler, R., Kass, D., & Somarriba, E. (1997). Shade management in coffee and cacao plantations. *Agroforestry Systems, 38*, 139–164.

Bigirimana, J., Adams, C. G., Gatarayiha, C. M., Muhutu, J. C., & Gut, L. J. (2019). Occurrence of potato taste defect in coffee and its relations with management practices in Rwanda. *Agriculture, Ecosystems and Environment, 269*, 82–87.

Cilas, C., Bouyjou, B., & Decazy, B. (1998). Frequency and distribution of *Antestiopsis orbitalis* Westwood (Hem., Pentatomidae) in coffee plantations in Burundi: implications for sampling techniques. *Journal of Applied Entomology, 122*, 601–606.

DaMatta, F. M. (2004). Exploring drought tolerance in coffee: a physiological approach with some insights for plant breeding. *Brazilian Journal of Plant Physiology, 16*, 1–6.

DaMatta, F. M., & Ramalho, J. D. C. (2006). Impacts of drought and temperature stress on coffee physiology and production: a review. *Brazilian Journal of Plant Physiology,18*, 55–81.

Foucart, G., & Brion, L. (1959). Contribution à l´étude de la punaise du caféier arabica au Rwanda-Urundi. *Rwanda Agricultural Research Institute* (ISAR). Rwanda.

Greathead, D.J. (1966). A taxonomic study of the species of *Antestiopsis* (Hemiptera, Pentatomidae) associated with *Coffea arabica* in Africa. *Bulletin of Entomological Research*, 56, 515–554.

Gueule, D., Fourny, G., Ageron, E., Le Fleche-Mateos, A., Vandenbogaert, M., Grimont, P. A., & Cilas, C. (2015). *Pantoea coffeiphila* sp. nov., cause of the ‘potato

taste'of arabica coffee from the African great lakes region. *International Journal of Systematic and Evolutionary Microbiology*, *65*, 23–29.

Kirkpatrick, T. W. (1937). Studies on the ecology of coffee plantations in East Africa. II. The autecology of *Antestia* spp. (Pentatomidae) with a particular account of a strepsipterous parasite. *Transactions of the Royal entomological Society of London*, *86*, 247–343.

Lawrence, M. A. (2016). 'ez'. *R package*. https://cran.r-project.org/web/packages/ez/ez.pdf.

Le Pelley, H. R. (1942). The food and feeding habits of antestia in Kenya. *Bulletin of Entomological Research*, *33*, 71–89.

Matsuura, Y., Hosokawa, T., Serracin, M., Tulgetske, G. M., Miller, T. A., & Fukatsu, T. (2014). Bacterial symbionts of a devastating coffee pest, the stinkbug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae). *Applied and Environmental Microbiology*, *80*, 3769–3775.

Mac Nally, R. (1996). Hierarchical partitioning as an interpretative tool in multivariate inference. *Australian Journal of Ecology*, *21*, 224–228.

Mac Nally, R., & Walsh, C. J. (2004). Hierarchical partitioning public domain software. *Biodiversity and Conservation*, *13*, 659–660.

McNutt, D. (1979). Control of *Antestiopsis* spp. on coffee in Uganda. *Proceedings of the National Academy of Sciences of the United States of America*, *25*, 5–15.

Mugo, H. M., Kimemia, J. K., & Mwangi, J. M. (2013). Severity of antestia bugs, *Antestiopsis* spp. and other key insect pests under shaded coffee in Kenya. *International Journal of Science and Nature*, *4*, 324–327.

Njihia, T. N., Torto, B., Murungi, L. K., Irungu, J., Mwenda, D. M., & Babin, R. (2017). Identification of kairomones of second instar nymphs of the variegated coffee bug *Antestiopsis thunbergii* (Heteroptera: Pentatomidae). *Chemoecology*, *27*, 239–248.

Njihia, T. N., Torto, B., Murungi, L. K., Irungu, J., Mwenda, D. M., & Babin, R. (2018). Ripe coffee berry volatiles repel second instar nymphs of Antestia bugs (Heteroptera: Pentatomidae: *Antestiopsis thunbergii*). *Chemoecology*, *28*, 91–100.

Ovalle-Rivera, O., Läderach, P., Bunn, C., Obersteiner, M., & Schroth, G. (2015). Projected shifts in *Coffea arabica* suitability among major global producing regions due to climate change. *PloS One*, *10*, e0124155.

Ovuka, M., & Lindqvist, S. (2000). Rainfall variability in Murang'a district, Kenya: meteorological data and farmers' perception. *Physical Geography*, *82*, 107–119.

R Development Core Team. (2016). R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. https://www.R-project. org/.

Railsback, S. F., & Johnson, M. D. (2014). Effects of land use on bird populations and pest control services on coffee farms. *Proceedings of the National Academy of Sciences*, *111*, 6109–6114.

Rao, M. R., Singh, M. P., & Day, R. (2000). Insect pest problems in tropical agroforestry systems: contributory factors and strategies for management. *Agroforestry Systems*, *50*, 243–277.

Ribeyre, F., & Avelino, J. (2012). Impact of field pests and diseases on coffee quality. In: Oberthür, T., Läderach, P., Pohlan, H.A.J., & Cock, J. H, editors. Specialty coffee: managing coffee. *International Plant Nutrition Institute*. Penang, Malaysia. pp. 151–176.

Teodoro, A., Klein, A. M., Reis, P. R., & Tscharntke, T. (2009). Agroforestry management affects coffee pests contingent on season and developmental stage. *Agricultural and Forest Entomology*, *11*, 295–300.

van der Meulen, H. J., & Schoeman, A. S. (1990). Aspects of the phenology and ecology of the antestia stink bug, *Antestiopsis orbitalis orbitalis* (Hemiptera: Pentatomidae), a pest of coffee. *Phytophylactica*, *22*, 423–426.

Walsh, C. J., & Mac Nally, R. (2015). Package 'hier.part'. Available at: https://cran.r-project.org/web/packages/hier.part/hier.part.pdf.

World Coffee Research. (2018). Drought tolerant and very good cup quality potential, but susceptible to major diseases. Found commonly in Kenya, Malawi, Uganda, Zimbabwe. https://varieties.worldcoffeeresearch.org/varieties/sl28.

CHAPTER FIVE

Prediction of insect pest distribution as influenced by elevation: Combining field observations and temperature-dependent development models for the coffee stink bug, *Antestiopsis thunbergii* (Gmelin)

Abstract

The antestia bug, *Antestiopsis thunbergii* (Gmelin 1790) is a major pest of Arabica coffee in Africa. The bug prefers coffee at the highest elevations, contrary to other major pests. The objectives of this study were to describe the relationship between *A. thunbergii* populations and elevation, to elucidate this relationship using our knowledge of the pest thermal biology and to predict the pest distribution under climate warming. *Antestiopsis thunbergii* population density was assessed in 24 coffee farms located along a transect delimited across an elevation gradient in the range 1,000–1,700 m asl, on Mt. Kilimanjaro, Tanzania. Density was assessed for three different climatic seasons, the cool dry season in June 2014 and 2015, the short rainy season in October 2014 and the warm dry season in January 2015. The pest distribution was predicted over the same transect using three risk indices: the establishment risk index (ERI), the generation index (GI) and the activity index (AI). These indices were computed using simulated life table parameters obtained from temperature-dependent development models and temperature data from 1) field records using data loggers deployed over the transect and 2) predictions for year 2055 extracted from AFRICLIM database. The observed population density was the highest during the cool dry season and increased significantly with increasing elevation. For current temperature, the ERI increased with an increase in elevation and was therefore distributed similarly to observed populations, contrary to the other indices. This result suggests that immature stage susceptibility to extreme temperatures was a key factor of population distribution as impacted by elevation. In the future, distribution of the risk indices globally indicated a decrease of the risk at low elevation and an increase of the risk at the highest elevations. Based on these results, we concluded with recommendations to mitigate the risk of *A. thunbergii* infestation.

Key wards: *Coffea arabica*, stink bugs, climate change, pest forecasting models, Antestia bugs.

Introduction

The antestia bug, *Antestiopsis thunbergii* (Gmelin 1790) (Hemiptera: Pentatomidae) is one of the major insect pests of Arabica coffee in eastern and southern Africa (Greathead, 1966; Babin et al., 2018). The pest feeds on coffee leaves, buds, flowers and berries leading to direct damage (McNutt, 1979). By feeding on young berries, the bug causes premature fruit fall and necrosis of the beans (Cilas et al., 1998). In addition, feeding lesions allow the fungi *Nematospora* spp. to colonise the beans, leading to endosperm rotting and a damage known as "zebra beans" (Ribeyre and Avelino, 2012). *Antestiopsis thunbergii* is also supposedly involved in the transmission of a bacteria *Pantoea coffeiphila* (Enterobacteriaceae), causing a flavour defect of coffee beverage known as 'potato taste' defect (PTD), which threatens coffee production in the African Great Lakes region (Matsuura et al., 2014; Gueule et al., 2015). The economic threshold for taking action against this pest is as low as one or two bugs per coffee tree on average, depending on the level of damage found in the country (Cilas et al., 1998; Bigirimana et al., 2012; Mugo et al., 2013). Contrary to most other coffee pests like the coffee berry borer, *Hypothenemus hampei* (Ferrari), *A. thunbergii* thrives in high elevation Arabica coffee. The pest is present in plantations between 1,000 and 2,100 m asl, but with a strong preference for plantations of the highest elevations (Greathead, 1966). Two closely related antestia bug species, *A. intricata* (Ghesquière and Carayon) and *A. facetoides* Greathead, are more common in coffee plantations at low-medium elevation (1,000-1,600 m asl) (Greathead, 1966; Abebe, 1987; Babin et al, 2018). A recent study by Azrag et al. (2017) suggested that *A. thunbergii* preference for higher elevations might be due to the pest's adaptation to cool habitats in the tropics.

Temperature is the most important environmental factor that affects insect distribution (Bale et al., 2002; Ahmed et al., 2016), and it is highly correlated with elevation (Zehnder et al., 2009). Variation in temperature affects insect population dynamics through insect physiology and behaviour (Estay et al., 2009). Indirect effects also are expected due to the impact of temperature on host plants and natural enemies (Bale et al., 2002). A recent study by Azrag et al. (2017) provided an insight into *A. thunbergii* thermal requirements using temperature-based development models. These models showed that the pest was able to survive and develop under a temperature range of 14.6-32.9°C. However, based on simulated life table parameters, growth of the rearing population was restricted to a temperature range of 19-25°C (Azrag et al., 2017). Such thermal requirements suggest an adaptation to temperatures cooler than expected for a tropical insect.

In tropical and temperate regions, change in distribution and abundance are expected for many insect pests as a result of global warming (Bale et al., 2002; Jaramillo et al., 2011; Mwalusepo et al., 2015). For example, in East Africa, *H. hampei* is currently an issue for coffee production at elevation of up to 1,500 m asl. By the year 2050, rising temperatures may lead to higher populations on Arabica coffee at elevations up to 1,800 m asl, leading to reduction in both production and quality of renowned Arabica coffees (Jaramillo et al., 2011; Cilas et al., 2016). In contrast, the abundance of some herbivorous insects is expected to decrease due to their vulnerability to high temperatures (Bale et al., 2002); yet, such scenarios have not been well documented.

Considering previous findings by Azrag et al. (2017), it becomes feasible and necessary to predict the risk of *A. thunbergii* infestation in coffee of the East African highlands. Therefore, the general objectives of this chapter were first to elucidate the relationship

between *A. thunbergii* current distribution and elevation, and second to assess the potential impact of temperature change on the future distribution of the pest. To achieve these objectives, we first analysed this relationship using the pest abundance surveyed along an elevation gradient. In a second step, we used simulated life table parameters, incorporating the full life history of the pest, obtained from temperature-dependent development models (Azrag et al., 2017) to generate risk indices based on temperature, using Insect Life Cycle Modelling software (ILCYM) (Tonnang et al., 2013). The indices were calculated and mapped on the same elevation transect using temperature data collected in the field, in order to compare index distribution to this obtained from the field survey. Finally, to predict the potential impact of temperature increase on the future distribution of *A. thunbergii*, we generated and mapped the risk indices on the transect with simulated temperature data for the year 2055 from the Representative Concentration Pathway warming scenario 4.5 (RCP 4.5).

Materials and methods

Study site

Our study targeted the Arabica coffee growing area of the south-eastern slope of Mt. Kilimanjaro in Tanzania (S3.28° E37.43° and S3.47° E37.50°). A transect approximately 11 km-long and 2 km-wide with a total surface area of around 22.2 km² was delimited over an elevation gradient of 1,000-1,700 m asl (Figure 5.1). This agricultural area is characterised by a bimodal rainfall regime, with a long rainy season starting from March to May and a short one from mid-October to December. The mean annual temperature ranges between 18 and 23°C (Mwalusepo et al., 2016) and mean annual rainfall between

1,500 and 2,000 mm at 1,500 m asl (Hemp, 2006). During the coolest period (June to August), the average temperature ranges between 18 and 20°C. In this area, agricultural landscape is dominated by agroforestry systems known as Chagga home gardens, where coffee is grown in small farms with banana and other food crops as well as fruit trees used as shade trees, such as *Persea americana* Mill. and *Mangifera indica* L. (Hemp, 2006).

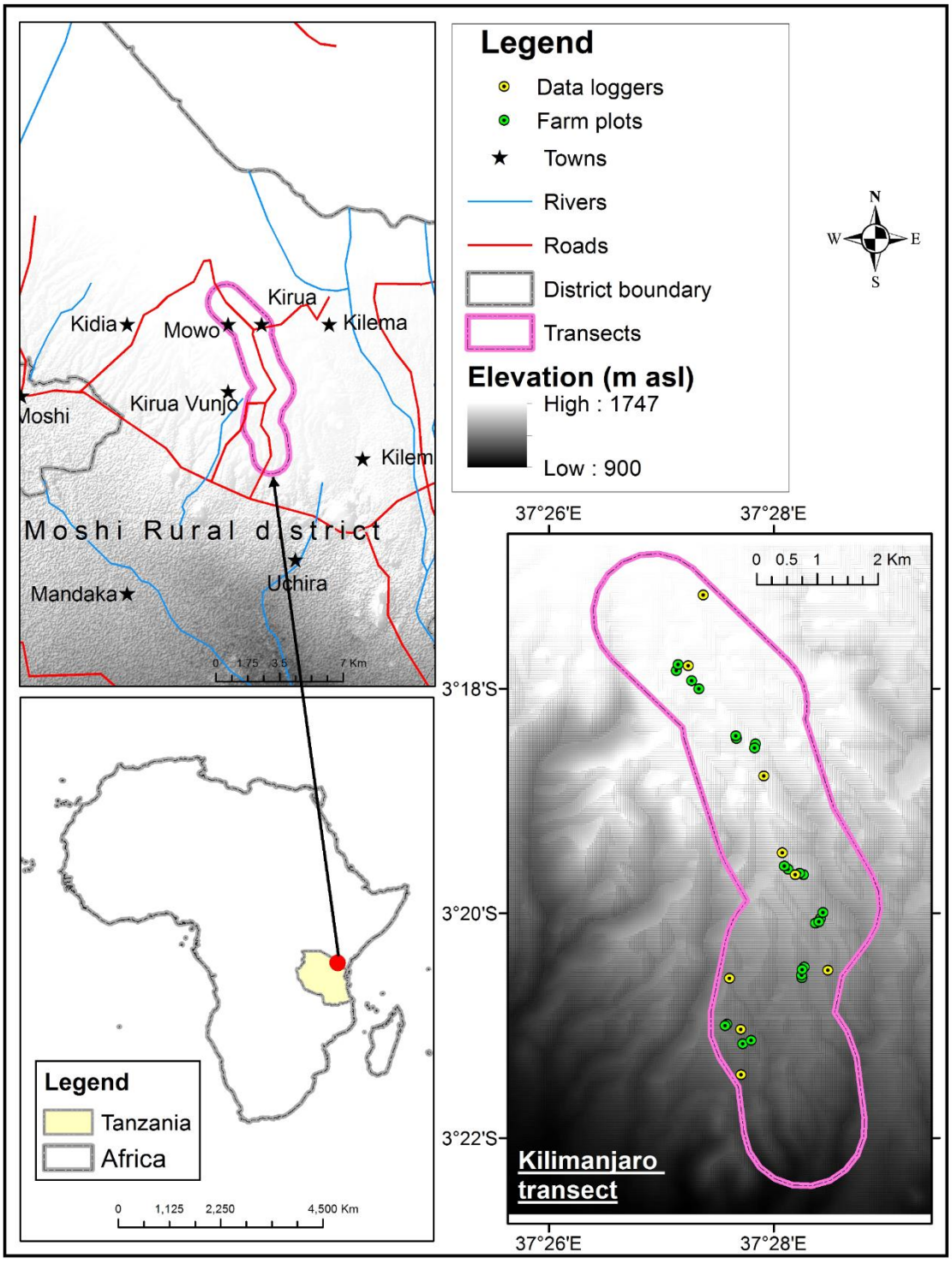


Figure 5.1 Location of the study transect over an elevation gradient on the south-eastern slope of Mt. Kilimanjaro, in Moshi district, Tanzania. The transect delimited in pink is approximately 11 km-long and 2 km-wide with a total surface area of around 22.2 km^2.

Field surveys

The antestia bug, *A. thunbergii* was identified as one of the two main pests of coffee in the studied area. The second major pest was the African coffee white stem borer, *Monochamus leuconotus* Pascoe (Coleoptera: Cerambycidae) (Gichuhi et al., 2017). The antestia bugs observed on the transect were identified as the subspecies *A. thunbergii bechuana* Kirkaldy, using the morphological identification key and distribution maps proposed by Greathead (1966). This subspecies differs from *A. thunbergii ghesquierei* Carayon, which has a slightly different coloration and a more western distribution in east Africa (Babin et al., 2018). The two other species of antestia bugs found on coffee in East Africa, *A. intricata* and *A. facetoides* were not found on the transect. Four field surveys were conducted spanning across three different climatic seasons, two during the cool dry season in June 2014 and 2015, one at the beginning of the short rainy season in October 2014 and one during the warm dry season in January 2015. *Antestiopsis thunbergii* populations were assessed in 24 coffee farms, selected in groups of 4 at approximately 1,100, 1,200, 1,300, 1,400, 1,500 and 1,600 m asl (Figure 5.1). The selected farms were representative of the area; they were usually small, with about 100 coffee trees, and the age of the trees was between 10 and 50 years. The farms were homogeneously planted with the same traditional Arabica coffee variety, originated from breeding of Bourbon-type and Kent-type old varieties. In each farm, 15 trees were randomly selected on both sides of a diagonal across the farm. Individuals of *A. thunbergii* were counted visually on the selected trees, irrespective of the development stage of the pest. Presence of eggs was noted, without counting.

Temperature data

Real-time air temperature was recorded hourly throughout the year 2013 as an activity of the CHIESA project (http://chiesa.icipe.org/) using climate data loggers (iButtons Hygrochron, Maxim Integrated, San Jose, USA). This data was then borrowed to the project for analyses presented in the present paper. Nine data loggers were deployed across the transect (Figure 5.1). Data loggers were hanged on tree branches at approximately 2 m above the ground to record air temperature under shade. Data logger geographical position and elevation were recorded using a handheld Global Positioning System (GPS).

For 2055 temperature estimates, we used simulated temperature dataset (AFRICLIM version 3.0) of the Representative Concentration Pathway scenario 4.5 (RCP 4.5) of the fifth assessment report of Intergovernmental Panel on Climate Change (IPCC-AR5). RCP 4.5 emission scenario was selected because it is a moderate scenario, which includes long term global emissions of greenhouse gases, land use and land cover in a global economic framework (Thomson et al., 2011). The simulated temperature data were downscaled to a resolution of 30″ (1 km) using General Circulation Models (GCMs) and Regional Climate Models (RCMs), with WorldClim grids as baseline. Data are well documented by Platts et al. (2015) and freely accessible at http://www.york.ac.uk/environment/research/kite/resources/. We downloaded the temperature dataset for the year 2055 as raster layers in tiff format. Then, we used point sampling tool in QGIS software (version 2.18.4) to extract temperature data from layers, using the geographical position of data loggers on the transect as coordinates.

Statistical analysis of the survey data

The relationship between *A. thunbergii* populations (both nymphs and adults) and elevation was determined through a regression analysis, using a generalised linear model with a Poisson distribution. R programming environment (R Core Team, 2016) was used for all calculations. The dependent variable was the total number of bugs per farm, i.e. the total number of bugs counted on 15 coffee trees. This variable was regressed against the elevation variable. The same regression analysis was computed for the 4 observation times. Poisson distribution assumes that the mean and variance of the count data are equal, and over-dispersion occurs when the variance is greater than the mean. This case was detected in our data and was corrected using "dispmod" package in R (Scrucca, 2018). With this method, the goodness of fit of Poisson regression model is assessed on the basis of the deviance (log-likelihood ratio statistic), which has a distribution approximating to chi-squared, and is represented as χ^2. To display the current distribution of the bug on the study transect, we mapped the bug density (mean number of bugs per tree) using the regression model obtained for each observation time, and elevation data computed from the digital elevation model (DEM) of the transect. Calculation and mapping were done using the "raster" package in R programming environment (Hijmans et al., 2017). To analyse the effect of season on *A. thunbergii* populations, generalised linear model with a Poisson distribution was fitted to the total number of bugs per plot as dependent variable and season as independent variable. Once a significant difference was detected, data were submitted to post hoc analysis for mean comparison, using Tukey test at $P = 0.05$.

Prediction of distribution based on temperature-dependent models

Demographic parameters' simulation and model validation

To predict the distribution of *A. thunbergii* populations as impacted by elevation, we used the temperature-dependent development models reported by Azrag et al. (2017). The laboratory colonies used in this study were initiated with individuals of the same subspecies, *A. thunbergii bechuana*, collected in the same area that was targeted targeted in the present study. Models were developed using life table data collected at 7 constant temperatures (18, 20, 23, 25, 28, 30 and 32°C). Mathematical functions were fitted to immature stage development time and rate, mortality rate, female fecundity and adult senescence, using the software Insect Life Cycle Modelling (ILCYM, version 3.0) (Tonnang et al., 2013). Using these models, *A. thunbergii* life table parameters were simulated for different constant temperatures, *viz.* 1) the gross reproductive rate (*GRR*), which is defined as the average number of daughters produced by a living female throughout her life time, 2) the net reproductive rate (R_o), which is the rate of multiplication per generation taking into account the mortality rate of immature stages, 3) the mean generation time (*T*) which is defined as the mean time (in days) between the birth of parents and the birth of offspring, 4) the doubling time (*Dt*), which is the time (in days) that is required for the population to double, 5) the intrinsic rate of increase (r_m), which is defined as the innate capacity of a population to grow, and 6) the finite rate of increase (λ), which is the average per capita multiplication factor per one time unit (Tonnang et al., 2013 ; Kroschel et al., 2013; Azrag et al., 2017) .

For the present study, an additional experiment was conducted at fluctuating temperature in order to validate the models developed by Azrag et al. (2017) at constant temperatures.

The validation experiment was carried out in an open insectary from April to October 2015 at International Centre of Insect Physiology and Ecology (*icipe*), Kenya. A total of 154 individuals of *A. thunbergii* were reared from egg to adult using green coffee berries and leaves as diet (see Ahmed et al. (2016) for a complete description of the rearing method). During the experiment, temperature was recorded every hour using a data logger (iButtons Hygrochron, Maxim Integrated, San Jose, USA). The daily mean temperature ranged between 17.4 and 28.1°C, while the daily minimum temperature ranged between 10.6 and 18.7°C, and the daily maximum temperature between 20.6 and 36.9°C. *Antestiopsis thunbergii* demographic parameters were calculated from the models using fluctuating temperature data. Then, the calculated parameters were compared to the values simulated from data collected at constant temperatures (Azrag et al., 2017). Consistency between simulated and calculated values of demographic parameters was good enough to validate the models and undertake risk index calculation.

Calculation and mapping of infestation risk indices

Antestiopsis thunbergii distribution as impacted by elevation was displayed using the following three indices, i) the establishment risk index (ERI), ii) the generation index (GI) and iii) the activity index (AI). The ERI gives the capacity of an insect to establish in a particular area based on temperature. When mapped, the ERI enables the visualisation of the geographical areas suitable for the insect's establishment and survival. The index is 1 when all immature stages of the pest survive throughout the year in the particular area. Otherwise, the number of days in which a single stage would not survive (100% mortality) are counted and divided by 365 (number of Julian days), and then subtracted from 1. The GI estimates the mean number of generations that an insect may produce

within a year, and it is estimated by the number of Julian days (365) divided by the estimated generation time (*T*). The AI assesses the potential distribution and abundance of the species; the AI is closely related to the finite rate of population increase (λ) (population growth rate), which takes into account the whole life history of the pest. It is calculated by taking the log of the products of estimated finite rates of increase calculated for each Julian day. For example, an AI value of 4 gives a potential population increase factor of 10, 000 in one year. These indices were computed and mapped on the same transect using "*index interpolator*" in ILCYM (Tonnang et al., 2013) based on i) the temperature-dependent development models and related demographic parameters reported by Azrag et al. (2017), ii) the digital elevation model (DEM) for the study area (elevation was used as a co-variable in ASCII format), and iii) the current and future temperature data (daily minimum and maximum temperatures). Indices were initially calculated for each geographical point where temperature data were recorded or simulated, and then interpolated on the surface of the digital elevation model using thin plate algorithm interpolation method (Tonnang et al., 2013).

In order to better characterise the changes in the pest's distribution between current and future temperatures, we randomly extracted risk index values from a total of 160 geographical points in groups of 20, located at every 100-m elevation across the transect, using point sampling tool in QGIS. The same geographical points were used to extract indices for current and future temperatures. Then, extracted data were tested for normality and a Wilcoxon test was applied to the data in R (version 3.3.0) (R Core Team, 2016) to compare the mean of the indices in current and future temperature conditions at each elevation.

Results

Effect of elevation and season on *A. thunbergii* populations

The impact of elevation on the total number of *A. thunbergii* per farm (total counts on 15 trees) was significant, with an increase in populations together with elevation for the four different evaluation periods (June 2014 (cool dry season): $\chi^2 = 20.159$, $df = 19$, $z = 2.553$; $P < 0.05$; June 2015 (cool dry season): $\chi^2 = 20.848$, $df = 22$, $z = 2.877$; $P < 0.01$; October 2014 (short rainy season): $\chi^2 = 19.065$, $df = 18$, $z = 2.302$, $P < 0.05$; January 2015 (warm dry season): $\chi^2 = 16.868$, $df = 18$, $z = 3.622$, $P < 0.0001$) (Figure 5.2). Total number of *A. thunbergii* per farm was very variable even for farms located at similar elevation and for the same evaluation period, especially for the highest elevations. For example, in June 2014, populations ranged between 4 and 48 bugs for 15 trees for elevation around 1,600 m asl. For the same elevation, in January 2015, population ranged between 5 and 32 bugs for 15 trees (Figure 5.2). Maps of Figure 5.3 confirm that population densities were the highest at the top of the transect (1,600-1,700 m asl), whatever the period. They exceeded 1.5 bug per tree at the top in June 2014 and January 2015, but were lower in October 2014 and June 2015, with about 1 bug per tree. At the bottom of the transect (1,000-1,100 m asl), densities were around 0.1 bug per tree and were slightly higher in June 2014, when compared to the other periods. In the cold season, populations of *A. thunbergii* reached 12.5 bugs per farm, which was significantly higher, compared to the warm dry season (6.6 bugs per farm) ($\chi^2 = 63.2$, $df = 68$, $P < 0.05$).

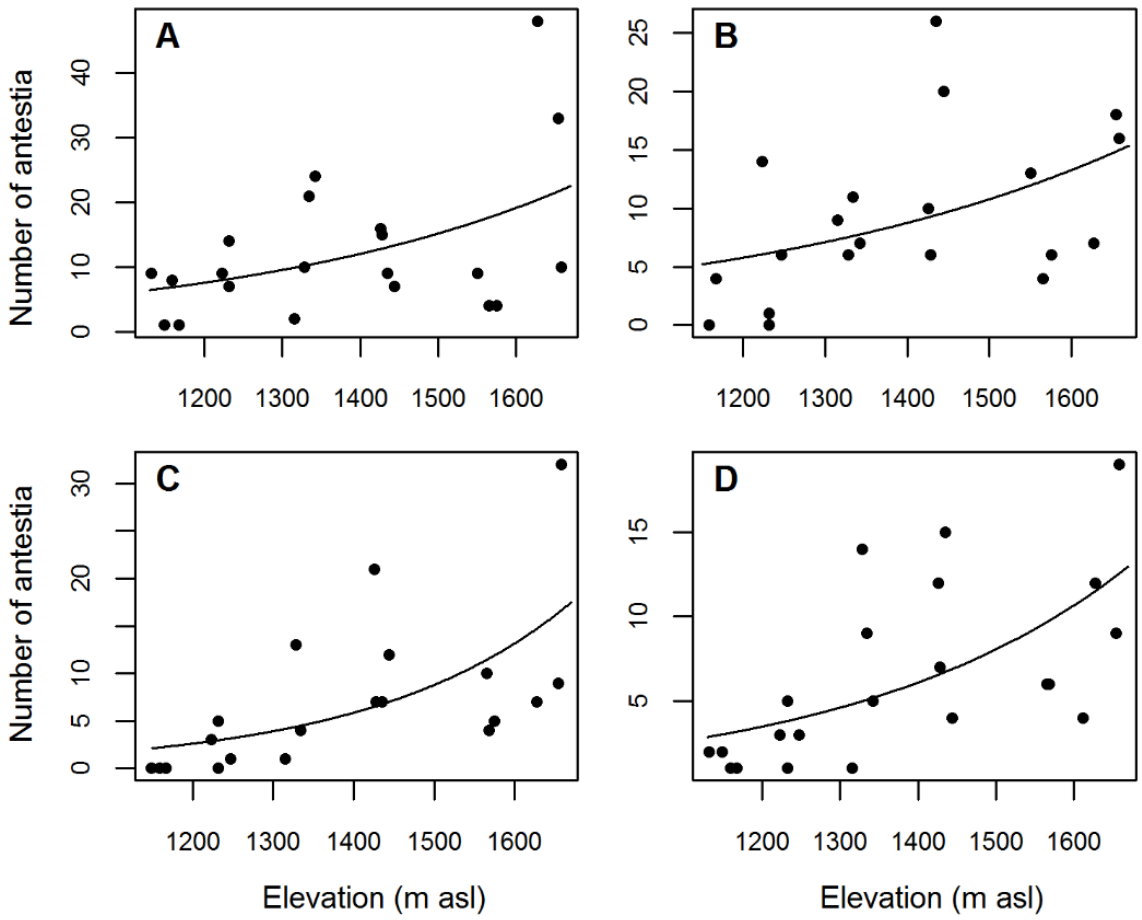


Figure 5.2 Observed *Antestiopsis thunbergii* populations (total number of bugs on 15 coffee trees) in relation to elevation on Mt. Kilimanjaro transect for the different evaluation periods. (A) June 2014, cool dry season, (B) October 2014, short rainy season, (C) January 2015, warm dry season, (D) June 2015, cool dry season.

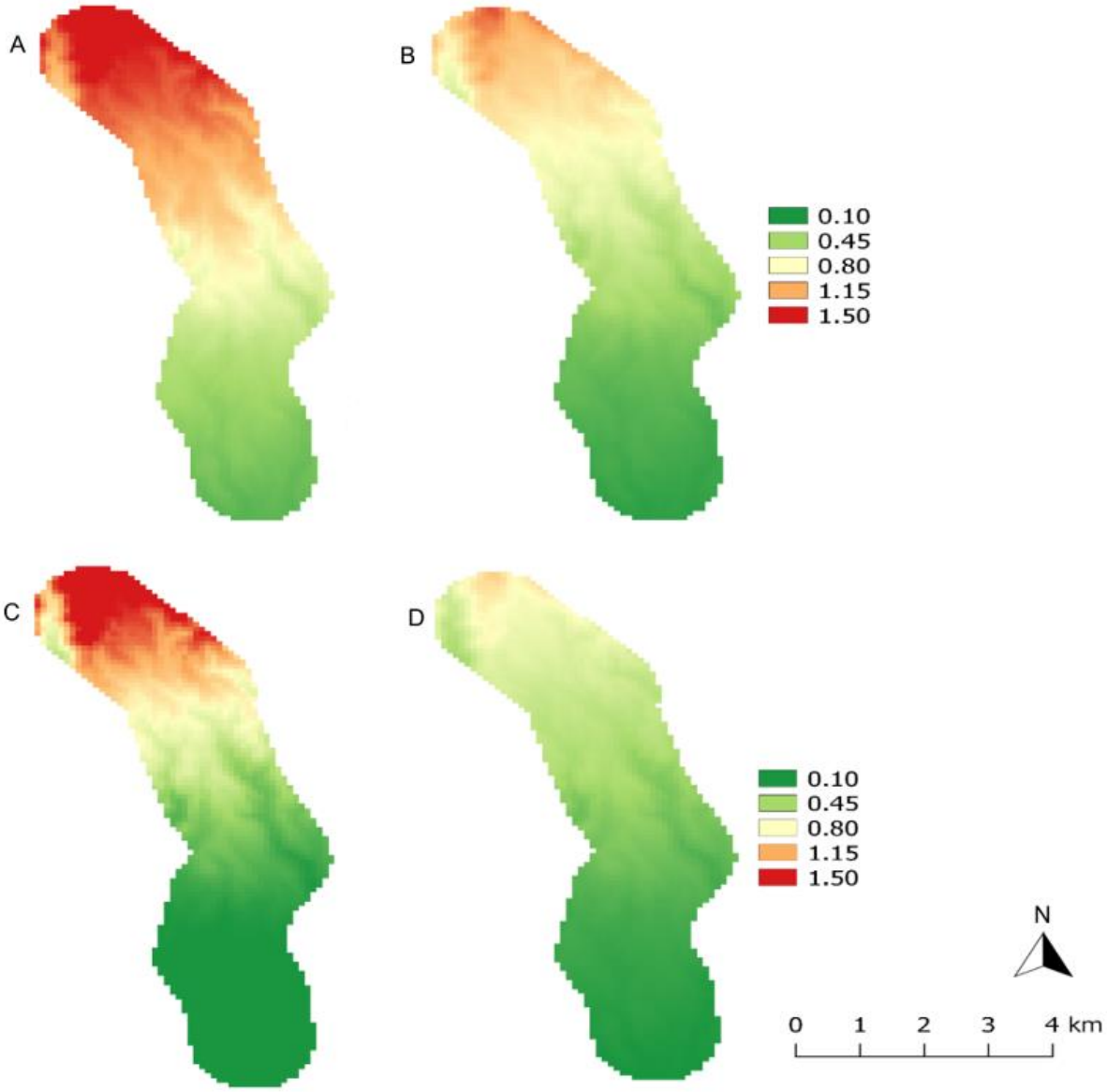


Figure 5.3 Distribution of *Antestiopsis thunbergii* populations (mean density per tree) over the elevation transect on Mt. Kilimanjaro, for the different evaluation periods. (A) June 2014, cool dry season, (B) October 2014, short rainy season, (C) January 2015, warm dry season, (D) June 2015, cool dry season.

Risk indices under current temperature

Mean current temperature measured with data loggers varied in the range of 18-23°C from the top to the bottom of the transect. Maps of risk index distribution showed that the establishment risk index is clearly linked to elevation with the lowest values (ERI ≈ 0.47) at the bottom of the transect and the highest values (ERI ≈ 0.59) at the highest elevations (Figure 5.4A). By contrast, the generation index is higher in the lower zone of the transect (GI ≈ 3.17) when compared to the top (GI ≈1.91) (Figure 5.4B). The activity index follows a similar trend with AI ≈ 4.84 at the bottom and AI ≈ 2.94 at the top of the transect (Figure 5.4C).

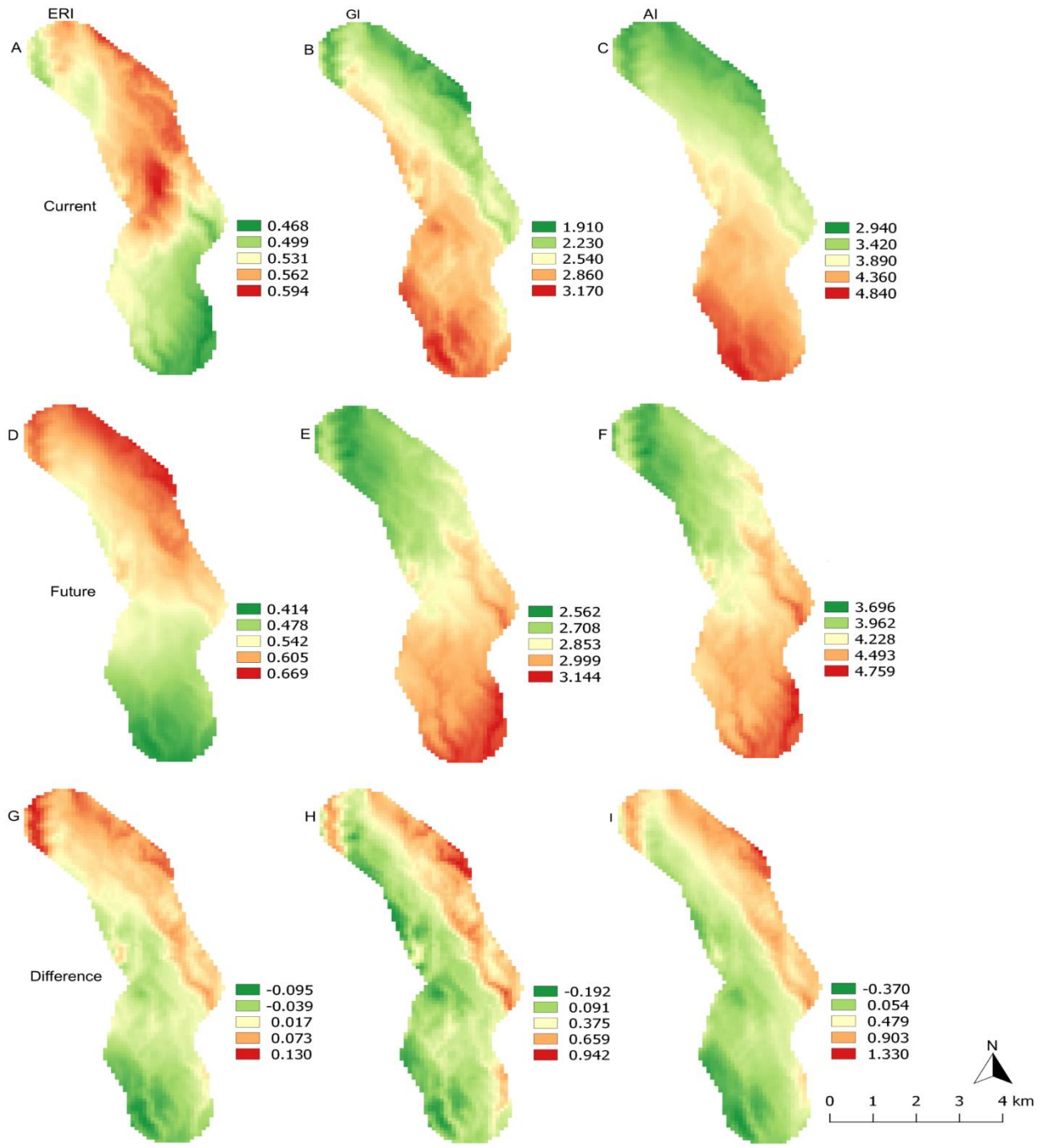


Figure 5.4 Distribution of establishment risk index (ERI), generation index (GI) and activity index (AI) of *A. thunbergii* on Mt. Kilimanjaro elevation transect calculated from the temperature dependent-development models under current (2013) and future (2055) temperature conditions. (A) ERI, (B) GI and (C) AI under current temperature; (D) ERI, (E) GI and (F) AI under future temperature projections; (G) ERI, (H) GI and (I) AI absolute difference between future and current temperature conditions.

Change in risk indices under future temperature

Minimum temperature predicted for 2055 will be higher than temperature we measured in 2013 across the transect by 0.1-0.3°C, whereas maximum temperature predicted for 2055 will be higher by 0.6-1.8°C (Table 5.1). Change in temperature varies with elevation but there is no clear pattern in the variations. Under future temperatures, the ERI will change across the transect with differences ranging between -0.09 and 0.13 at the bottom and top of the transect, respectively (Figures 5.4D and 5.4G). The ERI will significantly decrease at elevations between 1,000 and 1,100 m asl (1,000 m asl: $W = 210$, $P < 0.0001$; 1,200 m asl: $W = 206$, $P < 0.0001$), it will remain unchanged between 1,200 and 1,400 m asl, and will significantly increase between 1,500 and 1,700 m asl ($W = 210$, $P < 0.0001$ for 1,500, 1,600 and 1,700 m asl) (Figure 5.5A). The GI will change with differences between -0.19 and 0.94 from the bottom to the top of the transect (Figures 5.4E and 5.4H). It will remain unchanged at elevation around 1,000 m asl ($W = 138$, $P = 0.1089$) and will significantly increase for elevations between 1,100 and 1,700 m asl (1,100 m asl: $W = 202$, $P = 0.0001$; 1,200 m asl: $W = 210$, $P < 0.0001$; 1,300 m asl: $W = 171$, $P = 0.0068$; 1,400 m asl: $W = 203$, $P = 0.0001$; 1,500 m asl: $W = 209$, $P < 0.0001$; 1,600 and 1,700 m asl: $W = 210$, $P < 0.0001$) (Figure 5.5B). The AI will change in a range of -0.37-1.33 from the bottom to the top of the transect (Figures 5.4F and 5.4I). The AI will decrease at elevations of 1,000 and 1,100 m asl (1,000 m asl: $W = 162$, $P = 0.0166$; 1,100 m asl: $W = 160$, $P = 0.0200$) and will increase significantly for elevations between 1,200 and 1,700 m asl (1,200 m asl: $W = 201$, $P < 0.0001$; 1,300 m asl: $W = 169$, $P = 0.0084$; 1,400 m asl: $W = 203$, $P = 0.0001$; 1,500 m asl: $W = 210$, $P < 0.0001$; 1,600 m asl: $W = 210$, $P < 0.0001$; 1,700 m asl: $W = 210$, $P < 0.0001$) (Figure 5.5C).

Table 5.1 Predicted change in minimum and maximum temperatures (mean ± SD) between current (2013) and future (2055) climatic conditions on selected locations along the Kilimanjaro transect. The current temperatures were recorded using iButtons Hygrochron data loggers in the selected locations and future temperatures obtained from AFRICLIM 3.0 climatic projections of RCP 4.5 scenario.

Elevation (m)	**Coordinate (°)**		**Minimum temperatures (°C)**			**Maximum temperatures (°C)**		
	Longitude	Latitude	Current	Future	Difference	Current	Future	Difference
1081	37.461845	-3.357280	17.18±1.29	17.28±1.23	0.10±0.74	28.59±3.47	30.42±2.56	1.83±1.20
1249	37.474743	-3.341794	16.67±1.31	16.78±1.18	0.11±0.28	29.53±3.72	29.63±2.39	0.10±1.43
1419	37.469891	-3.327607	15.31±1.48	15.58±1.14	0.27±0.52	27.41±2.78	27.98±2.38	0.57±0.84
1533	37.465210	-3.312971	14.60±1.44	14.94±1.29	0.34±0.42	26.58±4.20	27.34±2.32	0.76±2.91
1705	37.456239	-3.286082	13.85±1.44	14.02±1.34	0.17±0.56	25.58±3.29	26.34±2.14	0.76±1.50

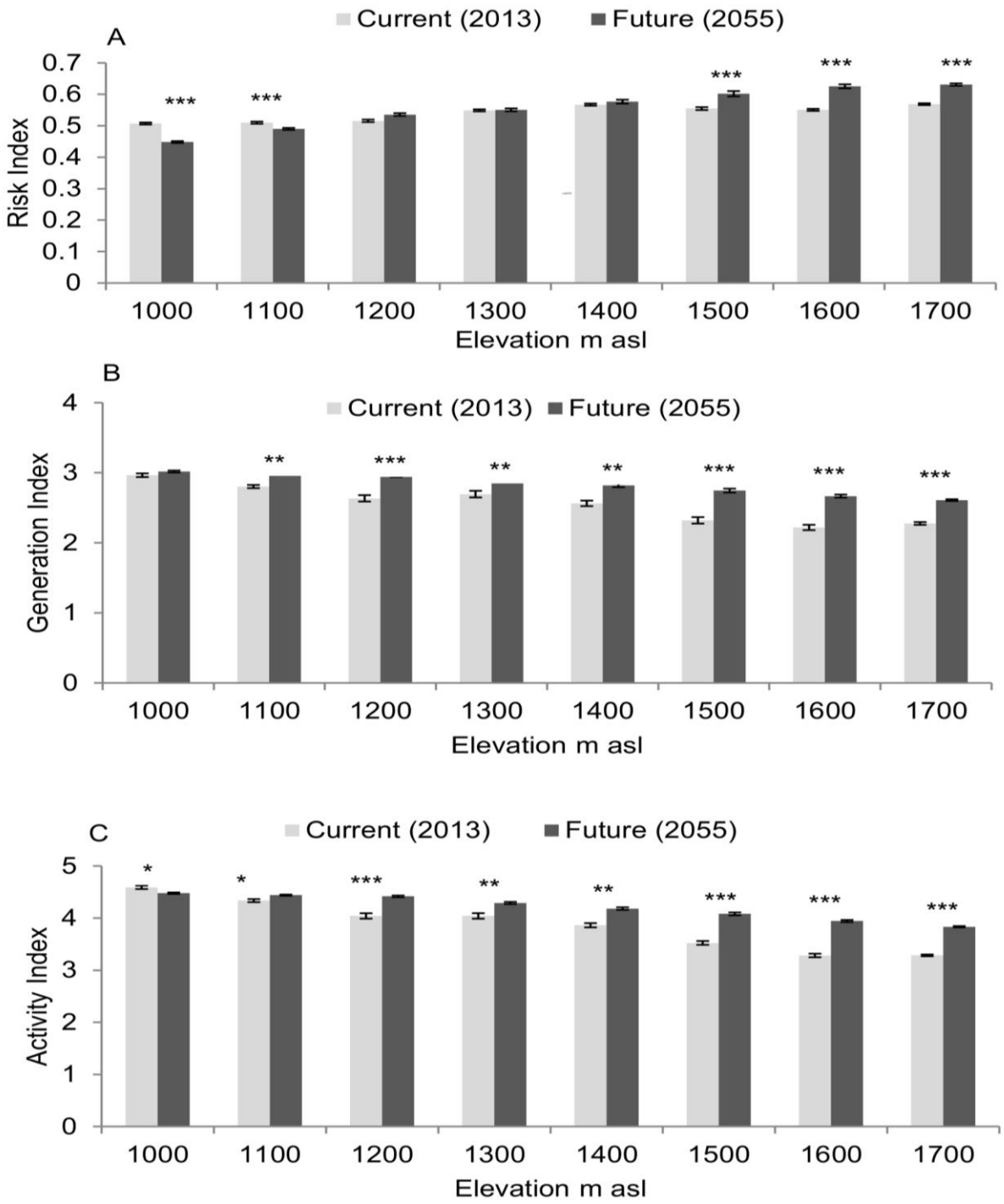


Figure 5.5 Change in risk indices for *A. thunbergii* populations of Mt. Kilimanjaro transect between current (2013) and future (2055) temperature conditions, plotted against elevations. (A) establishment risk index (ERI), (B) generation index (GI), (C) activity index (AI). * = $P < 0.05$, ** = $P < 0.001$, *** = $P < 0.0001$.

Discussion

Impact of elevation on population density from field observations

In the last decades, generalized linear models (GLMs) have been widely used in ecological studies because of their ability to deal with different error structures associated with occurrence data (Guisan et al., 2002). Also, they are more flexible and better suited to analysing ecological relationships such as the relationship between insect distribution and elevation (Guisan et al., 2002). In our study Poisson regression well predicted the relationship between *A. thunbergii* populations and elevation. Our results showed that *A. thunbergii* was present at all elevations of the study area with the highest population densities at the highest elevations, whatever the climatic seasons. Our results confirm those of previous studies reporting that *A. thunbergii* is present in coffee at elevations between 1,000 and 2,100 m asl, with a preference for the highest elevations, especially for the subspecies *A. thunbergii bechuana* (Greathead, 1966; Babin et al., 2018). In our study, a mean density of 1 to 1.5 bug per tree was reported for elevation between 1,600 and 1,700 m asl across the different climatic seasons. In most countries of East Africa, this density is considered as an economic threshold beyond which an intervention for controlling the pest is required (Cilas et al., 1998; Bigirimana et al., 2012; Mugo et al., 2013). Maximum density we obtained was a bit more than 3 bugs per tree at around 1,630 m asl. This is rather low when compared to maximal densities of 45 bugs per tree obtained for the subspecies *A. thunbergii ghesquierei* in Rwanda by Foucart and Brion (1959). Evaluation method used in that study was the "pyrethrum tests", a knock-down technique based on pyrethrum spraying. In our study, visual counts on coffee trees may have led to underestimated densities, since the bugs tend to hide between berries or on the underside

of the leaves. However, our results also suggest that densities of *A. thunbergii* could have been higher on the transect at elevation above 1700 m asl if coffee had been present.

In our study, high variation in *A. thunbergii* density was observed even between farms located at similar elevations. This indicates that, besides elevation, other crucial factors were involved in the infestation level. Some ecological traits of *A. thunbergii* suggest that the bug prefers cool environments; populations are usually more abundant in bushy coffee trees and in shaded plantations, especially at medium and low elevation (Kirkpatrick, 1937; Mugo et al., 2013). Our study area is characterised by agrosystems known as Chagga home gardens, where vegetation usually develops in four layers: big trees for fruits, wood and shade, banana trees, coffee trees, and maize and/or vegetables at ground level (Hemp, 2006). Such complex systems lead to a wide range of shade and microclimate conditions, which may explain the variation we obtained in the bug density for similar elevations.

Our results also showed that *A. thunbergii* populations varied between seasons, with the highest populations in June, during the coolest period of the year. During our field surveys, additional observations revealed that all the development stages, from eggs to adults were present on trees during this period, whereas eggs were not observed during the warmer seasons. These results are consistent with report from Van der Meulen and Schoeman (1990), who recorded higher populations in the cold season, for the same species in South Africa. The authors suggested that in addition to lower temperatures, coffee fruiting cycle might have been involved in the bug seasonal variations. More specifically, the presence of green berries on trees might have favoured immature stage survival and development. This explanation may be valid in our study since coffee trees

bore developing green berries in June, whereas from October to December, berries ripened and were harvested (personal observation).

Modelling infestation risk under current temperature

Several bioclimatic models such as BIOCLIM, CLIMEX and MaxEnt (Busby, 1991; Phillips, 2005; Sutherst et al., 2007), have been adapted for predicting the distribution and abundance of insect species, based on field and/or laboratory data (Beaumont et al., 2005; Jaramillo et al., 2011; Booth et al., 2014; Fand et al., 2014). These models use occurrence points of the species to predict, based on bioclimatic variables, its distribution over a broad geographic area (for example, at global, continental or regional scale). However, in our study, we used ILCYM software to predict the distribution of *A. thunbergii* at a local scale, namely over an elevation transect of a few dozen square kilometres. ILCYM software uses a deductive approach of modelling, incorporating the detailed knowledge of the pest life history from laboratory experiment to predict where the species can occur. Also, the software is able to predict the pest distribution at a very small geographical scale, which provides efficient information for risk mitigation strategies that need to be implemented at local scale. Nevertheless, the main limitation of ILCYM is that the software uses only temperature variable to predict the pest distribution and does not incorporate other climatic variables such as rainfall and relative humidity. Future versions of the software should consider such variables to improve prediction accuracy.

The first index, the ERI, characterises the area suitable for the survival and establishment of an insect. The ERI is computed based on survival of immature stages and has a range of 0 to 1. In our study, the ERI for current temperature ranged from 0.47 to 0.59. This

result suggests that *A. thunbergii* immature stages are able to survive all over the transect for half the year, at least. This result also indicates that the bug distribution may not be limited to elevation range of the transect. In fact, the relationship we observed between the ERI and elevation suggests that areas above 1,700 m may be even more favourable for *A. thunbergii* establishment than the areas covered by the transect. Such assertion is supported by previous studies reporting that *A. thunbergii* is more common in Arabica coffee at the highest elevations that can reach 2,100 m asl in East Africa (Greathead, 1966).

In the study by Azrag et al. (2017), the temperature-dependent mortality models showed that the constant temperature range of 22-24°C was optimal for the survival of all *A. thunbergii* immature stages. This range matches the mean annual temperatures for the elevations between 1000 and 1300 m asl, where the ERI was the lowest in our study. This contradictory result is linked to the fact that the ERI calculation uses daily minimum and maximum temperatures. Thus, extreme daily maximum temperatures observed at the lowest elevations of the transect may be lethal for the bug immature stages, leading to low ERI. This is supported by the study of Ahmed et al. (2016), who recorded a mortality rate of 89 % at 30°C for immature stages of the bug.

The second index, the GI, estimates the mean number of generations per year. The GI is computed based on the generation time (*T*) and does not take survival into account. In our study, the GI decrease with increasing elevation is linked to the relationship between insect development time and temperature. For temperatures allowing survival of at least some individuals, an increase in temperature invariably shortens insect development, as demonstrated by Azrag et al. (2017) at constant temperatures for *A. thunbergii* in a

temperature range of 18-32°C. In our study, GI ranged 1.9-3.2 generations per year from the top to the bottom of the transect. When maintained at constant temperature, the bug was able to have 2.6 generations at 19 °C to 4.4 generations at 25°C generations per year (Azrag et al., 2017). The field study by Van der Meulen and Schoeman (1990) reported up to 4 overlapping generations per year in South Africa. However, our results suggest that in our study area, it is unlikely that *A. thunbergiii* populations would be able to reach 4 generations per year considering the low risk of establishement at low elevation.

The third index, the AI, indicates the potential population growth and is an indicator of the pest severity and spread risk. The AI is computed based on the finite rate of increase (λ), which is the exponential of the intrinsic rate of increase (r_m). As such, the AI takes the whole life cycle of the pest into account. Our predictions revealed that the AI distribution was very similar to that of the GI with a decrease in values from the top to the bottom of the transect. This can be explained by the results obtained by Azrag et al. (2017) for simulated life table parameters. In this study, intrinsic rate of increase and generation time varied in an opposite trend with an increase of temperature in the range 18-23°C. This range matches with temperature range of the transect. It is then consistent that the AI and the GI showed similar distribution on the transect. Also, results from Azrag et al. (2017) suggest that the AI reached its highest value in the bottom of the transect and should have decreased for lower elevations, contrary to the GI. In our study, the AI ranged 2.94-4.84 from the top to the bottom of the transect. According to AI formula (Kroschel et al., 2013), these values mean that the bug population may potentially grow by a factor of ≈ 870 at the top and ≈ 69,200 at the bottom of the transect, if temperature was the only factor involved in population variation.

Comparison between risk indices and current distribution of the pest

In this study, the ERI and the pest populations had similar distribution and a close relationship to elevation on the transect. By contrast, the GI and the AI indicate an opposite relationship with elevation. The ERI is therefore the best index to predict *A. thunbergii* distribution at a local scale. Similar results have been reported in similar studies conducted on other pests at world scale, like the potato tuber moth *Phthorimaea operculella* (Zeller) (Kroschel et al., 2013) or fruit flies *Ceratitis rosa* Karsch and *Ceratitis quilicii* De Meyer (Tanga et al., 2018). In addition, our results suggest that immature stage survival is a crucial parameter of the pest distribution. Immature stage susceptibility to high temperature that may occur during the hottest hours of the day may be one of the main factors preventing the pest from establishing in plantations at low elevation. This is supported by the fact that *A. thunbergii* adopts behaviours aiming at avoiding highest temperatures: the pest is usually more active in the morning and in the evening and avoids direct sunlight during the day by hiding in the leaf cover or between berries (Babin et al., 2018). Susceptibility to desiccation, which is also linked to sunlight exposure, cannot be ignored, and more studies are needed to understand the impact of moisture on antestia bug development.

In our study, *A. thunbergii* was found all over the transect for the three different seasons. Clearly populations were small in the bottom of the transect, but some individuals were recorded around 1200 m asl even during the warm season which is not favourable. This suggests a permanent establishment of the bug throughout the year on the transect. A permanent establishment should match with an ERI close to 1. However, in our study, the ERI ranged 0.47-0.59. This contradictory result has already been reported in other studies

for other pests at world scale, where the ERI value for permanent establishment was either > 0.6 (Tanga et al., 2018) or > 0.8 (Kroschel et al., 2013). In our study, it seems that an ERI > 0.5 is enough to illustrate a permanent establishment of *A. thunbergii*. As already mentioned above, highly diversified coffee systems in our study area lead to a large variety of microclimates and it is likely that the bug finds favourable temperature conditions even at the lowest elevations. Another disputable result in our study is the mismatch between the high AI values we obtained, illustrating high potential growth rates for the bug populations and low densities globally observed in coffee. The issue of the counting method has already been mentioned but factors other than temperature were undoubtedly involved in the limitation of the bug population. The most important limiting factor for antestia bugs is probably the pressure of natural enemies, especially egg parasitoids. Most field surveys reported egg parasitism rates in the range of 40-95 % and rates exceeding 80% were not rare (Babin et al., 2018). An interesting study may be to assess the impact of temperature on egg parasitoid development.

Change in risk indices with temperature increase

Our predictions showed that by the year 2055, *A. thunbergii* will still be present in Arabica coffee plantations along the study area. However, the infestation risk will change with rising temperature. Unsurprisingly, at low elevation an increase in temperature will lead to a decrease in the ERI. This decrease is estimated at 4-12% at elevations between 1,000 and 1,100 m asl. A more surprising result in our study is the ERI increase at the top of the transect, which is estimated at 8-14% for elevation between 1,500 and 1,700 m asl. One of the reasons may be the reduction of periods of the year with low temperatures

lethal for *A. thunbergii* immature stages. Azrag et al. (2017) recorded high mortality rates for the bug nymphs, especially between the 2nd and 5th stages, for constant temperatures between 16 and 18°C. However, this result should be considered with caution since current and future temperatures are not measured the same way in our study.

As mentioned above, the GI reflects the impact of temperature on insect development time and does not take mortality into account. It is therefore not surprising that the GI is expected to increase all over the transect with rising temperature. By contrast, the AI is expected to decrease at the bottom of the transect. This result confirms our assumption that, for current temperature, the AI reached its highest value at the lowest elevations. An increase in temperature may therefore lead to a shift of the AI highest values from low to higher elevation.

Globally, our results suggest that an increase in the risk of infestation by *A. thunbergii* is expected for coffee at high elevations. Several studies showed that rising temperatures will highly influence coffee production in eastern Africa. For example, in Tanzania, suitable areas for coffee growing are expected to shift from 1,000-2,000 m asl to 1,300-2,300 m asl due to temperature increase of 1.9-2.6°C (Hemp, 2006; Davis et al., 2012; Craparo et al., 2015; Ovalle-Rivera et al., 2015). Therefore, a reduction in antestia bug infestation at low elevations as demonstrated in our study will have limited consequences for coffee production in the future. By contrast, our results suggest that *A. thunbergii* will be able to follow the shift in coffee production area and will threaten renowned Arabica coffee at the highest elevations, as it does today.

Conclusions and recommendations

In conclusion, the temperature-based models and related risk indices we used in this study allowed the prediction of *A. thunbergii* distribution as impacted by elevation. Immature stage susceptibility to extreme temperatures has proven to be a crucial factor limiting population growth. Other biophysical factors, such as shade, farmer practices, moisture, coffee phenology or natural enemies are undoubtedly involved in the pest population dynamics. These factors could explain the high variation in *A. thunbergii* populations we obtained from field observations. Therefore, we recommend further field studies to elucidate the relationships between these factors and *A. thunbergii* population dynamics. Such understanding would help refine predicting models for this major pest of coffee.

In the future, infestation by the bug will remain high at high elevation and the bug will be able to follow the shift of coffee areas to higher elevations. Coffee farmers of east Africa highlands have to be ready for that. Our results strengthen one of the current recommendations for the control of antestia bug: a good pruning of the coffee bushes. Good pruning practices enhance producing branches exposure to sun and improve productivity. In addition, these practices are detrimental for antestia bug development because they expose the pest to extreme temperature conditions, which can be lethal, especially for immature stages. Shading coffee is a valid recommendation to mitigate temperature increase and control some pests that prefer warmer conditions, like the coffee berry borer. For antestia bug, shade management recommendations should take elevation into account, since they can have opposite effect on pest infestation depending on the global climate of the target area.

Acknowledgments

We thank Dr. James Odanga for providing the current climatic data and all the staff of the coffee pest project at the International Centre of Insect Physiology and Ecology, especially Ephantus Guandaru and Dickson Mwenda for technical support in the laboratory. We are also grateful to the coffee farmers from the study area for allowing us to access their farm for surveys. Permission to carry research (surveys and insect field collections) by the "Climate Change Impacts of Ecosystem Services and Food Security in Eastern Africa" (CHIESA) project was granted by the Regional Administrative Secretary Kilimanjaro under reference FA/191/228/01/61.

References

Abebe, M. (1987). Insect pests of coffee with special emphasis on antestia, *Antestiopsis intricata*, in Ethiopia. *International Journal of Tropical Insect Science*, *8*, 977–980.

Ahmed, A. G., Murungi, L. K., & Babin, R. (2016). Developmental biology and demographic parameters of antestia bug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae), on *Coffea arabica* (Rubiaceae) at different constant temperatures. *International Journal of Tropical Insect Science*, *36*, 119–127.

Azrag, A. G., Murungi, L. K., Tonnang, H. E., Mwenda, D., & Babin, R. (2017). Temperature-dependent models of development and survival of an insect pest of African tropical highlands, the coffee antestia bug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae). *Journal of Thermal Biology*, *70*, 27–36.

Babin, R., Mbondji Mbondji, P., Mendesil, E., Mugo, H. M., Lee, J-H., Serracin, M., Rukazambuga, N. D. T. M., & Miller, T. A. (2018). The antestia bug complex in Africa and Asia. In: McPherson, J. E, editor. Invasive stink bugs and related species (Pentatomoidea): Biology, higher systematics, semiochemistry, and management. *CRC Press*. USA. pp. 465–493.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., & Good, J. E. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, *8*, 1–16.

Beaumont, L. J., Hughes, L., & Poulsen, M. (2005). Predicting species distributions: use of climatic parameters in BIOCLIM and its impact on predictions of species' current and future distributions. *Ecological Modelling*, *186*, 250–269.

Bigirimana, J., Njoroge, K., Gahakwa, D., & Phiri, N. A. (2012). Incidence and severity of coffee leaf rust and other coffee pests and diseases in Rwanda. *African Journal of Agricultural Research*, *7*, 3847–3852.

Booth, T. H., Nix, H. A., Busby, J. R., & Hutchinson, M. F. (2014). BIOCLIM: the first species distribution modelling package, its early applications and relevance to most current MAXENT studies. *Diversity and Distributions*, *20*, 1–9.

Busby, J. R. (1991). BIOCLIM–A bioclimatic analysis and predictive system. In: Margules, C., Austin, M, editors. Nature conservation: cost effective biological surveys and data analysis. *CSIRO*. Australia. pp. 64–68

Cilas, C., Bouyjou, B., & Decazy, B. (1998). Frequency and distribution of *Antestiopsis orbitalis* Westwood (Hem., Pentatomidae) in coffee plantations in Burundi: implications for sampling techniques. *Journal of Applied Entomology*, *122*, 601–606.

Cilas, C., Goebel, F., Babin, R., & Avelino, J. (2016). Tropical crop pests and diseases in a climate change setting—a few examples. In: Torquebiau, E, editor. Climate Change and Agriculture Worldwide. *Springer*. Netherlands. pp. 73–82.

Craparo, A. C. W., Van Asten, P. J. A., Läderach, P., Jassogne, L. T. P., & Grab, S. W. (2015). *Coffea arabica* yields decline in Tanzania due to climate change: global implications. *Agricultural and Forest Meteorology*, *207*, 1–10.

Davis, A. P., Gole, T. W., Baena, S., & Moat, J. (2012). The impact of climate change on indigenous arabica coffee (*Coffea arabica*): predicting future trends and identifying priorities. *PloS One, 7*, e47981.

Estay, S. A., Lima, M., & Labra, F. A. (2009). Predicting insect pest status under climate change scenarios: combining experimental data and population dynamics modelling. *Journal of Applied Entomology, 133,* 491–499.

Fand, B. B., Tonnang, H. E., Kumar, M., Bal, S. K., Singh, N. P., Rao, D. V. K. N., Kamble, A. L., Nangare, D. D., & Minhas, P.S. (2014). Predicting the impact of climate change on regional and seasonal abundance of the mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) using temperature-driven phenology model linked to GIS. *Ecological Modelling, 288,* 62–78.

Foucart, G., & Brion, L. (1959) Contribution à l´e´tude de la punaise du cafe´ier Arabica au Rwanda-Urundi. *Rwanda Agricultural Research Institute (ISAR)*. Rwanda.

Gichuhi, J. M., Ndegwa, P. N., Mugo, H. M., Guandaru, E. K., & Babin, R. (2017). Rearing method and developmental biology of the African coffee white stem borer, *Monochamus leuconotus* (Coleoptera: Cerambycidae). *Journal of Economic Entomology, 110,* 1120–1126.

Greathead, D. J. (1966). A taxonomic study of the species of *Antestiopsis* (Hemiptera, Pentatomidae) associated with *Coffea arabica* in Africa. *Bulletin of Entomological Research,* 56, 515–554.

Gueule, D., Fourny, G., Ageron, E., Le Fleche-Mateos, A., Vandenbogaert, M., Grimont, P. A., & Cilas, C. (2015). *Pantoea coffeiphila* sp. nov., cause of the ‘potato

taste'of arabica coffee from the African great lakes region. *International Journal of Systematic and Evolutionary Microbiology*, *65*, 23–29.

Guisan, A., Edwards, T. C, J., & Hastie, T. (2002). Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecological Modelling*, *157*, 89–100.

Hemp, A. (2006). The banana forests of Kilimanjaro: biodiversity and conservation of the Chagga homegardens. *Biodiversity and Conservation*, *15*, 1193–1217.

Hijmans, R. J., van Etten, J., Cheng, J., Mattiuzzi, M., Sumner, M., Greenberg, J. A., Lamigueiro, O. P., Bevan, A., Racine, E.B., Shortridge, A., & Hijmans, M. R. J. (2015). Package 'raster'. *R package*. ftp://slartibardfast.gtlib.gatech.edu/pub/CRAN/web/packages/raster/raster.pdf.

Jaramillo, J., Muchugu, E., Vega, F. E., Davis, A., Borgemeister, C., & Chabi-Olaye, A. (2011). Some like it hot: the influence and implications of climate change on coffee berry borer (*Hypothenemus hampei*) and coffee production in East Africa. *PloS One*, *6*, e24528.

Kirkpatrick, T. W. (1937). Studies on the ecology of coffee plantations in East Africa. II. The autecology of *Antestia* spp. (Pentatomidae) with a particular account of a strepsipterous parasite. *Transactions of the Royal entomological Society of London*, *86*, 247–343.

Kroschel, J., Sporleder, M., Tonnang, H. E., Juarez, H., Carhuapoma, P., Gonzales, J. C., & Simon, R. (2013). Predicting climate-change-caused changes in global temperature on potato tuber moth *Phthorimaea operculella* (Zeller) distribution

and abundance using phenology modeling and GIS mapping. *Agricultural and Forest Meteorology*, *170*, 228–241.

Matsuura, Y., Hosokawa, T., Serracin, M., Tulgetske, G. M., Miller, T. A., & Fukatsu, T. (2014). Bacterial symbionts of a devastating coffee pest, the stinkbug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae). *Applied and Environmental Microbiology*, *80*, 3769–3775.

McNutt, D. (1979). Control of *Antestiopsis* spp. on coffee in Uganda. *Proceedings of the National Academy of Sciences of the United States of America*, *25*, 5–15.

Mugo, H. M., Kimemia, J. K., & Mwangi, J. M. (2013). Severity of antestia bugs, *Antestiopsis* spp. and other key insect pests under shaded coffee in Kenya. *International Journal of Science and Nature*, *4*, 324–327.

Mwalusepo, S., Massawe, E., & Johansson, T. P. (2016). Spatially continuous dataset at local scale of taita hills in Kenya and Mount Kilimanjaro in Tanzania. *Data in Brief*, *8*, 1115–1119

Mwalusepo, S., Tonnang, H. E., Massawe, E. S., Okuku, G. O., Khadioli, N., Johansson, T., Calatayud, P. A., & Le Ru, B. P. (2015). Predicting the impact of temperature change on the future distribution of maize stem borers and their natural enemies along east African mountain gradients using phenology models. *PloS One*, *10*, e0130427.

Ovalle-Rivera, O., Läderach, P., Bunn, C., Obersteiner, M., & Schroth, G. (2015). Projected shifts in *Coffea arabica* suitability among major global producing regions due to climate change. *PloS One*, *10*, e0124155.

Phillips, S. J. (2005). A brief tutorial on Maxent. *AT&T Research*.https://biodiversityinformatics.amnh.org/open_source/maxent/Maxent_tutorial2017.pdf.

Platts, P. J., Omeny, P. A., & Marchant, R. (2015). AFRICLIM: high-resolution climate projections for ecological applications in Africa. *African Journal of Ecology*, *53*, 103–108.

R Development Core Team. (2016). R: A language and environment for statistical computing. Rfoundation for statistical computing, Vienna, Austria. https://www.R-project.org/.

Ribeyre, F., & Avelino, J. (2012). Impact of field pests and diseases on coffee quality. In: Oberthür, T., Läderach, P., Pohlan, H.A.J., & Cock, J. H, editors. Specialty coffee: managing coffee. *International Plant Nutrition Institute*. Penang, Malaysia. pp. 151–176.

Scrucca, L. (2018). Package 'dispmod'. *R package*. http://brieger.esalq.usp.br/CRAN/web/packages/dispmod/dispmod.pdf.

Sutherst, R. W., Maywald, G. F., & Kriticos, D. J. (2007). CLIMEX version 3: user's guide. https://www.hearne.software/getattachment/0343c9d5-999f-4880-b9b2.1c3eea908f08/ClimexUser-Guide.aspx.

Tanga, C. M., Khamis, F. M., Tonnang, H. E., Rwomushana, I., Mosomtai, G., Mohamed, S. A., & Ekesi, S. (2018). Risk assessment and spread of the potentially invasive *Ceratitis rosa* Karsch and *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio sp. Nov. using life-cycle simulation models: implications for phytosanitary measures and management. *PloS One*, *13*, e0189138.

Thomson, A. M., Calvin, K. V., Smith, S. J., Kyle, G. P., Volke, A., Patel, P., Delgado-Arias, S., Bond-Lamberty, B., Wise, M. A., Clarke, L. E., & Edmonds, J. A. (2011). RCP4. 5: a pathway for stabilization of radiative forcing by 2100. *Climatic Change*, *109*, 77–94.

Tonnang, H. E., Juarez, H., Carhuapoma, P., Gonzales, J. C., Mendoza, D., Sporleder, M., Simon, R., & Kroschel, J. (2013). ILCYM – Insect Life Cycle Modeling. A software package for developing temperature-based insect phenology models with applications for local, regional and global analysis of insect population and mapping. *International Potato Center*. Lima, Peru. p. 193.

van der Meulen, H. J., & Schoeman, A. S. (1990). Aspects of the phenology and ecology of the antestia stink bug, *Antestiopsis orbitalis orbitalis* (Hemiptera: Pentatomidae), a pest of coffee. *Phytophylactica*, *22*, 423–426.

Zehnder, C. B., Stodola, K. W., Joyce, B. L., Egetter, D., Cooper, R. J., & Hunter, M. D. (2009). Elevational and seasonal variation in the foliar quality and arthropod community of *Acer pensylvanicum*. *Environmental Entomology*, *38*, 1161–1167.

CHAPTER SIX

General conclusions

Preceding chapters in this thesis have described the distribution and life history traits of three major insect pests of Arabica coffee in East Africa, namely, the Antestia bugs, *Antestiopsis* spp., the coffee berry borer, *Hypothenemus hampei* and the African coffee white stem borer, *Monochamus leuconotus*. Despite their significant effect on coffee production (Tapley, 1960; Jaramillo et al., 2009; Azrag et al., 2017), it appears that the recommended control measures against these pests are not effective enough to limit the damages they cause on coffee plantations. This might be a consequence of the insufficient knowledge on the biology and ecology of these pests, which were poorly documented in the past. Developing control measures against coffee pests in the future needs to incorporate the life history traits and ecology in order to result in effective control strategies adapted to specific agro-ecological zones of coffee growing in east Africa. Thus, an extra effort is needed in order to better understand the biology, ecology, and future distribution of these pests. It is in order to contribute to this effort that the work presented in this thesis was conducted. This section summarises the key findings from all chapters by bringing together the life history traits, seasonal variation and factors that affect the populations, and future potential distribution of the three pests, and highlights research areas for the future.

Findings from chapter two described the relationship between temperature, and *H. hampei* life history parameters, and provided the thermal thresholds for the pest's development. For the first time, the complete life cycle of *H. hampei*, from egg to adult,

was monitored by direct observation, using a novel observation method. The results showed a complete development at temperatures between 18 and 30°C. Linear and nonlinear models revealed that all immature stages of *H. hampei* could develop at temperature ranges of 13-32°C. Wang 2 function predicted that 23°C is an optimum temperature for all immature stage survival. This temperature is similar to the mean annual temperatures for elevations between ≈1,100-1,400 m asl, where *H. hampei* causes the highest damage to Arabica coffee in east Africa under present climate conditions (Jaramillo et al., 2011). Considering the impact of global warming, shifting ranges by *H. hampei* populations to higher elevations are expected by the year 2050, with an increase in the number of generations per year (Jaramillo et al., 2009; 2011). However, the relationships between *H. hampei's* distribution and elevation, with the temperature being that main factor was not clearly elucidated. Understanding this relationship could help to develop control strategies adapted to specific agro-ecological zones where coffee is grown. Therefore, findings from chapter two with the incorporation of oviposition model and validation under fluctuating temperatures can help better understand the distribution of *H. hampei* over an elevation gradient and predict the potential areas for its future establishment.

For the first time, the complete life cycle of *M. leuconotus* was studied and the population growth parameters were simulated at different constant temperatures. The egg incubation period ranged between 10.8 and 44.8 days in the temperature range of 15-30°C. However, the results showed that the larva stage needs at least one year to complete its development to pupa at temperatures below 25°C. This was shorter than findings of Tapley (1960), who reported approximately two years (20 months) for the larval development under field

conditions. Linear and nonlinear functions predicted a wide range of temperature (10-40°C) for the *M. leuconotus* development, with 23-23.9°C being the optimum temperatures for immature survival. Linking this to the field infestation in east Africa, it has been reported that *M. leuconotus* infestation is higher in full shaded coffee plantations at elevations between 1,500 and 1,800 m asl (Jonsson et al., 2015). At these elevations, the average temperature is lower than 23°C, especially in full shaded coffee plantations. Thus, the reason behind the high infestation of *M. leuconotus* in shaded plantations is not clear. The life table parameters showed that the highest population growth of *M. leuconotus* occurs at temperatures between 26 and 28°C. Thus, the rising in temperature might increase the infestation of this pest by shortening its life cycle, which may result in potential population increase. Therefore, the results presented here can help better understand the life history of *M. leuconotus* and predict its distribution over the elevation gradient in the light of climate change.

To mitigate the effect of rising temperature on coffee plantations, growing coffee under shade has already become a widespread practice by coffee farmers (Beer et al., 1998; Albertin & Nair, 2004). Shade reduces the daily extreme temperatures on coffee trees, hence provides a suitable microclimate for the production of beans. However, findings from chapter four showed that high shade density influences the populations of antestia bug by increasing the infestation level of *A. thunbergii* and reducing those of *A. facetoides*. These results were aligned with a previous study by Mugo et al. (2013), who reported high infestation level of *A. thunbergii* in shaded coffee compared to full sun plantations on the slope of Mt. Elgon, Kenya. Providing coffee with shade trees will still be a valid recommendation to mitigate the negative effect of climate change. High shade

can be applied especially in areas such as the Aberdare range, in Kenya, where *A. facetoides* and *H. hampei* are the main problems in coffee production beside the rising temperature. However, to minimise infestation risk by *A. thunbergii*, findings of chapter five recommend good pruning practices that improve coffee productivity and expose *A. thunbergii* immature stages to extreme temperatures that can be lethal. Also, coffee pruning offers favourable conditions for parasitoids of *A. thunbergii* by favouring access to different developmental stages of the pest, especially eggs (Mugo et al., 2013; Babin et al., 2018).

Despite the positive and negative effects of shade on *A. thunbergii* and *A. facetoides*, repectively, the seasonal variation of both species in coffee plantations had a similar trend with a population peak between July and August. Understanding this dynamic could help farmers to target both species in coffee plantations at the same time, especially when applying control measures such as insecticide sprayings. A number of studies (Kirkpatrick, 1937; van der Meulen & Schoeman, 1990; Azrag et al., 2017) pointed out that food availability could play a more significant role in the ecology and population dynamics of *A. thunbergii* in comparison to climatic factors such as temperature. This was proven by findings of chapter four which reveal that the availability of the green berries on coffee trees was the key factor explaining the high level of populations of both species. Interestingly, when the green berries became ripe, the populations of *A. thunbergii* and *A. thunbergii* decreased in coffee farms. Thus, supporting the findings of Njihia et al. (2017; 2018) that showed that the responses of second instar nymphs of *A. thunbergii* are attracted to odours from mature green berries but avoided odours from

riped ones. These two findings provide trapping and prevention tools to control antestia bugs in coffee plantations.

Chapter five of this thesis highlighted current and future distribution of *Antestiopsis thunbergii* as influenced by elevation in order to assess the risk under climate warming. Findings showed that population density of *A. thunbergii* was higher at higher elevations under current climate conditions, a prove that this pest prefers cooler habitats. This was in agreement with the report of Kirkpatrick (1937) who have shown that *A. thunbergii* mostly attacks coffee plantations at higher elevations. Although it is a tropical pest, the reason for its preference to cool habitats has eluded researchers. However, it has been demonstrated that, susceptibility of immature stages to extreme temperatures is one of the key factors that limit the population (Azrag et al., 2017), especially at lower elevations, where daily temperatures can be as high as 30°C. Population densities of *A. thunbergii* varied even on farms located at the same elevation that were sampled in the same season. The reasons behind this could be linked to the variation in shade intensity as highlighted in chapter four. Thus, farmers' practices and the phenology of coffee varieties need to be investigated further. In contrast, results from chapter four showed that *A. facetoides* have preferences for low elevations (below 1,600 m asl). This suggests a preference for warmer climate by *A. facetoides* compared to *A. thunbergii*. Since *A. thunbergii* and *A. facetoides* are two closely related species which sometimes co-occur together on the same plantations, future studies should focus on the thermal biology *A. facetoides* and investigate reason behind its preference for low elevations compared to *A. thunbergii*.

In the light of global warming, rising temperature will have a potential impact on the distribution of *A. thunbergii*. The infestation risk will be higher at the highest elevations

(1,500-1,800 m asl) in the future, with an increase of one generation making it possible for the *A. thunbergii* to have at least two generations in a year. In addition, the renowned Arabica coffee production will also be directly influenced by increases in temperature, which will result in yield reduction and shifting of suitable production areas to higher elevations (Davis et al., 2012; Ovalle-Rivera et al., 2015). It is possible that *A. thunbergii* will be able to follow the shift in coffee production areas and will continue to threaten the production of Arabica coffee at the highest elevations, as it does today. In the case of *H. hampei* the most devastating pest of coffee worldwide, it is expected to expand its range up to 1,800 m asl, as a result of rising temperature (Jaramillo et al., 2009; 2011). Taking all of these results into consideration, the potential threat to Arabica coffee production in east Africa is expected in the future especially at elevations between 1,500 and 1,800 m asl. Therefore, mitigation of the risk of climate change on coffee production is needed to minimise the pests risk.

In conclusion, this thesis brings valuable contributions to the understanding of how global warming will potentially affect *A. thunbergii* distribution and added to our knowledge of the life history traits of *H. hampei* and *M. leuconotus*. Thus, future research could focus on using these results to map the impact of climate change on these pests and developing control measures adapted for specific agro-ecological zones. Also, the effect of microclimate on the infection of coffee pests must be carried out in order to develop some efficient control strategies.

However, this thesis has some limitations: modelling the life history traits of insect species using ILCYM requires data quantity and quality. Collecting this data is often demanding and time consuming especially for insects that have a long-life cycle such as

M. leuconotus. In chapter two, deformation of *M. leuconotus* larvae, pupae and adults, as well as the high mortality rate of immature stages, was observed. This might be due to inbreeding in the colony that was used for the experiments. As a result, the number of females obtained for fecundity assessment at different constant temperatures were relatively low. Besides, the use of artificial diet accelerated the development time of immature stages compared to those observed in coffee wood. Therefore, the life table parameters obtained for *M. leuconotus* might not necessarily be a reflection of what happens in the field and thus should be considered with caution. Another limitation from this work is that the software (ILCYM) used to predict the future distribution of *A. thunbergii* considers temperature only as a factor that influences the distribution and abundance of the pest. This is because the software uses the life history of the insect species which is assessed through laboratory experiments in incubators and measuring other climatic factors such as relative humidity and rainfall for the insect development is rather difficult. The effects of these factors should be quantified and included in the model for to enhance its accuracy for the detection of *A. thunbergii*'s distribution and abundance under field conditions. Despite the homogeneity of coffee farms that were selected to assess the seasonal variation of antestia bugs and factors that affects their population dynamics, controlling farmers practices was challenging, especially pruning and pesticides applications that might have influenced the results obtained. In addition, shade level (low, moderate and high) on coffee farms was visually measured and this method has lower accuracy compared to other methods such as measuring the shade using leaf area index.

References

Albertin, A., & Nair, P. K. R. (2004). Farmers' perspectives on the role of shade trees in coffee production systems: An assessment from the Nicoya Peninsula, Costa Rica. *Human Ecology*, *32*, 443–463.

Azrag, A. G. A., Murungi, L. K., Tonnang, H. E., Mwenda, D., & Babin, R. (2017). Temperature-dependent models of development and survival of an insect pest of African tropical highlands, the coffee antestia bug *Antestiopsis thunbergii* (Hemiptera: Pentatomidae). *Journal of Thermal Biology*, *70*, 27–36.

Babin, R., Mbondji Mbondji, P., Mendesil, E., Mugo, H. M., Lee, J-H., Serracin, M., Rukazambuga, D.N.T., & Miller, T.A. (2018). The antestia bug complex in Africa and Asia. In: McPherson JE, editor. Invasive stink bugs and related species (Pentatomoidea): Biology, higher systematics, semiochemistry, and management. *CRC Press*. USA: pp. 465–493.

Beer, J., Muschler, R., Kass, D., & Somarriba, E. (1998). Shade management in coffee and cacao plantations. *Agroforestry Systems*, *38*, 139–164.

Davis, A. P., Gole, T. W., Baena, S., & Moat, J. (2012). The impact of climate change on indigenous arabica coffee (*Coffea arabica*): predicting future trends and identifying priorities. *PloS One*, *7*, e47981.

Jaramillo, J., Chabi-Olaye, A., Kamonjo, C., Jaramillo, A., Vega, F. E., Poehling, H. M., & Borgemeister, C. (2009). Thermal tolerance of the coffee berry borer *Hypothenemus hampei*: predictions of climate change impact on a tropical insect pest. *PloS One*, *4*, e6487.

Jaramillo, J., Muchugu, E., Vega, F. E., Davis, A., Borgemeister, C., & Chabi-Olaye, A. (2011). Some like it hot: the influence and implications of climate change on coffee berry borer (*Hypothenemus hampei*) and coffee production in East Africa. *PloS One*, *6*, e24528.

Jonsson, M., Raphael, I. A., Ekbom, B., Kyamanywa, S., & Karungi, J. (2015). Contrasting effects of shade level and altitude on two important coffee pests. *Journal of Pest Science*, *88*, 281–287.

Kirkpatrick, T. W. (1937). Studies on the ecology of coffee plantations in East Africa. II. The autecology of *Antestia* spp. (Pentatomidae) with a particular account of a strepsipterous parasite. *Transactions of the Royal Entomological Society of London*, *86*, 247–343.

Mugo, H. M., Kimemia, J. K., & Mwangi, J. M. (2013). Severity of antestia bugs, *Antestiopsis* spp. and other key insect pests under shaded coffee in Kenya. *International Journal of Science and Nature*, *4*, 324–327.

Njihia, T. N., Torto, B., Murungi, L. K., Irungu, J., Mwenda, D. M., & Babin, R. (2018). Ripe coffee berry volatiles repel second instar nymphs of Antestia bugs (Heteroptera: Pentatomidae: *Antestiopsis thunbergii*). *Chemoecology*, *28*, 91–100.

Njihia, T. N., Torto, B., Murungi, L. K., Irungu, J., Mwenda, D. M., & Babin, R. (2017). Identification of kairomones of second instar nymphs of the variegated coffee bug *Antestiopsis thunbergii* (Heteroptera: Pentatomidae). *Chemoecology*, *27*, 239–248.

Ovalle-Rivera, O., Läderach, P., Bunn, C., Obersteiner, M., & Schroth, G. (2015). Projected shifts in *Coffea arabica* suitability among major global producing regions due to climate change. *PloS One*, *10*, e0124155.

Tapley, R. G. (1960). The white coffee borer, *Anthores leuconotus* Pasc., and its control. *Bulletin of Entomological Research*, *51*, 279–301.

van der Meulen, H. J., & Schoeman, A. S. (1990). Aspects of the phenology and ecology of the antestia stink bug, *Antestiopsis orbitalis orbitalis* (Hemiptera: Pentatomidae), a pest of coffee. *Phytophylactica*, *22*, 423–426.