

**Impacts of temperature variation on performance, life-history and flight ability of the false codling moth,
Thaumatotibia leucotreta (Lepidoptera: Tortricidae)**

by

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DECLARATION

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SUMMARY

The sterile insect technique (SIT), the process of mass-rearing, sterilizing and releasing sterile insects, can be used to combat economically important pests by suppressing their population numbers as part of an integrated pest management programme. The success of SIT programmes depends upon the production of high-quality, competitive insects for field release. In SIT programmes, the influence of temperature variation during larval development and chilling during storage and their effects on the field performance of adult mass-reared insects are poorly understood but may be a significant avenue for increasing programme efficacy. The use of different temperatures to rear, handle and immobilise insects allows increased quantities of insects to be collected, handled, irradiated, transported and released. Unfortunately, the use of different temperature regimes in the rearing, storage, handling and shipping of insects have poorly understood impacts on the field performance of mass-reared insects. I mainly studied the impact of different developmental temperatures on larvae and treatment temperatures on adults, examining adult performance in the false codling moth *Thaumatotibia leucotreta* (Meyrick). After larvae were reared at 15, 20 or 25 °C for their full developmental period, the effect of different acute (2 h) temperature treatments (10, 15 or 20 °C) during the adult stage on traits of (i) cold tolerance, (ii) fecundity and (iii) longevity were determined. In addition, I assessed the flight performance of adults in both laboratory and field conditions after they were exposed to chilling (2 °C) for 16 h during the adult stage. The cold tolerance of adults was not influenced by larval acclimation temperature but was affected by sex and adult treatment temperature. Adult fecundity and longevity were affected by larval acclimation temperature, adult treatment temperature and the interaction of these factors with sex. In flight assays, adults exposed to 2 °C for 16 h performed better in colder environments, both in the laboratory and the field, than adults not subjected to pre-release cold treatment. The benefits of chilling for improved field recapture rates, however, depended on the specific ambient temperature upon release. These results suggest a complex, and in some cases sex-dependent, interplay of short- and longer-term temperature history across developmental stages for these traits. Further studies of how these and other traits might respond to artificial manipulation, coupled with information on how any induced trait variation impacts field performance, are essential for the SIT and pest management, with far-reaching implications for understanding thermal adaptation of ectotherms.

OPSOMMING

Die steriele insek tegniek (SIT), 'n proses waartydens insekte in massa geteel, gesteriliseer en vrygelaat word, kan aangewend word om pesbevolkings te beheer en peste van ekonomiese belang te beveg as deel van 'n geïntegreerde pesbeheer program. Die sukses van SIT-programme hang egter af van die produksie van insekte met goeie gehalte wat kan kompeteer met wilde insekte in die veld. Die invloed van temperatuurvariasies tydens larwale ontwikkeling en die effek van verkoeling tydens berging op die prestasie van volwasse insekte in die veld is onduidelik, maar kan gebruik word om die effektiwiteit van SIT-programme te verbeter. Motte word teen verskillende temperature geteel, hanteer en geïmmobiliseer om hul getalle vir versameling, hantering, irradiasi, vervoer en vrystelling te verhoog. Ongelukkig kan hierdie wisselende temperature tydens produksie, hantering en verskeping ook 'n ongekende effek op die prestasie van volwasse insekte vanuit 'n insektarium hê. Ek het hoofsaaklik die invloed van temperatuurbehandeling tydens ontwikkeling en volwasse fase op die volwasse kompeteerbaarheid en prestasie van die valskoddingmot *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) ondersoek. Larwes is teen 15, 20 en 25 °C vir die volledige ontwikkelingsperiode geteel, en die effek van akute (2 uur) temperatuurbehandelings (teen 10, 15 en 20 °C) tydens die volwasse stadium is daarna op i) kouetoleransie, ii) fekunditeit en iii) lanklewendheid bepaal. Verder het ek ook die effek van kouebehandeling (teen 2 °C vir 16 ure) op die vlugvermoë van volwasse insekte in die laboratorium en in die veld ondersoek.

Die kouetoleransie van volwassenes is hoofsaaklik deur geslag en volwasse temperatuur behandeling beïnvloed maar nie deur die ontwikkelingstemperatuur nie. Alhoewel die ontwikkelingstemperatuur en temperatuurbehandeling 'n beduidende effek op eierlegging en langlewendheid van die volwasse mot het, is die invloed van hierdie faktore afhanklik van die mot se geslag. Verder het lae-temperatuur behandeling (2 °C vir 16 uur) van volwasse motte 'n betekenisvolle hoër getal hervangste in beide die laboratorium en veld tydens koeler omgewingstoestande opgelewer in vergelyking met motte wat nie aan die kouebehandeling blootgestel was nie. Die verbetering in hervangstes van volwasse motte in die veld wat aan kouebehandeling bloot gestel was is afhanklik van die temperatuur waaraan die volwasse motte blootgestel word tydens loslaat in die veld. Die resultate dui op 'n komplekse geslagafhanklike wisselwerking tussen kort- en lang-termyn temperatuurgeskiedenis oor al die ontwikkeling stadiums van betrokke eienskappe wat die effektiwiteit van SIT mag beïnvloed. Verdere navorsing met betrekking tot die invloed van kunsmatige manipulasie asook informasie oor die effek van variasie op sekere eienskappe ten einde fiksheid van volwasse motte in die veld te verbeter is noodsaaklik om die effektiwiteit van SIT en

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PREFACE

This thesis is presented as a compilation of four chapters. Each chapter is introduced separately and Chapters 2 and 3 are written according to the style of the journals ***Agricultural and Forest Entomology*** to which they were submitted for publication.

- Chapter 1** General introduction and project aim
- Chapter 2** Published article
‘Sex-dependent thermal history influences cold tolerance, longevity and fecundity in false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae)’
- Chapter 3** Submitted article
‘Chilling enhances low-temperature flight performance in false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae)’
- Chapter 4** General discussion and conclusions

Table of contents

Declaration.....	i
Summary.....	ii
Opsomming	iii
Acknowledgements.....	iii
Preface	vi
List of Figureures.....	x
List of tables	xii
Chapter 1. General introduction	1
1.1. Thermal biology of insects	1
1.1.1. Phenotypic plasticity and thermal acclimation	2
1.1.2. Costs and benefits of thermal acclimation	4
1.1.3. Physiological mechanisms underlying thermal acclimation responses	5
1.2. Sterile insect technique	6
1.3. False codling moth (<i>Thaumatotibia leucotreta</i>).....	8
1.3.1. Biology of <i>Thaumatotibia leucotreta</i>	8
1.3.2. Temperature effects on <i>Thaumatotibia leucotreta</i>	10
1.3.3. <i>Thaumatotibia leucotreta</i> as a phytosanitary pest	12
1.3.4. Implementation of the sterile insect technique for <i>Thaumatotibia leucotreta</i>	13
1.3.5. Challenges encountered with sterile moths in a commercial sterile insect technique programme	14
1.4. Study aims	16
1.5. References	17
Chapter 2. Sex-dependent thermal history influences cold tolerance, longevity and fecundity in false codling moth, <i>Thaumatotibia leucotreta</i> (Lepidoptera:Tortricidae).....	26
2.1. Introduction.....	26
2.2. Materials and methods.....	29
2.2.1. Insect rearing.....	29
2.2.2. Acclimation (larval developmental temperatures)	29

2.2.3.	Moth temperature treatments.....	29
2.2.4.	Critical thermal minimum.....	29
2.2.5.	Fecundity.....	30
2.2.6.	Longevity	30
2.2.7.	Statistical analysis	30
2.3.	Results.....	31
2.3.1.	Cold tolerance	31
2.3.2.	Fecundity.....	33
2.3.3.	Longevity	35
2.4.	Discussion	37
2.5.	References	40
Chapter 3.	Cold treatment enhances low-temperature flight performance in false codling moth, <i>Thaumatotibia leucotreta</i> (Lepidoptera: Tortricidae).....	45
3.1.	Introduction.....	45
3.2.	Materials and methods.....	47
3.2.1.	Insect rearing.....	47
3.2.2.	Treatments	48
3.2.2.1.	<i>Laboratory flight performance</i>	48
3.2.2.2.	<i>Field flight performance</i>	49
3.2.3.	Statistical analyses	50
3.2.3.1.	<i>Laboratory assays</i>	50
3.2.3.2.	<i>Field assays</i>	51
3.3.	Results.....	52
3.3.1.	Cold treatment effects on flight activity in the laboratory	52
3.3.2.	Effects of cold temperature treatment on field recapture rates	53
3.4.	Discussion	56
3.5.	References	59
Chapter 4.	General discussion and conclusion	65
4.1.	Effects of acclimation and cold-hardening.....	66
4.2.	Implications for sterile insect technique programmes	67
4.3.	Future work.....	69
4.3.1.	Acclimation and test temperatures.....	69
4.3.2.	Effects of cold-treatment on other traits	70
4.3.3.	Effect of temperature treatment on F1 generation	70

4.3.4.	Enhancing the sterile insect technique	70
4.3.5.	Other approaches.....	71
4.4.	Conclusion	71
4.5.	References	72

LIST OF FIGURES

Figure. 1.1. Relationship between body temperature and performance in ectotherms, including insects (adapted from Angilletta, 2009) showing the optimum temperature range for performance, the 80% performance breadth (B_{80}). The critical limit at high temperatures is known as the critical thermal maximum (CTmax), whereas that at low temperatures, is known as the critical thermal minimum (CTmin). Topt indicates the optimum performance temperature.	2
Figure. 1.2. Schematic representation of the reaction norms adapted from Fusco & Minelli (2010). A and B represent plastic reaction norms, whereas C depicts a non-plastic reaction norm. A is a polyphenic character, whereas C is monophenic.	3
Figure. 1.3. Life cycle of the false codling moth, <i>Thaumatotibia leucotreta</i> , indicating the duration to temperature range of each life stage.	9
Figure. 1.4. Temperature tolerance of different life stages of <i>Thaumatotibia leucotreta</i> (adapted from Boardman <i>et al.</i> , 2012). Empty cells represent a lack of information. Values in brackets indicate ramping rates. Superscripted numbers indicate sources: 1: Johnson & Neven, 2010; 2: Daiber, 1979a; 3: Blomefield, 1978; 4: Daiber, 1979b; 5: Boardman <i>et al.</i> , 2012; 6: Daiber, 1979c; 7: Daiber, 1975; 8: Daiber, 1980; 9: Stotter & Terblanche, 2009; 10: Terblanche <i>et al.</i> , 2017. CTmin, critical thermal minimum; CTmax: critical thermal maximum; LDT: lower developmental threshold; ULT50: upper lethal temperature resulting in 50% mortality; LLT50: lower lethal temperature resulting in 50% mortality; SCP: supercooling point, i.e. the temperature at which body fluids freeze (after Boardman <i>et al.</i> , 2012).	12
Figure. 1.5. Comparison of sterile and wild <i>Thaumatotibia leucotreta</i> males in the Olifants River Valley from 2011 to 2013 (data obtained from XSIT).	16
Figure. 2.1. Effects of thermal acclimation (Acc) and temperature treatment on the critical thermal minimum of female and male <i>Thaumatotibia leucotreta</i> moths. Means with the same letter are not significantly different.	32
Figure. 2.2. Effects of different mating pairings, thermal acclimation and temperature treatment on the fecundity of <i>Thaumatotibia leucotreta</i> moths (total egg production was scored as cumulative eggs laid per pair over five days). Normal (N) individuals were acclimated at 25 °C and received no temperature treatment. Treated (T) individuals were acclimated and exposed to a temperature treatment (M: male; F: female). Means with the same letter are not significantly different (compared across all groups).	34
Figure. 2.3. Influence of sex, thermal acclimation (Acc) and temperature treatments on the survival time (longevity) of adult <i>Thaumatotibia leucotreta</i> moths. The generalised linear model (GLZ) is displayed along with the predicted upper and lower 95% confidence limits (UCL and LCL, respectively) and the different colours represent the different thermal acclimation groups.	37

- Figure.3.1.** Results of laboratory flight performance assays of cold-treated (2 °C) and control (25 °C) *Thaumatomibia leucotreta* female (F) and male (M) adults as measured at different test temperatures. Flight performance was measured by three possible responses: successful flight (sustained flight), landing in the surrounding water (partial flight) and non-dispersal (no flight).53
- Figure. 3.2.** Results from the random forest analyses, displaying the effect of the different independent variables contributing to the flight performance of adult *Thaumatomibia leucotreta* scored as a percentage increase in the model's mean-squared error (MSE). The higher the value (MSE), the more important the variable.54
- Figure. 3.3.** Summarised results of field recapture rates (number of male moths) of *Thaumatomibia leucotreta* to estimate flight performance. Each data point represents the moths recaptured on a specific release date. Recapture rates of moths are shown in relation to average maximum night temperatures either as absolute numbers (A) or as a ratio of the control moths (B).....55

LIST OF TABLES

Table 1.1. Physiological and cellular mechanisms underlying thermal acclimation responses in ectotherms	6
Table 2.1. Results of two separate generalised linear models (Gaussian distribution with identity link) for the effects of acclimation, treatment temperature and their interaction on critical thermal minimum for mass-reared <i>Thaumatotibia leucotreta</i> . Both the full (saturated) and minimum adequate models are presented here. Statistically significant effects are shown in bold. s.e.m.: standard error of the mean.	32
Table 2.2. Results of generalised linear models (Gaussian distribution, with identity link) on the fecundity of adult <i>Thaumatotibia leucotreta</i> moths for each pairing combination, where normal (N) individuals were acclimated at 25 °C and received no temperature treatment. Treated (T) individuals were acclimated and exposed to temperature treatment (M: male; F: female). Statistically significant effects are shown in bold. s.e.m.: standard error of the mean.	33
Table 2.3. Results of linear mixed effects models (binomial distribution) assessing survival time (in days) of adult <i>Thaumatotibia leucotreta</i> from the experimental treatments, with sex, thermal acclimation and temperature treatments as factors. s.e.m.: standard error of the mean.	35
Table 2.4. Survival time (days), represented by the time at which either 90, 50 or 10% of the population survives (LT90, LT50 and LT10 values, respectively) of adult <i>Thaumatotibia leucotreta</i> moths from the experimental treatments, with sex, thermal acclimation and temperature treatment as factors. Lower and upper 95% confidence limits (LCL and UCL, respectively) of model fits are shown to allow for post-hoc comparisons.....	36
Table 3.1. Additional climatic data and environmental conditions measured associated with orchard conditions for 72 hours after release.....	50
Table 3.2. Summarised output of laboratory results of adult <i>Thaumatotibia leucotreta</i> . A multinomial model was used to test the effects of cold temperature treatment, test temperature sex and replicate on the flight performance of adult moths. SE: standard error of the mean. Text in bold indicates significant relationships.	52
Table 3.3. Summarised results of a general linear model assessing flight performance (in terms of recapture rates) of adult <i>Thaumatotibia leucotreta</i> in field tests with average maximum night temperature, treatment and their interaction. SE: standard error of the mean. Text in bold indicate significant relationships. Degrees of freedom = 28.	54

CHAPTER 1. GENERAL INTRODUCTION

1.1. Thermal biology of insects

Climate has direct and indirect impacts on insects. Temperature, amongst other abiotic factors, is an important climatic factor that influences the development, growth, life history and fitness of insects, primarily by governing the rate of metabolic and biochemical reactions (Angilletta, 2009; Porter *et al.*, 1991; Walther *et al.*, 2002). Furthermore, it can directly stimulate or restrict insect activities, such as dispersal, feeding, mating and resource competition. The phenology and survival of insect species in adverse environmental conditions are also subject to temperature (Jaworski & Hilszczański, 2013). Indirect influences of temperature include its effects on plant growth (maturation rate, structure and timing of flowering), food quality and phenology which is relate to insects as food and habitat (Jaworski & Hilszczański, 2013). However, determining the impact of temperature variation on insect life cycles is complex, as it depends on multiple factors, including the timing and duration of that variation and how much it differs from optimal growth conditions. Different temperatures experienced over the course of an insect's life may breach specific physiological thresholds, impacting its performance in terms of, for example, locomotion, feeding/assimilation, growth, development, reproduction success and survival (Angilletta, 2009; Marshall & Sinclair, 2012).

A schematic representation of the above-mentioned impacts is the thermal performance curve, which forms a useful framework for illustrating several key concepts on the thermal adaptation of ectotherms, including insects, generally. The curve indicates the relationship between an insect's performance and its body temperature (T_b) (Angilletta, 2009). The temperature at which an insect is able to achieve maximal performance is known as the optimal performance temperature (T_{opt}) (Figure. 1.1) (Angilletta, 2009); an insect's performance is therefore limited at a certain point at both high and low extreme temperatures. These points are known critical thermal limits. The critical thermal limit at high temperatures is known as the critical thermal maximum (CT_{max}), whereas that at low temperatures, where insects typically enter a non-lethal coma, is referred to as the critical thermal minimum (CT_{min}). Exposure to temperatures outside these limits results in a loss of function, which, if sustained, results in death. The difference in temperature between these thermal limits is known as the thermal range, throughout which an insect can function. The ideal temperature range differs between and within species, depending on their thermal history (temperature history a species was exposed to for a certain time frame) (Verhoef *et al.*, 2014). Different

performance traits can have different curves with specific optima occurring within the same species (e.g. jumping vs running in crickets; Lachenicht *et al.*, 2010).

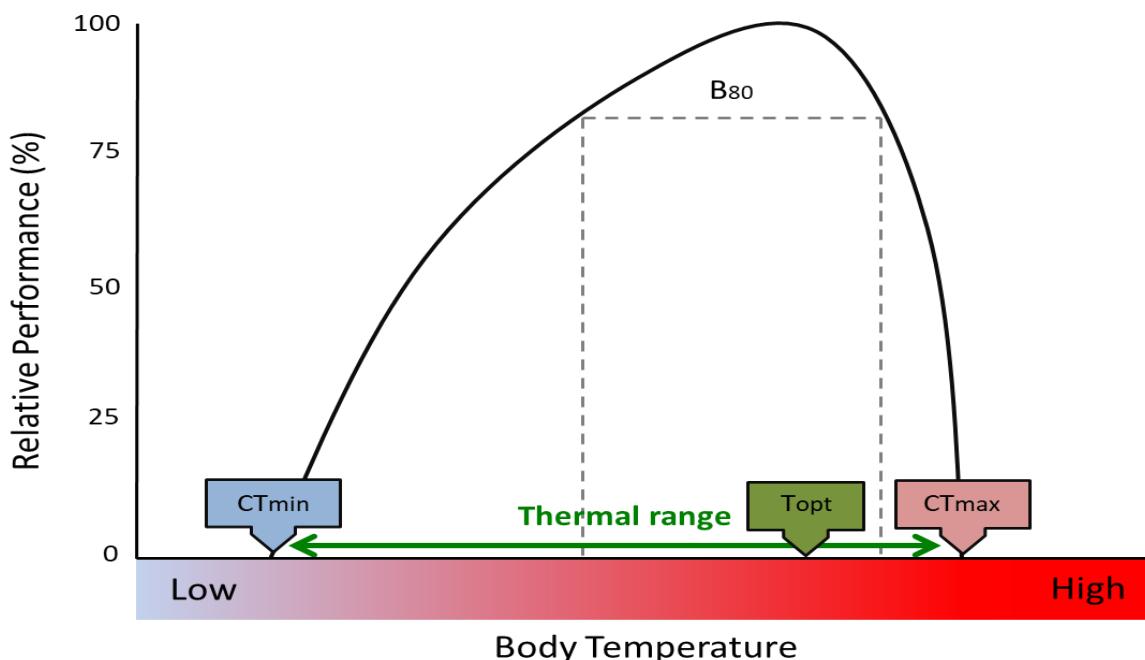


Figure. 1.1. Relationship between body temperature and performance in ectotherms, including insects (adapted from Angilletta, 2009) showing the optimum temperature range for performance, the 80% performance breadth (B_{80}). The critical limit at high temperatures is known as the critical thermal maximum (CTmax), whereas that at low temperatures, is known as the critical thermal minimum (CTmin). Topt indicates the optimum performance temperature.

1.1.1. Phenotypic plasticity and thermal acclimation

The overall relationship between temperature and other climatic factors and an insect's performance is variable to a certain extent and is known as phenotypic plasticity. Many insect species change phenotypes (e.g. behaviour and stress resistance) in response to environmental conditions. Plasticity can also be defined as how much the environment modifies phenotypic expression of a genotype, allowing individuals to survive stressful thermal conditions (Chown & Nicolson, 2004; Ju *et al.*, 2013; Sgrò *et al.*, 2016). This principle relies on the ability of a genotype to be expressed in a range of phenotypes in reaction to environmental variation, and is thus also known as a genotype-by-environment reaction or the reaction norm (Fordyce, 2006; Terblanche & Chown, 2006).

The reaction norm represents specific phenotypes that are formed by a single genotype when a species is exposed to different environments (Figure. 1.2, A & B) (Fusco & Minelli, 2010). Plastic phenotypic characters are the result of an association with the values of one or more environmental parameters (Figure. 1.2). A plastic character presents a response with a substantial range (Figure. 1.2, A & B), whereas non-plastic characters denote an

environmentally invariant value or flat reaction norm (monophenism, Figure. 1.2, C). When two or more distinct phenotypes are produced by an environmental cue, it is known as polyphenism (West-Eberhard, 2003).

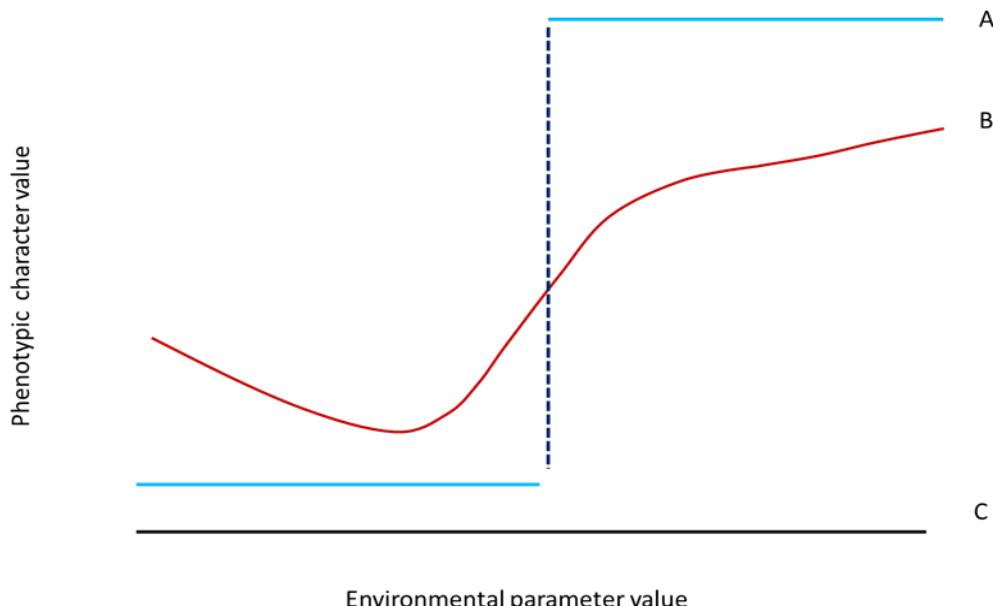


Figure. 1.2. Schematic representation of the reaction norms adapted from Fusco & Minelli (2010). A and B represent plastic reaction norms, whereas C depicts a non-plastic reaction norm. A is a polyphenic character, whereas C is monophenic.

Phenotypic discontinuity, which portrays polyphenism, can result from an actual discontinuity in the reaction norm (as in reaction norm A), imitating an induced threshold-like switch due to the environment from one pathway to another or to the influence of discontinuities of important environmental factors to which a species is exposed. This results in the expression of phenotypes of an otherwise constant reaction norm (Nijhout, 2003).

Phenotypic plasticity takes several forms and has multiple complex terms, covering aspects of the timing, magnitude and persistence of the induced change. For example, canalisation is a form of developmental plasticity defined as the ability of a population to produce the same phenotype regardless of variability in its environment or genotype (i.e. there is a single, stable output despite many inputs) (Liefting *et al.*, 2009). On the other hand, hardening or acclimation refers to increased tolerance to environmental stress, especially in response to changes in a single abiotic variable, such as the expression of heat shock proteins caused by rapidly increasing or decreasing temperatures (Fusco & Minelli, 2010). Some plastic responses vary in terms of the length of time for which they are expressed, whereas some responses are developmentally fixed (Fordyce, 2006).

Depending on exposure temperatures and time scales, insects' responses to climatic conditions are frequently considered as acclimatisation, acclimation or hardening. Acclimation usually takes place under fairly well-controlled conditions, whereas acclimatisation refers to plastic responses over longer time frames, typically during seasonal change, but may involve climate or abiotic factors changing simultaneously (e.g. photoperiod and temperature) (Denlinger & Lee, 2010). On the other hand, hardening or rapid stress responses can be achieved over a short period of exposure to stressful or extreme temperatures, whereby a non-lethal cold shock may increase an insect's cold tolerance (Lee *et al.*, 1987; Shreve *et al.*, 2004). It is generally thought that rapid hardening-type responses are reversible, whereas developmental plastic responses can be fixed or reversible depending on the trait in question. However, few studies have reported on the persistence of plastic responses (e.g. Weldon *et al.*, 2011).

Responses to environmental stress in insects are defined in terms of the trait of the environmental phenotype induced by an environmental parameter (Whitman & Agrawal, 2009). This is especially evident in the attainment of stress tolerance of insects during developmental acclimation to diverse conditions (Sgrò *et al.*, 2016). Classical examples include the first report of rapid cold-hardening in insects tested on the flesh fly, *Sarcophaga crassipalpis* (Diptera) (Lee *et al.*, 1987), and the monarch butterfly, *Danaus plexippus* (Lepidoptera) (Larsen & Lee, 1994). Flies that were subjected to 30 min of chilling at 0 °C before being exposed to -10 °C had double the survival rate of flies that were not chilled. Chilling of *D. plexippus* adults at 4 °C for 1 h led to a rapid increase in cold hardiness to sub-freezing temperatures, with an increase of 65% in flight ability at -4 °C. Even greater results were obtained when adults were exposed to more benign temperatures over a longer period (Larsen & Lee, 1994). *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) larvae showed a decrease in their critical thermal minimum with increased cooling rate, whereas that of adults only increased with a faster cooling rate, suggesting higher plasticity in the larval stage (Terblanche *et al.*, 2017).

1.1.2. Costs and benefits of thermal acclimation

Thermal acclimation involves both benefits and costs, meaning that improved performance under a certain environmental condition will occur at the expense of performing worse under an opposite environmental condition (Kristensen *et al.*, 2008). Sub-optimal temperatures during rearing had an adverse effect on the fecundity and fertility of *Drosophila melanogaster* (Diptera: Drosophilidae), but it nonetheless improved heat resistance and longevity (Hoffmann *et al.*, 2003). *Drosophila melanogaster*, reared at 15 and 25 °C, respectively, exhibit variances in recapture volumes, with pronounced fitness costs and benefits arising from the thermal

history subject to the environment into which the flies were released (Kristensen *et al.*, 2008). Flies acclimated at cold conditions and released at low temperatures were benefited, as they were the only flies able to find resources. On the other hand, flies that were not acclimated under cold conditions and released in a warm environment were 36 times more likely to discover food sources than those acclimated at cold temperatures (Kristensen *et al.*, 2008). Comparable results were observed in codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), suggesting that these effects could be widespread in different species (Chidawanyika & Terblanche, 2011). Recapturing of adults acclimated in cooler temperatures was significantly greater in colder environments compared to that of adults acclimated in warmer temperatures, and more heat- than cold-acclimated adults were trapped when released into warm conditions (Chidawanyika & Terblanche, 2011).

A study on *D. melanogaster* from different geographical and latitudinal areas showed that insects from a temperate strain, which were kept below their thermal minimum for 16 h, recovered faster than those from a tropical strain, which may be an indication of genetic adaption (Ayrinhac *et al.*, 2004). It can therefore be postulated that phenotypic plasticity may contribute to the geographical distribution of insects.

1.1.3. Physiological mechanisms underlying thermal acclimation responses

Insects are vulnerable to temperature changes due to their ectothermic physiology and small body size (Stevenson, 1985). This may lead to thermal sensitivities at cellular, systemic and organism levels that place constraints on normal functionality (Rome *et al.*, 1992). Furthermore, insects' metabolic rate is dependent on the environmental temperature. Their biological systems can therefore be regarded as sensitive to environmental change, although studies have shown that insects' metabolism exhibits adaptability to thermal variation (Neven, 2000).

Temperature changes directly influence enzymatic reactions, affecting the binding of a substrate to an enzyme, thereby disturbing the metabolic compensation of a species (Hochahcka & Sommero, 1984). These adaptions to changing temperatures evolved from physiological mechanisms, including changes in biochemistry and up-regulation of stress proteins, as well as cell function and gene expression (Kristensen *et al.*, 2008; Kristensen *et al.*, 2010; Meyer, 1978; Rock & Shaffer, 1983; Teets & Denlinger, 2013; Vermeulen *et al.*, 2014). One of the most well-known metabolic responses is the elicitation of heat shock proteins (Colinet *et al.*, 2010; Neven, 2000) after long-term cold exposure, as well the production of cryoprotectant molecules, depression of metabolism and alteration in gene expression (Angilletta, 2009; Bale, 1996; Hayward *et al.* 2014; Sgrò *et al.*, 2016; Sinclair, 1999); an increase in polyols and polyphosphates has also been recorded (Meyer, 1978).

These responses, including acclimation, adaption and hardening, which allow ectothermic species to tolerate extreme temperatures, are multifactorial, involving several physiological systems and biochemical adjustments (Overgaard & Macmillan, 2017). Another temperate effect known as fluctuating thermal regime (FTR) has been shown to increase the survival of some insects. This may be due to the fact that the period between stressful temperatures permits damage to be repaired as a result of low temperature (Marshall & Sinclair, 2012). The physiological and cellular mechanisms underlying thermal acclimation responses are summarised in Table 1.1 (reviewed in Overgaard & Macmillan, 2017).

Table 1.1. Physiological and cellular mechanisms underlying thermal acclimation responses in ectotherms

Physiological mechanism	Physiological and plastic response
Preservation of contractile function at low temperatures	Sustained muscle force production and ability to move in the cold and reduced critical thermal minimum (CTmin).
Changed thermal sensitivity of ion channel kinetics	Electrical properties of action potentials maintained and reduced CTmin.
Changed membrane structure	Sustained membrane protein function barriers and synaptic signalling, leading to shorter chill coma recovery time, reduced CTmin and increased survival rate.
Deployment of ion carriers in nerve and muscle cells	Ion balance in nervous system locally maintained and muscle force more thermally stable, leading to shorter chill coma recovery time and higher survival rate.
Regulation of paracellular penetrability	Reduced leak rates of water, ions and other solutes in the cold, leading to shorter chill coma recovery time and higher survival rate.
Decreased Na ⁺ gradients and water balance	Reduced rates of Na ⁺ and water migration, leading to shorter chill coma recovery time and higher survival rate.
Preservation of haemolymph K ⁺ clearance	Reduced extracellular K ⁺ concentration, leading to shorter chill coma recovery time and higher survival rate.
Safeguarding and reparation of macromolecule stability	Maintenance or restoration of protein conformation and membrane structure, leading to shorter chill coma recovery time, lower CTmin and higher survival rate.
Reticence of apoptotic signalling	Reduced apoptotic cell death despite ion and water balance disruption, leading to higher survival rate.

1.2. Sterile insect technique

Sustainable agriculture is under pressure, as lepidopteran pests have become one of the most problematic financial and socio-economic burdens on the production of food (Oerke, 2006; Simmons *et al.*, 2010). In addition, concerns for human health and consumer safety are

continuously raised as harmful pesticides are regularly discovered in groundwater (Odendaal *et al.*, 2015). Internationally, the sterile insect technique (SIT), as a measure of an integrated area-wide pest management strategy, has proven to be an effective approach for controlling insect disease vectors and agricultural crop pests, thus contributing to a safer and cleaner environment (Simmons *et al.*, 2010).

Sterile insect release is a specialised integrated pest control system used for the management of various pest species. This technique involves the mass-rearing of insects and subsequent irradiation to render individuals sterile for release purposes. These released insects mate with wild insects of the same species, resulting in a decline of the target insect population (Dowell *et al.*, 2005). This technique is mostly deployed against insects that feed on high-value crops or attack the fruiting tissues of a crop, resulting in significant economic losses (Sutter *et al.*, 1998). The success of the SIT necessitates an understanding of the target species' ecology, including the population density and how it varies or fluctuates over seasons (Lindquist *et al.*, 1974). The frequency of sterile releases depends on the species and the average longevity of the sterile insects. The longevity of sterile insects involves these insects remaining alive as long as their wild counterparts. If the longevity of the sterile insects declines, the frequency of release needs to increase to ensure an optimum over-flooding ratio (Dowell *et al.*, 2005).

The deployment of sterile insect releases began around 1930 after a cattle parasite, the New World screwworm, *Cochliomyia hominivorax* (F.) (Diptera: Calliphoridae), was successfully reared on an artificial diet, sterilised and released. After eradication of this parasite in Curacao (Caribbean Islands) in 1954 and Florida (USA) in 1959, the application of the technique became a more common pest management practice (Baumhover, 2002).

Area-wide management programmes aimed at the codling moth, *Cydia pomonella*, in Canada, the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), in the USA and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), in Australia have a prominent SIT component, demonstrating the viability and efficacy of combining sterile insect releases with other control strategies (Simmons *et al.*, 2010). More recent SIT programmes include the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), in California (USA), the European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae), in Argentina, the African sugar-cane borer, *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae), in South Africa and the mosquito species *Aedes albopictus* (Linnaeus) (Diptera: Culicidae) in Mexico and elsewhere (Anguelov *et al.*, 2012; Ant *et al.*, 2012; Saour, 2014; Walton & Conlong, 2016). The SIT was also successfully used to eradicate tsetse fly, *Glossina austeni* (Diptera: Glossinidae), from Unguja Island, Zanzibar (Vreysen *et al.*, 2000).

Combining the SIT with the incompatible insect technique, a technique where *Wolbachia* (Rickettsiales: Anaplasmataceae), a genus of gram-negative bacteria, is introduced into a pest population through sterile males, resulted in the decline of many disease vectors and invasive species (Nikolouli *et al.*, 2018; Zhang *et al.*, 2016). *Wolbachia* induces alteration of the paternal nuclear material, resulting in the failure of progeny to develop. These bacteria are conveyed by the cytoplasm of the eggs, influencing reproduction of their hosts, comprising the initiation of reproductive discordancy, parthenogenesis, and feminisation (Nikolouli *et al.*, 2018; Zhang *et al.*, 2016).

However, knowledge concerning the effect of temperature on adult insects used in sterile release programmes is limited and in-depth knowledge of the effects of temperature on the species concerned is crucial, as it can contribute to rearing protocols increasing the survival ability of released insects (Bloem *et al.*, 2004; Boersma & Carpenter, 2016; Chidawanyika & Terblanche, 2011). Understanding temperature effects on insects will assist in any pest management programme. Although most rearing facilities use constant temperatures, it would help to understand the reason for poor performance of insects under cooler temperate conditions. This study paid particular attention to the effects of temperature on the adult false codling moth.

1.3. False codling moth (*Thaumatotibia leucotreta*)

The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is a pest from sub-Saharan Africa (Blomefield, 1978). This species attacks many cultivated, deciduous, sub-tropical and tropical plants but prefers citrus as its main host (Economides, 1979). Other host crops include nuts, grapes, wild Figure, guava, pomegranate, plum, peach, apricot, avocado, mango and peppers (Bloem *et al.*, 2003).

1.3.1. Biology of *Thaumatotibia leucotreta*

The life cycle of *T. leucotreta* consists of eggs, larvae, pupae and adult moths (Figure. 1.3). From egg to adult, *T. leucotreta* passes through five larval instars and the developmental rate is influenced by temperature and food quality. Eggs are laid on the peel of the host fruit. After the larva hatches it gnaws through the rind. A hole with a diameter of about 1 mm is made and the entrance becomes visible due to the presence of frass and discolouration of the surrounding rind (Daiber, 1979a; b). The mature larva exits the fruit just before the pre-pupal stage and drops to the ground on a silk thread before constructing a cocoon made from silk and/or soil particles (Grout & Moore, 2015). The fifth instar larva inside the cocoon develops from a pre-pupa into a pupa. The development of the pupa and the duration of the cocoon stage are determined by the average ambient temperature and relative humidity (Daiber,

1979c; Grout & Moore, 2015). The relative humidity also has a significant impact on the successful development of a pupa into an adult (Daiber, 1979c).

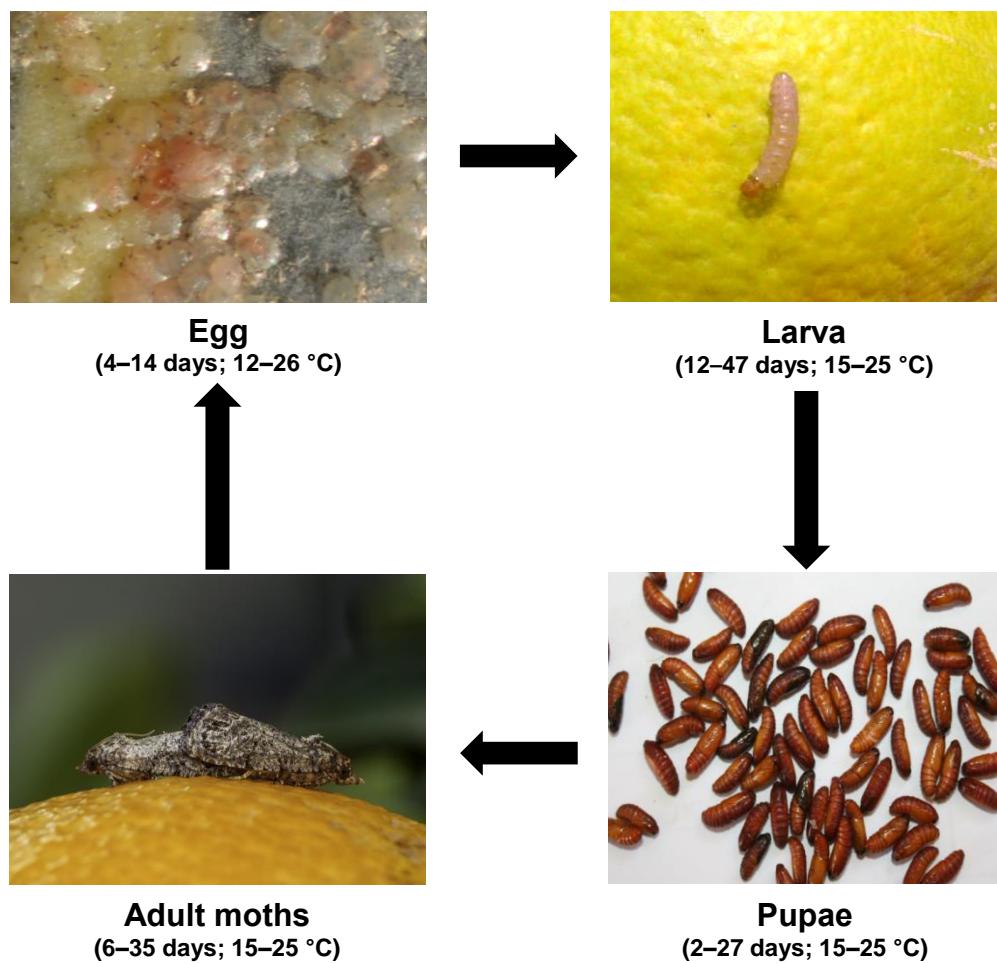


Figure. 1.3. Life cycle of the false codling moth, *Thaumatotibia leucotreta*, indicating the duration to temperature range of each life stage.

The adult is an inconspicuous nocturnal moth seldom noticed (Grout & Moore, 2015). Its colour varies between shades of grey with a curl of grey scales on the dorsal surface of the body (Grout & Moore, 2015). The male can be distinguished by its slender body and black setae on its legs. The female releases a pheromone after nightfall until daybreak. Males mate with females as soon as they are located but only fly when temperatures are above 10 °C. The mated female lays her eggs at irregular intervals between 17:00 and 23:00 on a host plant and deposits three to eight eggs per fruit, with a total number of up to 800 eggs throughout her lifespan (Daiber, 1980; Stotter & Terblanche, 2009; USDA, 2014). There are five to six overlapping generations per year with no winter diapause (Grout & Moore, 2015; Terblanche, 2014). Depending on the environment or rearing conditions, *T. leucotreta* requires 600–800 degree days for completing one generation (Daiber, 1979a, b, c; Daiber, 1980).

1.3.2. Temperature effects on *Thaumatomibia leucotreta*

The effects of temperature on the development of *T. leucotreta* differ for each life stage. For eggs, hatching takes 4–8 days in the summer and 8–14 days in the winter (Stofberg, 1954). The lower threshold temperature where egg development ceases is between 11 and 12 °C. Eggs are killed when they are exposed to temperatures below 0 °C for 48–72 h (Daiber, 1979a). At 25 °C, the development of the different larval instars (1–5) to pupae lasts approximately 12 days versus 47 days at a constant temperature of 15 °C (Daiber, 1979b). The lower developmental threshold (LDT) (the temperature where development ceases) for *T. leucotreta* larvae is between 11 and 12 °C (Daiber, 1979b).

The average duration of the development from pupa to emergence of the adult is 13 days for females and 14 days for males at a constant temperature of 15 °C. This can last between 2 and 27 days at different temperatures. The LDT for *T. leucotreta* pupae is 11.9 °C (Daiber, 1979c). The lifespan of an adult male at a constant temperature of 15 °C is 34.1 days, whereas a female will live for 48 days at the same constant temperature (Daiber, 1980). At constant temperatures higher than 15 °C (20 and 25 °C), the lifespan of an adult is significantly shorter at 25 °C: 13.7 and 15.8 days for males and females, respectively. The LDT for adult *T. leucotreta* differs between males and females as well as for the specific degree of maturity in adulthood. The LDT for *T. leucotreta* males is 8 °C compared to 9.5 °C for females (Daiber, 1980).

The effect of temperature on pre-oviposition, the age at which females start laying eggs, and the number of eggs laid is significant. At 10 °C, a female reaches an age of 23 days before she has laid 50% of her eggs; this age increases to 26 days at 15 °C and decreases to 6 days at 25 °C (Daiber, 1980). The average number of eggs laid at 10 °C is only 0.4 eggs per female. This increases as the temperature increases. At 15 °C, a female *T. leucotreta* lays 86.9 eggs on average and the number of eggs increases to around 456 eggs per female at 25 °C (Daiber, 1980).

Higher temperatures stimulate reproduction at the expense of longevity, whereas lower temperatures are especially detrimental for both reproduction and lifespan. Intermediate temperature (20 °C) is associated with a comparatively long lifespan at the cost of reproduction (Daiber, 1980). The temperature tolerance of each developmental stage of *T. leucotreta* differs, which may influence the following life stage. For pest management, it is important to understand the effects of temperature on the developmental stages of the target species whereas survival and activity limits are regulated by experimental protocols assessing these limits (Terblanche *et al.*, 2017). Studies conducted on *T. leucotreta* have indicated that larval mortality below -14 °C is 100% after 1 h (Boardman *et al.*, 2012). The upper and lower

developmental threshold for eggs were established to be between 41 - 45 °C and 11.7 - 11.9 °C, respectively (Figure. 1.4). Subject to the feeding status, the supercooling point, where *T. leucotreta* larvae freeze, is -15.6 °C, whereas larvae enter a chill coma around 3–7 °C (reviewed in Boardman *et al.*, 2012). Depending on the exposure time, larvae can survive a broader temperature range (lower lethal temperature: -14 to -6 °C; upper lethal temperature: 36–52 °C), (Terblanche *et al.*, 2017). Larval activity limits are mostly influenced by ramping rate (intensity of a temperature assay), more than adult activity limits are, and the estimates of thermal activity thresholds (critical thermal maximum and minimum) vary between life stages across all ramping rates.

For *T. leucotreta* larvae, larval body water and lipid content remain unaffected in reaction to fluctuating thermal regime (Boardman *et al.*, 2013). However, such regimes only act as a protective measure when associated with constant low temperature (15 °C), possibly owing to an increase of heat shock protein 70 (HSP70). Larvae exposed to a constant benign environment suffer long-term fitness consequences, such as low pupation rates, probably because reserves for continuing their life cycle were depleted, although they were capable of surviving the thermal stress (Boardman *et al.*, 2013). This indicates that fluctuating thermal regimes do not necessarily result in high larval mortality in *T. leucotreta*, which might be beneficial information for rearing insects for a pest management programme (Boardman *et al.*, 2013).

The effects of time, temperature and their interaction on the survival of adult *T. leucotreta* were determined at -4.5 °C by means of lower lethal temperature assays (Stotter & Terblanche, 2009). These assays indicated that sub-zero temperatures reduce the probability of survival by 50% after exposure of 2 h, which varied significantly to -0.5 °C after 10 h (lower lethal temperature: -14 to -6 °C; upper lethal temperature: 32–48 °C) (Figure. 1.4) (Stotter & Terblanche, 2009; Terblanche *et al.*, 2017).

Greater life-stage related variances in the critical thermal minimum are observed when cold tolerance assays were conducted at slower more ecologically relevant cooling rates (0.06 °C per min), whereas the opposite is apparent in life stage-related differences of the critical thermal maximum (Figure. 1.4) (Miller, 1978; Terblanche *et al.*, 2017). As larvae are the active feeding stage, most studies have been conducted on this stage of *T. leucotreta*'s development, but little is still known about the temperature tolerance of adults and pupae.



	Eggs	Larvae	Pupae	Adult
	Temperature			
CT min		6.7°C ⁵ 10.5°C ¹⁰ (0.06°C ⁻¹) 8°C ¹⁰ (0.1°C ⁻¹) 6°C ¹⁰ (0.25°C ⁻¹)		2.2°C ¹⁰ (0.06°C ⁻¹) 2.5°C ¹⁰ (0.1°C ⁻¹) 3.8°C ¹⁰ (0.25°C ⁻¹)
CT max			42.5°C ¹⁰ (0.06°C ⁻¹) 42.5°C ¹⁰ (0.01°C ⁻¹) 45.5°C ¹⁰ (0.1°C ⁻¹)	42.5°C ¹⁰ (0.06°C ⁻¹) 42.5°C ¹⁰ (0.01°C ⁻¹)
LDT	11.7-11.9°C ²	11.6 -12.5°C ⁴	11.9°C	6.4-15°C ^{7,8}
ULT 50	41-45°C 1.5-2.5h ¹	38.5-45°C 2-2.5h ¹ 51.3°C 2h ¹⁰		42.3°C ¹⁰
LLT 50		-12°C 2h ⁵ -14°C 2h ¹⁰		0.5°C 10h ⁹ -4.5°C 2h ⁹ -7.7°C 2h ¹⁰
SCP		-15.6°C ⁵		
Mortality	0°C 2-3d ^{2,3}			

Figure 1.4. Temperature tolerance of different life stages of *Thaumatomibia leucotreta* (adapted from Boardman *et al.*, 2012). Empty cells represent a lack of information. Values in brackets indicate ramping rates. Superscripted numbers indicate sources: 1: Johnson & Neven, 2010; 2: Daiber, 1979a; 3: Blomefield, 1978; 4: Daiber, 1979b; 5: Boardman *et al.*, 2012; 6: Daiber, 1979c; 7: Daiber, 1975; 8: Daiber, 1980; 9: Stotter & Terblanche, 2009; 10: Terblanche *et al.*, 2017. CTmin: critical thermal minimum; CTmax: critical thermal maximum; LDT: lower developmental threshold; ULT50: upper lethal temperature resulting in 50% mortality; LLT50: lower lethal temperature resulting in 50% mortality; SCP: supercooling point, i.e. the temperature at which body fluids freeze (after Boardman *et al.*, 2012).

1.3.3. *Thaumatomibia leucotreta* as a phytosanitary pest

During the mid-1970s, the first incidence of *T. leucotreta* was confirmed in Citrusdal, Western Cape, South Africa, an important citrus exporting region. By the end of the decade, *T. leucotreta* had spread throughout the Olifants River Valley, becoming a devastating citrus pest in this region with developed resistance against registered insecticides (Hofmeyr *et al.*, 2015; Hofmeyr & Pringle, 1998). Furthermore, stricter regulations were progressively imposed upon exporters, resulting in a zero tolerance and cold treatment of fruit against the pest. A novel sustainable approach to *T. leucotreta* was therefore required. After a combined multi-

institutional research project, an SIT programme was launched in 2002 by Citrus Research International, the Food and Agriculture Organization in collaboration with the International Atomic Energy Agency, and the United States Department of Agriculture – Agricultural Research Service, as part of an integrated area-wide pest management programme to control *T. leucotreta* (Hofmeyr *et al.*, 2015).

1.3.4. Implementation of the sterile insect technique for *Thaumatotibia leucotreta*

The SIT programme for *T. leucotreta* in South Africa was developed over a five-year period with a subsequent five-phase development plan. In 2002, initial radiation biology and sterility studies were concluded. In the first phase, it was determined that 150 Gy resulted in 100% sterility when a treated female was crossed with a non-treated male. On the other hand, there was some residual fertility when treated *T. leucotreta* males were mated to untreated females. Inbred F1 sterility was tested and determined as 100% sterile when the F1 progeny were inbred and outcrossed with counterparts from the insectarium (Carpenter *et al.*, 2004). The second phase included field cage studies conducted in navel orange orchards. Results were similar to those of the first phase, confirming a dose of 150 Gy at a ratio of 1 wild moth to 10 sterile moths was sufficient to protect citrus trees from *T. leucotreta* damage above the economic threshold (Hofmeyr *et al.*, 2015).

The third phase commenced in 2005 with a commercial pilot project of 35 adjoining navel orange orchards surrounded by natural vegetation with no known host plants of *T. leucotreta*. Except for a strict sanitation programme, whereby all infested fruits were removed once a week, no other control measures were applied to suppress *T. leucotreta*. Twice a week, 1000 24-hour-old sterile unsexed moths were released per hectare for 29 weeks until June 2006, when harvest occurred. The released moths were marked with fluorescent powder to enable identification of sterile and wild male moths trapped in traps equipped with a synthetic sex pheromone (Hofmeyr *et al.*, 2015).

The SIT programme involved releases of between 1000 and 2000 sterile male and female adults per hectare per week, aiming to provide a minimum of 10 sterile males per wild male (Hofmeyr *et al.*, 2015). This ratio maximised the prospect that a sterile adult will mate with a wild adult, resulting in no viable offspring and subsequent population decline (Carpenter *et al.*, 2004). Trap catches by means of a sex pheromone of both sterile and wild males were monitored on a weekly basis. A food colorant, included in the diet of the reared larvae and which coloured adult moth intestines pink, enabled differentiation between sterile and wild catches (Hofmeyr *et al.*, 2015).

The efficacy of the programme was assessed with trap captures and fruit drop surveys. Five trees adjoining each trap were inspected weekly. Trap counts were recorded and dropped fruit were inspected to determine infestation levels. The mean crop loss to *T. leucotreta* infestation during the trial period was 0.1 and 2.1 infested fruit per tree per week in the SIT and control sites, respectively, representing an infestation reduction of 95% (Hofmeyr *et al.*, 2015). Following these results, as the fourth phase, the Citrus Growers' Association of Southern Africa commercialised the project. Citrus Research International created a new subsidiary and XSIT (Pty) Ltd., a commercial initiative to control *T. leucotreta*, was started in 2007. A mass-rearing facility of 2000 m² was constructed to produce up to 21 million moths per week. The last phase commenced in 2007 when the first commercial releases of sterile moths began on 1500 ha of citrus orchard (Hofmeyr *et al.*, 2015). This programme is presently operating in the Western, Eastern and Northern Cape provinces of South Africa, servicing more than 18000 ha of citrus and table grape orchards.

1.3.5. Challenges encountered with sterile moths in a commercial sterile insect technique programme

Several biological factors influence the success of an SIT programme. These include sexual reproduction of the species, available mass-rearing methods, fitness of insects after sterilisation, inherent characteristics of the species and methods available for monitoring released insects (Lance & McInnis, 2005).

The competitiveness of any sterile insect is crucial for effective control. To maintain high-quality competitive insects for optimum sterile-to-wild ratios in the field, biological factors that influence sterile quality should be monitored. These factors directly influence the mating ability and subsequent fecundity of sterile insects, which are critical to ensure sufficient competitiveness in the field. An initial SIT programme on the olive fruit fly, *Bactrocera oleae*, had to be abandoned due to poor competitiveness of sterile males, as diurnal mating rhythms of the sterile males resulted in asynchronous mating activity between the wild and sterile populations, which led to poor results (Ant *et al.*, 2012).

The addition of food supplements, such as yeast hydrolysate and guarana powder, to the diet of different tephritid genera enhanced male reproductive success in the days following emergence (Kaspi & Yuval, 2000). Moreover, exposing adults to various aromas may similarly increase adult performance. After exposing *Ceratitis capitata* males to the aroma of ginger root oil before release, the mating success of treated males significantly increased in relation to the control, suggesting that the application of ginger root oil in pre-release containers can enhance the effectiveness of such an SIT programme (Shelly *et al.*, 2004).

The longevity of sterile insects, in combination with active mating after release, determines the frequency of sterile releases, but the decline of this trait may be caused by mass-rearing and handling techniques (Lance & McInnis, 2005). Despite these factors, temperature in rearing and post-release environmental conditions is one of the most important factors affecting an SIT programme, as it influences temporal growth patterns, survival and reproduction (Sørensen *et al.*, 2012).

To ensure sustainable quality of mass-reared insects, the use of appropriate techniques to handle, store and ship reared insects is essential, because a storage regime could be either beneficial or harmful (Leopold, 2007). The use of cold temperatures to immobilise moths is standard procedure in most SIT programmes, as this allows an increased number of moths to be confined in a given container size for collection, handling, irradiation, transport and release. However, chilling and long cold-temperature storage may impact the field performance of some mass-reared moths, leading to a possible lack of competitiveness compared to their wild counterparts (Boersma & Carpenter, 2016). This has a noticeable effect on the efficacy of SIT programmes, which depend on appropriate interaction between mass-reared and wild insects in terms of dispersal, reproductive performance and activity thresholds (Boersma & Carpenter, 2016). To ensure the effectiveness of an SIT programme, understanding of the performance (and possible limits) of mass-reared insects is essential, as the rearing process, particularly thermal conditions during development, can have a significant effect on a wide range of traits once the moth is released (Sørensen *et al.*, 2012; Terblanche, 2014).

Sterile *T. leucotreta* adults produced by current mass-rearing techniques have an undesirably high minimum threshold temperature for activity (10–15 °C) (Stotter & Terblanche, 2009). Flight activity is diminished during the final (cooler) phase of the citrus-growing season, reducing the fitness of sterile adults and, consequently, the efficacy of the SIT programme (Stotter & Terblanche, 2009) with recaptures of sterile *T. leucotreta* declining considerably from week 23-36 as temperatures during the night start to decrease (Figure. 1.5).

Similar problems have been encountered in other mass-rearing programmes, such as that with the Mediterranean fruit fly (*C. capitata*), where a weakening of their mating performance was noted at higher altitudes and cooler temperatures. When flies were reared under cooler conditions, their ability to adapt to extreme habitats increased (Boller *et al.*, 1981). Zapien *et al.* (1983) found that the mating competitiveness of laboratory-reared *C. capitata* flies failed to attract wild females, especially on cloudy days, thus lacking any ability to compete with their wild counterparts in low light intensity environments.

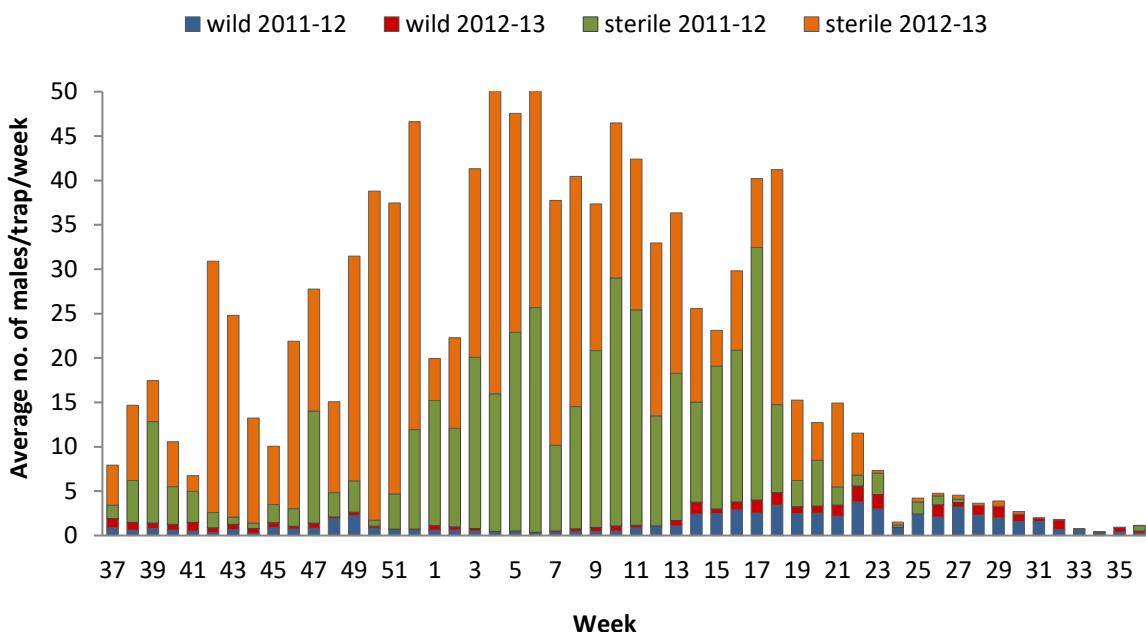


Figure. 1.5. Comparison of sterile and wild *Thaumatotibia leucotreta* males in the Olifants River Valley from 2011 to 2013 (data obtained from XSIT).

Field cage tests done with the Caribbean fruit fly (*Anastrepha suspense*) documented that laboratory-reared flies complete their mating activities in the early afternoon long before the wild flies start theirs at dusk. Exposure to continuous optimum environments in terms of temperature, light and relative humidity could result in individuals adapting to these environments, but it could also result in poor adaptability to fluctuating conditions (Cayol, 2000). Although environmental temperatures negatively affect insect performance, phenotypic plasticity (i.e. modifications in traits caused by prior stress exposure) may offset these effects as seen in the reaction norm in Figure. 1.2, improving the effectiveness of the SIT.

1.4. Study aims

Temperature during the rearing process affects the life traits of *T. leucotreta* adults and temperature treatment of adults impacts their flight ability under different temperature regimes. A study on the effect of different temperature conditions imposed on *T. leucotreta* larvae and adults would provide a valuable contribution to knowledge on the influence of different thermal conditions on adult moths in an insect-rearing facility and how these factors influence the efficacy of an SIT programme. The aim of this study was therefore to investigate the effect of temperature on diverse traits of adult *T. leucotreta* moths. Different temperature variations were applied to the larval stage of *T. leucotreta* and short-term temperature treatments were applied to adults in order to induce and measure acclimation responses.

The objectives of this study can therefore be summarised as follows:

- To determine the outcome of thermal conditions on life traits, including low temperature activity thresholds, fecundity, longevity and flight performance of adult moths.
- To determine whether temperature treatments can be used to improve the flight performance of mass-reared *T. leucotreta* in the context of improving the SIT programme.

The results are discussed in the context of the species' population dynamics and pest management with the SIT as the main component.

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CHAPTER 2. SEX-DEPENDENT THERMAL HISTORY INFLUENCES COLD TOLERANCE, LONGEVITY AND FECUNDITY IN FALSE CODLING MOTH, *THAUMATOTIBIA LEUCOTRETA* (LEPIDOPTERA:TORTRICIDAE)

This chapter has been published in *Agricultural and Forest Entomology*, Volume 20, Issue 1, pp 41-50, titled 'Sex-dependent thermal history influences cold tolerance, longevity and fecundity in false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae)' by Nevill Boersma, Leigh Boardman, Martin Gilbert and John S. Terblanche (2018). DOI: 10.1111/afe.12227.

2.1. Introduction

Temperature directly influences insect population dynamics and, consequently, their ecology and evolution. Life-history and phenological traits (e.g. growth rate, body size, time to maturation, or dispersal and migration events) (Chown & Gaston, 2010) and field performance traits, such as mating and temperatures permissive of flight or dispersal distances (Esterhuizen *et al.*, 2014; Kristensen *et al.*, 2008; Terblanche, 2014), are significant for understanding pest population dynamics and performance of individuals under variable field conditions. Insects' performance, behaviour and survival under thermal extremes are, however, not static. In contrast, these are influenced by multiple extrinsic and intrinsic factors at various taxonomic and hierarchical levels (reviewed in Chown & Nicolson, 2004; Denlinger & Lee, 2010; Hoffmann *et al.*, 2003). Other behavioural, physiological or life-history traits that may be of environmental relevance to survival, such as water balance, diapause induction and termination or immune responses, may also be affected by temperature regimes (Kleynhans *et al.*, 2014; Sinclair *et al.*, 2013; Xu *et al.*, 2012). Consequently, understanding the effect of time–temperature interactions at various stages of insect development on diverse traits, both immediately and over delayed time frames (e.g. across generations), plays a vital role in understanding the insects' adaptive capacity, their response patterns to climate change and risks that certain pests may pose to crop production (Hoffmann *et al.*, 2003; Shreve *et al.*, 2004; Terblanche *et al.*, 2015). Understanding these interactions will likely contribute to improved forecasting of future climate change impacts and the predictive management of pest outbreaks (Bebber *et al.*, 2013; Sgrò *et al.*, 2016).

The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is indigenous to sub-Saharan Africa. This species is a pest of cultivated crops and indigenous plants and established in the Paarl region, Western Cape province, South Africa, around 1969

(Hofmeyr *et al.*, 2015). By the mid-1970s, *T. leucotreta* had expanded its range northwards by several hundred kilometres to Citrusdal, an important citrus exporting region. The pest problem was exacerbated when *T. leucotreta* developed resistance to several insecticides and when stricter regulations were imposed on exporters (Hofmeyr & Pringle, 1998). An integrated, sustainable approach to pest management of this species became critical and resulted in the initiation of a collaborative, multi-institutional sterile insect technique (SIT) research project during 2002 to control *T. leucotreta* as part of an integrated area-wide pest control programme (Hofmeyr *et al.*, 2015). The SIT is based on the mass production of high-quality insects for sterilisation and release purposes. These sterile insects mate with their wild counterparts, resulting in the decline of the target population (Dowell *et al.*, 2005). This programme is currently active in the Western, Eastern and Northern Cape provinces of South Africa.

Lepidopterans are amongst the greatest pests of agricultural crops globally, imposing a huge financial and socio-economic burden on sustainable food production (Simmons *et al.*, 2010). The use of potentially harmful pesticides that are increasingly being detected in groundwater in South Africa raises concerns for long-term human health and safety (Odendaal *et al.*, 2015). Internationally, the SIT, as part of area-wide integrated pest management strategies, is regarded as an effective approach for controlling insect disease vectors and agricultural crop pests (Simmons *et al.*, 2010). The area-wide management programmes aimed at the codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), in Canada and the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), in the USA have a prominent SIT component and have demonstrated the viability and efficacy of combining sterile insect releases with other population control tactics (Simmons *et al.*, 2010).

The SIT programme for *T. leucotreta* in South Africa involves the release of both sterile males and females on a weekly basis at a rate of 1000–2000 moths per hectare of orchard to suppress the wild population. The programme aims to provide at least 10 sterile males for each wild male in the orchard (Hofmeyr *et al.*, 2015). This ratio is used to maximise the probability of a wild moth mating with a sterile moth in the field, resulting in reduced viable or fertile offspring and an eventual population decline (Carpenter *et al.*, 2004; Hofmeyr *et al.*, 2015). The released and wild populations are monitored at weekly intervals by means of male recaptures with sex pheromone traps. Sterile males are differentiated from their wild counterparts by means of their pink fat bodies, a colour-change caused by the addition of artificial food colorant in the larval diet during mass-rearing (Hofmeyr *et al.*, 2015).

The mating ability of the released moths and subsequent fecundity are critical to ensure sufficient suppression of the wild moth population. Understanding the performance (and possible limits) of mass- or laboratory-reared insects provides fundamental baseline

information for the effective use of an SIT programme (Sørensen *et al.*, 2012; Terblanche, 2014). It is becoming increasingly clear, however, that the rearing process, with particular reference to the thermal conditions during development (even if only modified by a few degrees and for a short period) can have a persistent effect on various traits upon release in the field. For example, sub-optimal rearing temperatures negatively affected the fecundity and fertility of *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) but provided increased longevity and heat resistance (Hoffmann *et al.*, 2003). In the field, *D. melanogaster* acclimated or reared at 15 and 25 °C showed significant differences in recapture rates at a food bait station, suggesting pronounced fitness costs and benefits to thermal history, depending on the environment into which the flies were released (Kristensen *et al.*, 2008). In colder areas, only cold-acclimated flies were able to find resources, but when conditions were warmer, flies not reared under cold conditions were up to 36 times more likely to find food than the former (Kristensen *et al.*, 2008). Similar effects have been documented in *C. pomonella*, suggesting that these effects are pervasive across diverse insect taxa (Chidawanyika & Terblanche, 2011). Recapture rates of moths acclimated at cooler temperatures were significantly higher under cooler conditions compared to that of heat-acclimated moths. Conversely, heat-acclimated moths were caught in higher numbers than cold-acclimated moths when released into a hot environment (Chidawanyika & Terblanche, 2011).

Such effects, or thermal legacies, may have a marked impact on the efficacy of an SIT programme that relies on suitable interaction between wild and mass-reared individuals in terms of mate-finding, dispersal, activity thresholds and reproductive behaviours. No such information is, however, presently available for *T. leucotreta*, despite its importance for the ongoing pest management of the species. Mass-reared sterile *T. leucotreta* adult moths currently have an undesirably high minimum activity threshold temperature of approximately 10–15 °C, indicating poor cold tolerance relative to the conditions in which they are expected to perform (Stotter & Terblanche, 2009). This means that moth flight activity could be reduced during the latter part of the citrus-growing season, affecting the efficacy of the SIT programme negatively. The aim of this study was therefore to investigate the effect of larval acclimation and short adult temperature treatments on the low temperature activity thresholds, fecundity and longevity of *T. leucotreta* moths. The results are discussed in the context of the species' population dynamics and pest management.

2.2. Materials and methods

2.2.1. Insect rearing

A laboratory population of *T. leucotreta* from the XSIT (Pty) Ltd mass-rearing facility (Citrusdal, Western Cape, South Africa) was used in this study. The population originated eight years ago from wild moths collected in orchards in Citrusdal and is periodically augmented with wild insects to avoid inbreeding depression or genetic divergence from natural populations. The population was maintained at 25 °C (12:12 D:L) on a maize-based diet. Relative humidity was not strictly controlled and varied between 40 and 60%.

2.2.2. Acclimation (larval developmental temperatures)

Freshly inoculated glass jars, each containing 280 g artificial feed and 1000–1200 eggs, were obtained from the commercial colony and incubated at 15, 20 or 25 °C in Peltier temperature cabinets (PTC-1 Sable Systems International, Las Vegas, USA). These cabinets were connected to a Peltier 3 controller (Sable Systems International) to monitor internal ambient temperatures. The pupae of each acclimation group were sorted according to sex, placed in separate Petri dishes (70 mm diameter) and incubated in temperature-controlled cabinets at 25 °C until eclosion occurred.

2.2.3. Moth temperature treatments

At eclosion, 50 males and 50 females (12–24 h old) from each acclimation group were placed into separate Petri dishes (70 mm diameter) and treated at 10, 15 or 20 °C for 2 h on a Perspex thermal stage (30 × 20 × 15 cm Perspex container) connected to a water bath (Grant GD200-R2, Grant Instruments, Cambridge, UK; accuracy: ± 0.1 °C). Temperatures in the Petri dishes were confirmed using a thermocouple (K-type, 36 standard wire gauge, Omega Engineering, Inc., Stamford, USA) attached to a digital thermometer (Fluke II, Fluke Corporation, Everett, USA).

2.2.4. Critical thermal minimum

Ten males and ten females for each of the different acclimation and temperature treatment combinations were individually placed into the tubes of an organ pipe device, connected to a programmable circulating bath (Grant GD200-R2, Grant Instruments) (accuracy: ± 0.1 °C) to test 10 individuals at a time. The water bath was filled with a solution of propylene glycol and water (1:1 ratio) to allow sub-zero temperature operation. The critical thermal minimum (CT_{min}) was visually determined for each group (acclimation and temperature treatment combinations) of 10 moths placed into a 60 ml non-airtight plastic vial, located in the watertight plastic containers and subjected to decreasing temperatures, commencing at 25 °C and

reduced at a fixed rate of 0.1 °C per minute. Although a reduction rate of 0.06 °C per minute is considered to be the most ecologically relevant (Terblanche *et al.*, 2007), a reduction rate of 0.1 °C per minute was used to represent facility conditions where more rapid cooling is typically used to immobilise moths. Regular inspection of water baths and control vials with a data logging thermometer (accuracy: ± 0.1 °C) confirmed that the desired temperature during treatments was achieved and maintained. Initially, moth condition and behaviour were assessed at each 0.5 °C increment. As soon as the temperature reached 10 °C, the moths were assessed at each 0.1 °C decrease. Temperatures were recorded for each individual as soon as the moth reached CT_{min}, which was scored as a loss of co-ordinated response to a gentle mechanical stimulus (prodding with a fine paintbrush).

2.2.5. Fecundity

Moths from each acclimation and treatment combination were paired, viz. treated male \times treated female, treated male \times untreated female and untreated male \times treated female. Untreated moths were obtained from the XSIT colony, reared at 25 °C but not subjected to any temperature treatment. Each of five pairs of moths for each acclimation and treatment combination was placed into a Petri dish (diameter of 45 mm, height of 15 mm) and supplied with water-saturated cotton wool. For five days, each of these pairs was transferred to a new dish every 24 h. Dishes were kept at 25±1 °C with a day–night photo period of 12 h and 75% relative humidity (confirmed using a relative humidity and temperature data logger, 101A, MadgeTech, Warner, USA). Fecundity was assessed by recording the number of eggs deposited in the Petri dishes.

2.2.6. Longevity

Ten males and ten females from each of the different acclimation and temperature treatment combinations were individually placed in small Petri dishes (diameter of 60 mm, height of 15 mm). The moths were incubated at 25 °C and 75% relative humidity (confirmed using a relative humidity and temperature data logger, 101A, MadgeTech) in the laboratory with water supplied in cotton wool. Mortality was recorded daily until all moths had died.

2.2.7. Statistical analysis

Statistical analyses for CT_{min}, fecundity and longevity were performed in R software (v. 3.1.3, R Foundation for Statistical Computing, 2008, Vienna, Austria; packages ‘stats’, ‘nnet’, ‘MASS’ and ‘car’) and Figures were drawn in Statistica (v. 12; Statsoft Inc., Tulsa, USA). The data distribution, degrees of freedom and residual deviance of both the generalised linear model (CT_{min} and fecundity data) and for linear mixed effects (longevity data) were inspected and verified so that model assumptions were not violated. For CT_{min}, we considered a fully

saturated generalised linear model (with a Gaussian distribution) with acclimation, treatment temperature and sex as well as all possible interaction effects on CTmin. Thereafter, we fitted the minimum adequate model following Crawley's method (Crawley, 2007) and present both sets of results herein. To assess the effects of acclimation temperature, treatment temperature, sex and their interactions on fecundity, a generalised linear model, using a Gaussian distribution with an identity link function, was used after log transforming the data. Post-hoc pairwise comparisons for both the CTmin (each sex separately) and fecundity were conducted using Tukey's contrast test. Using a linear mixed effects model, the effects of time, sex, acclimation and treatment temperature on longevity were determined. Post-hoc tests for these analyses involved the inspection of 95% confidence intervals.

2.3. Results

2.3.1. Cold tolerance

CTmin was significantly affected by moth sex ($t = 2.929, P < 0.001$) and temperature treatment ($t = 2.381, P < 0.01$) (Table 2.1), whereas other factors, such as the interaction between sex and acclimation ($t = 1.864, P = 0.064$), sex and temperature treatment ($t = 1.967, P > 0.05$) or acclimation and temperature treatment ($t = 1.330, P = 0.185$) (Table 2.1) were not significant. Females showed no significant differences in terms of CTmin between the acclimation temperatures of 15 and 25 °C when treated at 15 or 20 °C (Figure. 2.1). However, a significant difference was observed at the 10 °C treatment (6.4 and 5.8 °C, respectively). The 20 °C acclimation group differed significantly from both the 15 and 25 °C acclimation groups, except at the 10 °C treatment, where acclimation groups 20 and 25 °C showed similar results (5.7 and 5.8 °C, respectively). When comparing acclimation groups, females from larvae acclimated at 20 °C had the lowest CTmin regardless of treatment temperature. Of the females acclimated at 20 °C, those treated at 15 °C possessed the lowest CTmin (4.8 °C). No significant differences were observed between acclimation and treatment temperatures in adult males (Figure. 2.1). The CTmin values for adult males were 5.9, 6.4 and 6.3 °C, when treated at 10, 15 and 20 °C, respectively.

Table 2.1. Results of two separate generalised linear models (Gaussian distribution with identity link) for the effects of acclimation, treatment temperature and their interaction on critical thermal minimum for mass-reared *Thaumatotibia leucotreta*. Both the full (saturated) and minimum adequate models are presented here. Statistically significant effects are shown in bold. s.e.m.: standard error of the mean.

Model	Parameter	Estimate ± s.e.m.	t-value	P-value
Full model	Intercept	7.688 ± 1.240	6.198	<0.001
	Sex	-3.292 ± 1.754	-1.877	0.062
	Acclimation	-0.100 ± 0.061	-1.646	0.102
	Treatment	-0.089 ± 0.080	-1.115	0.266
	Sex × Acclimation	0.160 ± 0.086	1.864	0.064
	Sex × Treatment	0.222 ± 0.113	1.967	0.051
	Acclimation × Treatment	0.005 ± 0.004	1.330	0.185
	Sex × Acclimation × Treatment	-0.010 ± 0.006	-1.791	0.075
Minimal adequate model	Intercept	5.508 ± 0.182	30.228	<0.001
	Treatment	0.027 ± 0.011	2.381	0.01
	Sex	0.271 ± 0.093	2.929	<0.001

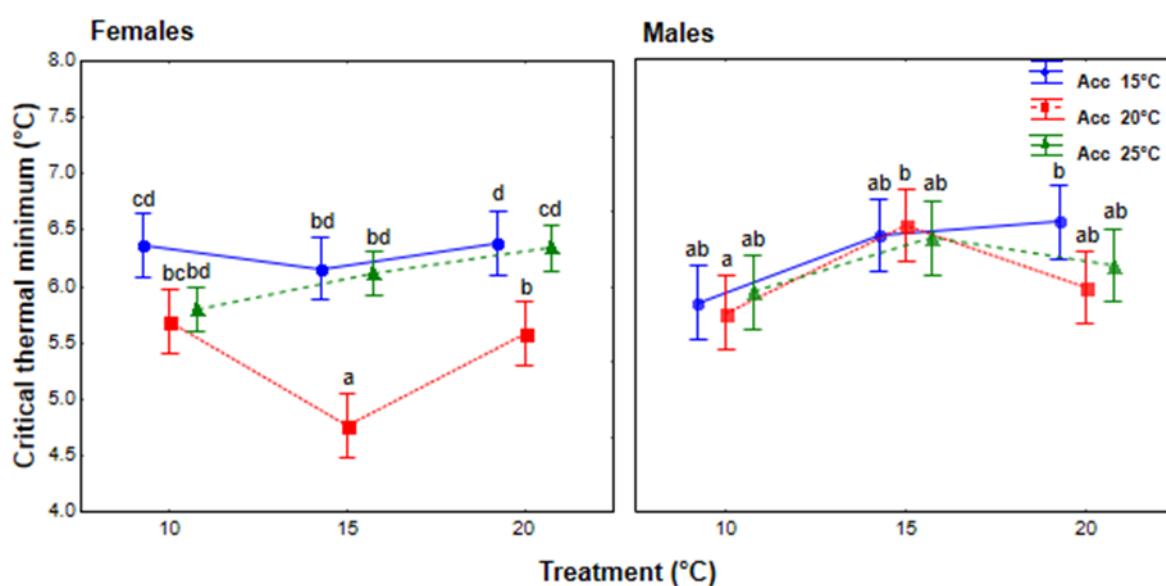


Figure 2.1. Effects of thermal acclimation (Acc) and temperature treatment on the critical thermal minimum of female and male *Thaumatotibia leucotreta* moths. Means with the same letter are not significantly different.

2.3.2. Fecundity

The effects of acclimation and temperature treatment on fecundity were investigated using three different moth pairings: non-treated male \times treated female (NM \times TF), treated male \times non-treated female (TM \times NF) and treated male \times treated female (TM \times TF) (Figure. 2.2). Acclimation temperature, treatment temperature or the interaction between them did not significantly affect the fecundity of the groups where treated males were included (Table 2.2).

When only males had been treated (TM \times NF), the acclimation temperature, treatment temperature and their interaction did not significantly affect the fecundity of the adult moths ($t = 1.208, P > 0.05$; $t = 1.231, P > 0.05$; $t = -1.608, P > 0.05$, respectively) (Table 2.2). Similarly, when both males and females had been treated (TM \times TF), these factors had no significant effects on the fecundity of the adult moths ($t = 1.482, P > 0.05$; $t = 1.245, P > 0.05$; $t = -1.170, P > 0.05$, respectively) (Table 2.2).

Table 2.2. Results of generalised linear models (Gaussian distribution, with identity link) on the fecundity of adult *Thaumatotibia leucotreta* moths for each pairing combination, where normal (N) individuals were acclimated at 25 °C and received no temperature treatment. Treated (T) individuals were acclimated and exposed to temperature treatment (M: male; F: female). Statistically significant effects are shown in bold. s.e.m.: standard error of the mean.

Pairing combination	Parameter	Estimate (\pm s.e.m)	t-value	P-value
TM \times NF	Intercept	5.133 \pm 0.174	1.177	0.246
	Acclimation	0.037 \pm 0.009	1.208	0.234
	Treatment	0.045 \pm 0.011	1.231	0.226
	Acclimation \times Treatment	-0.003 \pm 0.001	-1.608	0.115
NM \times TF	Intercept	2.615 \pm 0.183	1.553	0.128
	Acclimation	0.133 \pm 0.009	2.087	<0.05
	Treatment	0.213 \pm 0.011	2.681	<0.01
	Acclimation \times Treatment	-0.01 \pm 0.001	-2.389	<0.05
TM \times TF	Intercept	1.877 \pm 0.201	0.603	0.55
	Acclimation	0.163 \pm 0.01	1.482	0.146
	Treatment	0.202 \pm 0.012	1.245	0.22
	Acclimation \times Treatment	-0.009 \pm 0.001	-1.170	0.249

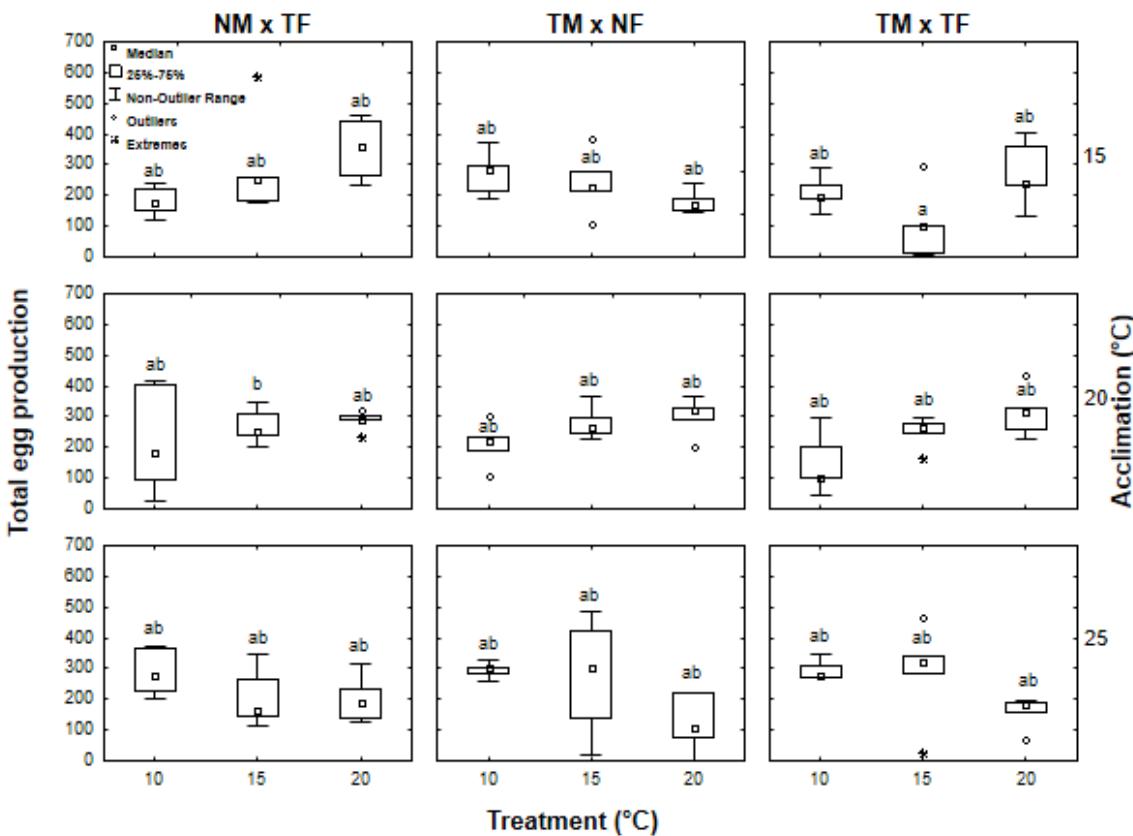


Figure. 2.2. Effects of different mating pairings, thermal acclimation and temperature treatment on the fecundity of *Thaumatotibia leucotreta* moths (total egg production was scored as cumulative eggs laid per pair over five days). Normal (N) individuals were acclimated at 25 °C and received no temperature treatment. Treated (T) individuals were acclimated and exposed to a temperature treatment (M: male; F: female). Means with the same letter are not significantly different (compared across all groups).

However, when only females had been treated (NM × TF) acclimation temperature, treatment temperature and their interaction significantly affected the fecundity of the adult moths ($t = 2.087, P < 0.05$; $t = 2.681, P < 0.05$; $t = -2.389, P < 0.05$, respectively) (Table 2.2). The results therefore indicate that when TM × NF and TM × TF are compared to NM × TF, the contribution of treated males to the fecundity of the adult moths was much less than that of females, indicating that females respond more strongly to prior thermal acclimation than males do.

Comparable effects were observed when treated females were included in pairings. The fecundity of groups acclimated at 15 and 20 °C increased as temperature treatment increased. However, when the moths were acclimated at 25 °C, their fecundity decreased as treatment temperature increased (Figure. 2.2). When only males were treated, groups acclimated at 20 °C showed an increase in fecundity as treatment temperature increased. When comparing pairings with treated males, treatment temperature showed similar patterns across acclimation groups. After treatment at 10 °C, pairings including males acclimated at 25 °C prior to treatment had the highest fecundity. Fecundity was lower when males had been acclimated

at 15 °C and lowest at 20 °C (Figure. 2.2). The opposite was observed in males treated at 20 °C. For both TM × NF and TM × TF, fecundity was highest when males had been acclimated at 20 °C and lowest at 25 °C. In all pairings treated at 10 °C, fecundity was highest when adults had been acclimated at 25 °C prior to treatment. However, when moths were treated at 20 °C, those acclimated at 25 °C had the lowest fecundity. The least variation between acclimation groups was observed when moths had been treated at 15 °C.

2.3.3. Longevity

Longevity, scored as survival probability over time, was tested against the interactions of sex, acclimation and treatment temperature. The influence of these factors had highly significant effects on the longevity of adults (Table 2.3). However, sex was important to survival over time as a distinct factor.

Acclimation had a positive effect on the probability of adult *T. leucotreta* living longer ($Z = 2.017$, $P < 0.01$). However, the effect of treatment was considerably more pronounced. Treatment temperature significantly reduced the probability of adult *T. leucotreta* living longer ($Z = -8.588$, $P < 0.001$) (Table 2.3). Although temperature treatment influenced the longevity of *T. leucotreta*, distinct patterns were not observed in either sex. For example, in the case of males acclimated at 20 °C, those treated at 20 and 10 °C respectively shared LT90, LT50 and LT10 values, but the LT90 and LT50 values of those that had been treated at 15 °C were much higher (Table 2.4). Although a similar phenomenon was observed in females acclimated at 15 °C, this did not appear to be a trend when considering other combinations (Table 2.4). Similarly, even though males and females exhibited similar patterns across acclimation groups when treated at 15 °C, this was not true for the 10 and 20 °C treatments (Table 2.4).

Table 2.3. Results of linear mixed effects models (binomial distribution) assessing survival time (in days) of adult *Thaumatotibia leucotreta* from the experimental treatments, with sex, thermal acclimation and temperature treatments as factors. s.e.m.: standard error of the mean.

Parameter	Estimate (\pm s.e.m)	Z-value	P-value
Intercept	5.083 ± 0.917	5.54	<0.001
Sex	5.658 ± 1.46	3.875	<0.001
Acclimation	0.091 ± 0.045	2.017	<0.01
Treatment	-0.086 ± 0.01	-8.588	<0.001
Sex × Acclimation	-0.245 ± 0.07	-3.489	<0.001
Sex × Survival time	-0.158 ± 0.069	-2.290	<0.01
Survival time × Acclimation	0.0007 ± 0.002	0.373	0.709
Survival time × Sex × Acclimation	0.005 ± 0.003	1.743	0.081

Table 2.4. Survival time (days), represented by the time at which either 90, 50 or 10% of the population survives (LT90, LT50 and LT10 values, respectively) of adult *Thaumatotibia leucotreta* moths from the experimental treatments, with sex, thermal acclimation and temperature treatment as factors. Lower and upper 95% confidence limits (LCL and UCL, respectively) of model fits are shown to allow for post-hoc comparisons.

Individual			LT90			LT50			LT10		
Sex	Acclimation (°C)	Treatment (°C)	Model (days)	LCL	UCL	Model (days)	LCL	UCL	Model (days)	LCL	UCL
Male	25	20	25	23	27	18	17	19	12	10	13
		15	27	25	29	18	17	19	9	7	11
		10	29	28	31	24	23	25	18	17	20
	20	20	24	23	26	18	17	19	12	10	13
		15	32	30	34	22	21	23	12	10	14
		10	24	23	26	18	17	19	12	10	13
	15	20	27	25	29	20	18	21	12	10	14
		15	26	24	27	20	19	21	15	13	16
		10	28	27	30	23	22	23	17	15	18
Female	25	20	29	28	31	23	22	24	18	16	19
		15	32	30	35	20	19	22	8	6	10
		10	30	29	32	24	23	25	19	17	20
	20	20	22	20	24	15	14	16	8	6	10
		15	32	31	35	24	22	25	15	13	17
		10	29	28	31	23	22	24	16	14	18
	15	20	25	23	27	16	15	18	8	6	10
		15	27	26	29	23	22	24	19	17	20
		10	25	23	27	17	16	18	9	7	10

The combination of acclimation and sex had a significant impact on the longevity of adults, specifically females. This is illustrated in Figure 2.3, where the longevity of females was significantly affected by acclimation across all treatments. Females lived longest when acclimated at 25 °C, whereas lifespan diminished when adult females had been acclimated at 15 °C, therefore suggesting a positive correlation between acclimation and longevity. Females acclimated at 25 °C had an average LT50 value of 22.3 days, whereas those acclimated at 15 °C had an average LT50 value of 18.7 days (Table 2.4).

Unlike for adult females, there was no correlation between acclimation temperature and longevity for males, for which all acclimation groups had an average LT90 of 27 days (Table 2.4). However, when considering LT10 values, more variation between acclimation groups existed. Overall, when comparing temperature treatment, the variation between acclimation temperatures was lowest when adult males were treated at 20 °C.

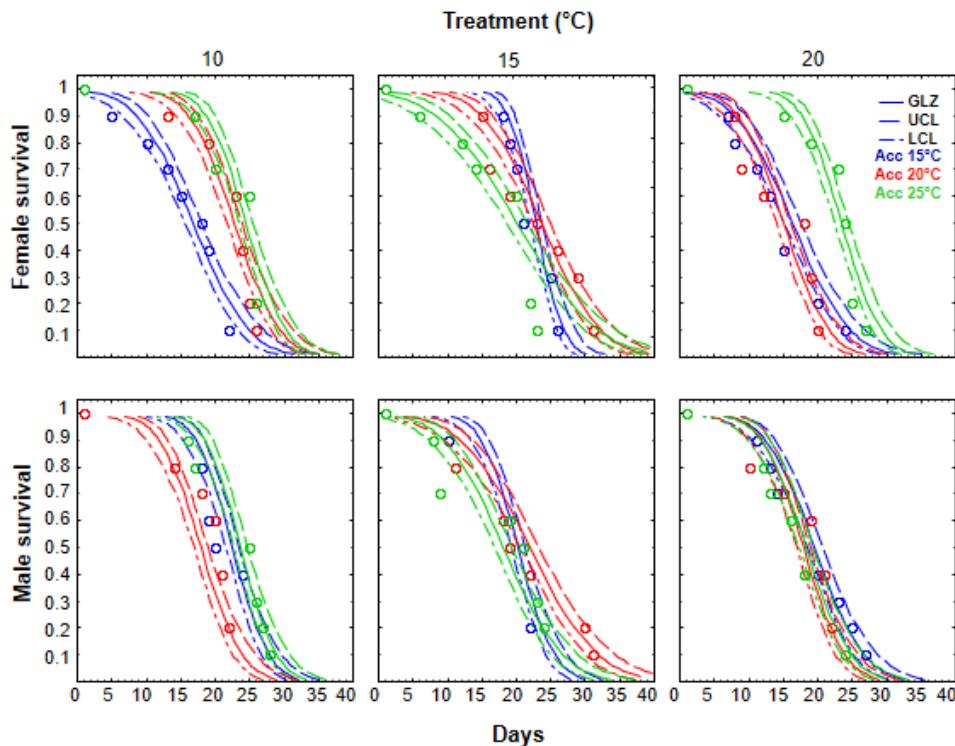


Figure 2.3. Influence of sex, thermal acclimation (Acc) and temperature treatments on the survival time (longevity) of adult *Thaumatomotibia leucotreta* moths. The generalised linear model (GLZ) is displayed along with the predicted upper and lower 95% confidence limits (UCL and LCL, respectively) and the different colours represent the different thermal acclimation groups.

2.4. Discussion

Multiple intrinsic factors (e.g. body condition, hormones and morphology) and extrinsic factors (e.g. environmental conditions into which an insect is released) affect the field performance of sterile insects in an SIT programme (e.g. Dowell *et al.*, 2005; Sørensen *et al.*, 2012). For example, the exact nature of the temperature treatment (intensity and/or duration and controlled fluctuations to benign temperatures) used in rapid chilling for handling and shipping sterile insects prior to field release might influence field performance of mass-reared insects in subtle but distinct ways (Chidawanyika & Terblanche, 2011; Terblanche *et al.*, 2008; Terblanche, 2014).

Consistent, optimised production and provision of high-quality sterile moths that can compete successfully in the field is critical to the success of area-wide pest management (Calkins & Parker, 2005). During each step of production, careful consideration of the temperatures and duration when handling and releasing sterile insects is crucial to prevent potential quality degradation. SIT programmes require the release of sterile insects capable of satisfactory dispersal throughout the wild insect range, competitive flight performance and optimal post-

release longevity to maintain satisfactory over-flooding ratios between wild and released insects.

Diverse aspects of production, handling, irradiation and release protocols may have a detrimental impact on the efficacy of sterile insects in the field (e.g. Carpenter *et al.*, 2004; López-Martínez *et al.*, 2014; Mudavanhu *et al.*, 2014). However, the role of thermal history on field population dynamics or as a means to manipulate or improve mass-reared insects upon release into diverse environmental conditions is not well understood for a broader suite of traits and species nor is it often the focus of investigation, despite the fact that temperature is a relatively easy parameter to alter (Terblanche, 2014) and has practical relevance to climate change forecasting of insect agricultural pests (Bebber *et al.*, 2013; Sgrò *et al.*, 2016; Terblanche *et al.*, 2015).

The present study extended this type of work to the specific case of laboratory mass-reared *T. leucotreta* by enquiring whether temperature history during development interacts with acute thermal conditions experienced by the adult moth. It explored the potential value of experimentally manipulating temperature for integration into the current SIT programme and tested the impacts of such thermal variation on a suite of potentially useful traits, namely cold activity limits, fecundity and longevity. In agreement with a range of studies on other insect species, immediate thermal conditions experienced by the adult insect can have a profound impact on performance for the fecundity and longevity traits scored (reviewed in Sinclair *et al.*, 2012; Sørensen *et al.*, 2012). Somewhat surprisingly, the impact of thermal rearing history on CTmin in *T. leucotreta* was more restricted. Typically CTmin is regarded as a highly plastic, labile trait in insects (Hoffmann *et al.*, 2013; Terblanche *et al.*, 2015), which correlates well with geographic distribution and climate variability (Andersen *et al.*, 2015), demonstrating local climatic adaptation in several cases (e.g. Kleynhans *et al.*, 2014; Sinclair *et al.*, 2012).

Nevertheless, some thermally insensitive insect species have been reported for either supercooling points, CTmin or chilling survival (e.g. Andrew *et al.*, 2013; Slabber *et al.*, 2007). Therefore, the outcomes we obtained are not entirely surprising. It seems that either CTmin in *T. leucotreta* conforms with those in these studies (i.e. genuine lack of plasticity in CTmin), that moths lack a pronounced acute acclimation response in agreement with prior work on adult *T. leucotreta* cold-hardening responses (Stotter & Terblanche, 2009) or that CTmin is not a particularly useful marker of low-temperature performance and acclimation responses (discussed in Andersen *et al.*, 2015; Sinclair *et al.*, 2012). Future work comparing different metrics of cold tolerance (e.g. CTmin, cold survival and chill coma recovery time) across a range of acclimation conditions would be especially useful since work on *Drosophila* suggests

that the underlying genes, mechanisms and responses of these cold tolerance-related traits may be independent (Colinet & Hoffmann, 2012; Rako *et al.*, 2007; Ransberry *et al.*, 2011).

One notable outcome from the results was that much of the larval acclimation responses were sex-dependent. In the cases of CT_{min}, fecundity and longevity, female moths responded more strongly to thermal history across the range of temperatures tested than males did. Sex-dependent thermal acclimation effects are common across a range of insect species and traits (Esterhuizen *et al.*, 2014; Fischer & Fiedler, 2000; Johnstone *et al.*, 2017; Rako & Hoffmann, 2006; Roux *et al.*, 2010). This suggests that there will be an effect on moth fitness following larval acclimation, or when the adults are treated at different temperatures, which, in turn, might have significance for population dynamics modelling of the species in the wild. Sex plays a key role in the longevity of adult moths and is therefore a significant factor governing survival over time, in addition to acclimation and treatment effects. Females lived longer than males did when acclimated and treated at certain conditions, whereas a less prominent effect was seen on males. This might be of value to the SIT, as a cohort could be acclimated at certain conditions, thereby ensuring that sterile females will be available for longer periods for mating with wild males.

The prominent effects of sex on thermal acclimation of females were seen in all cold tolerance, fecundity and longevity results. The pronounced effects of sex in our results may be explained by the female heterogametic sex chromosome system of lepidopterans, with WZ females and ZZ males (Sahara *et al.*, 2012). The W-chromosome is maternally inherited and contains repetitive elements that are not subject to recombination during meiosis. In some animals, repetitive elements can respond to environmental cues and may contribute to plasticity (Schmidt & Anderson, 2006; Śliwińska *et al.*, 2016). In *Drosophila melanogaster*, with an XX/XY system (heterogametic male), the Y-chromosome is associated with adaptive phenotypic variation (Lemos *et al.*, 2008). Thus, in a WZ/ZZ system, the lepidopteran W-chromosome may contribute to phenotypic plasticity in a similar way to the *Drosophila* Y-chromosome, potentially through epigenetic effects on autosomal genes. Determination of single nucleotide polymorphisms or genes involved and chromosomal mapping are presently limited by the lack of an annotated genome for *T. leucotreta*.

In conclusion, these results indicate a complex interplay between thermal history during larval development and adult thermal treatment on lower critical activity limits, fecundity and longevity. This study also highlights that much of the thermal acclimation response influences female performance to a much greater extent than that of males. Moth sex is therefore an important consideration in population dynamics or SIT modelling and potential programme efficacy. This suggests that females might have greater significance in an SIT programme

than previously considered, as they live longer and are more flexible in their physiological trait performance. Further investigation is necessary to determine whether the detected change in longevity might persist in the field, as this may allow a reduction in release frequency, as sterile females might still provide adequate control through thermal manipulation prior to release. In a broader context for SIT or pest management, careful consideration should be given to the outcome of prior and immediate environmental conditions on specific traits for maximising field performance of mass-reared *T. leucotreta*.

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CHAPTER 3. COLD TREATMENT ENHANCES LOW-TEMPERATURE FLIGHT PERFORMANCE IN FALSE CODLING MOTH, *THAUMATOTIBIA LEUCOTRETA* (LEPIDOPTERA: TORTRICIDAE)

This chapter has been submitted to *Agricultural and Forest Entomology*, titled 'Cold treatment enhances low-temperature flight performance in false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae)' by Nevill Boersma, Leigh Boardman, Martin Gilbert and John S. Terblanche.

3.1. Introduction

The sterile insect technique (SIT) has been effectively used to suppress insect pests, some of which are vectors of human, plant and animal disease (Krafsur, 1998; Lindquist *et al.*, 1992; Simmons *et al.*, 2010). This technique uses mass rearing and non-lethal irradiation to sterilise insects in the laboratory. After sterilisation, insects are released to mate with wild conspecifics, resulting in non-viable offspring and leading to a decline in the target pest population (Dowell *et al.*, 2005; Vreysen & Robinson, 2010). Sterile insects have been used in integrated programmes against various pests since the beginning of the 20th century. The most widely recognised successful application of this technique is probably the eradication of the screwworm (*Cochliomyia hominivorax*) (Diptera), a parasitic fly that feeds on healthy flesh of animals (Klassen & Curtis, 2005). Other examples include the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), in the USA and codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), in Canada (Judd *et al.*, 2005).

A major challenge to the success of SIT is the competitiveness and field performance of mass-reared insects (Bloem *et al.*, 1998; Bloem *et al.*, 2004; Enserink, 2007; Judd *et al.*, 2006; Simmons *et al.*, 2010; Sørensen *et al.*, 2012; Terblanche & Chown, 2007). The SIT aims to achieve a high percentage of mating between the released sterile and natural populations to reduce reproduction to a level below the economic threshold. The production of high-quality and competitive individuals is therefore required to achieve this aim (Parker, 2005). In an SIT programme, thermal conditions during or after insect development may affect various traits, such as body size, fecundity, longevity and flight performance (Boersma & Carpenter, 2016; Boersma *et al.*, 2018; Sørensen *et al.*, 2012; Terblanche, 2014). Typically, SIT programmes use optimal rearing temperatures to maximise production regardless of post-release environmental conditions, but this approach may negatively impact the dispersal potential of the mass-reared insects upon release (Bloem *et al.*, 2004; Chidawanyika & Terblanche, 2011; Sørensen *et al.*, 2012; Matveev *et al.*, 2017).

Treatment at cold temperatures is often used as a standard procedure to immobilise insects before release. Such cold treatment eases the collection, handling, irradiation, transportation and release of these insects. However, chilling and long cold-temperature storage may adversely impact the field performance if negative effects persist after rewarming and release, possibly leading to poorer performance or competitiveness than that of their wild counterparts (Boersma & Carpenter, 2016). On the other hand, insects cold-treated prior to release could disperse and perform better under cooler field conditions through plastic physiological adjustments (i.e. beneficial acclimation). Beneficial acclimation is widely debated in the evolutionary physiology literature (Chidawanyika & Terblanche, 2011; Chown & Terblanche, 2007; Judd & Gardiner, 2006) but is valuable to SIT programmes seeking to manipulate insect field performance (Sørensen *et al.*, 2012). Beneficial acclimation gives an organism a performance advantage in a particular environment over another that was not acclimated to that particular environment (Leroi *et al.*, 1994; Wilson & Franklin, 2002). The physiological response to the environmental stress could enhance the fitness of insects during subsequent sub-optimal conditions, since they are able to continue performing vital activities, such as mating, feeding and flying (Fasolo & Krebs, 2004; Powell & Bale, 2005).

Acclimation can occur over a longer pre-exposure to sub-optimal temperatures whilst hardening, a rapid response to extreme or acute environments, can be achieved over a shorter period (Colinet *et al.*, 2015; Denlinger & Lee, 2010; Ju *et al.*, 2013; Shreve *et al.*, 2004). No clear distinction between acclimation and cold hardening has been established, however, as acclimation in one species might be regarded as cold hardening in another (Teets & Denlinger, 2013). For example, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) acclimated in two minutes, whereas *B. oleae* (Rossi) required several days (Fletcher & Zervas, 1977; Meats, 1976; see discussion in Weldon *et al.*, 2011). Treatment temperature and exposure time therefore have species-specific physiological effects (Colinet *et al.*, 2015; Denlinger & Lee, 2010; Ju *et al.*, 2013; Shreve *et al.*, 2004). Furthermore, since insects' tolerance to environmental changes depends upon the timing and duration of their exposure to prior environmental stress (Sgrò *et al.*, 2016), thermal history in SIT programmes may affect the competitiveness between field and sterile mass-reared individuals, since multiple behaviour and locomotor performance traits can be strongly modified by thermal history (e.g. Chidawanyika & Terblanche, 2011; Esterhuizen *et al.*, 2014; Kristensen *et al.*, 2008). Because of a complex range of responses to thermal stress, further investigation is needed to provide an insight into any uncertainties regarding these responses of field released insects (Terblanche, 2014).

The false codling moth, *Thaumatotibia leucotreta* (Meyrick), is a phytosanitary pest of citrus fruit in southern Africa (Bloem *et al.*, 2004). An SIT programme in citrus orchards was

established in Citrusdal, Western Cape, South Africa, in 2007. Sterile male and female *T. leucotreta* adults were released during the growing and harvesting season, from spring to early winter (Hofmeyr *et al.*, 2005). Since the initiation of the programme, the wild *T. leucotreta* population has declined 10-fold. Pre-harvest crop losses due to *T. leucotreta* have also been reduced by 93% and post-harvest rejection by 38% (Hofmeyr *et al.*, 2015). Despite the broad success of the commercial SIT programme, moth performance at low temperatures remains a challenge (Stotter & Terblanche, 2009).

The thermal history of mass-reared *T. leucotreta* has a marked impact on a range of traits, such as longevity, fecundity and cold tolerance, with the extent of the impact varying between the sexes (Boersma *et al.*, 2018). Sterile *T. leucotreta* adults have a minimum activity threshold temperature of approximately 10–15 °C, indicating reduced cold tolerance relative to the conditions in which they must perform during colder months (Stotter & Terblanche, 2009). Similar effects have been observed in other SIT programmes involving the codling moth in Canada (Judd *et al.*, 2004). Thermal preconditioning is viewed as a possible solution to poor performance post-release at low temperatures in a variety of species (Fay & Meats, 1987; Chidawanyika & Terblanche, 2011; Sørensen *et al.*, 2012; Terblanche, 2014).

We aimed to determine the effect of thermal history on the flight performance of *T. leucotreta* under different thermal conditions. We focused on the effect of pre-release adult cold treatment (i.e. short-term exposure to cold temperatures) on the subsequent laboratory and field flight performance of the moths. Specifically, we tested the hypothesis that short-term exposure of sterile adult *T. leucotreta* to low temperatures prior to release will enhance flight performance, and hence recapture rates, under post-release cold conditions. This would indicate that plastic thermal physiology benefits flight performance that is dependent on the prevailing thermal conditions. The implications of these results are discussed in the context of enhancing flight performance of *T. leucotreta* in an SIT programme.

3.2. Materials and methods

3.2.1. Insect rearing

The *T. leucotreta* culture was established in 2006. This laboratory population from the XSIT (Pty) Ltd mass-rearing facility (Citrusdal, Western Cape, South Africa) was established from wild moths collected in orchards in Citrusdal. The colony was augmented with wild insects at several intervals to prevent inbreeding depressions or genetic divergence from natural populations. The population was maintained at 25 ± 1 °C under a 12:12 (L:D) photoperiod on a maize-based diet. Relative humidity was not strictly controlled and ranged between 40 and 60% (101A, MadgeTech, Warner, New Hampshire, USA).

3.2.2. Treatments

Moths for both laboratory and field flight performance tests were obtained from the above-mentioned commercial colony. Moths were collected within 12 h post eclosion. For laboratory assays, both male and female moths were used, as previous research showed significant differences between the sexes with regards to field traits, such as cold tolerance, fecundity and longevity (Boersma *et al.*, 2018). For field flight performance, however, only males were tested, as pheromone traps were used to monitor flight in the field.

Immediately after emergence, all moths were irradiated at 150 Gy in a panoramic cobalt-60 source (dose rate: 3.75 Gy/min; caesium-137 source (37 kBq reverence) with cobalt-60 (740 kBq) irradiator), after which they were kept at 25 °C for 20 min before they were either cold-treated overnight at 2±1 °C for 16 h in an environmental chamber (Binder climate chamber; KBW 240 Binder, Tuttlingen, Germany) or kept as a control group at 25±1 °C in a temperature-controlled room (confirmed using a temperature data logger, 101A, MadgeTech). A cold-treatment temperature of 2 °C was chosen to ensure that moths were kept below their critical thermal minimum (CT_{min}) (~6 °C) (Terblanche *et al.*, 2017) but above sub-zero temperatures to prevent ice formation. The chosen treatment temperature was deemed appropriate, as the current handling temperature of the SIT operation (8–10 °C) has revealed poor recapture rates under both warm and cold environmental conditions (Boersma & Carpenter, 2016; Stotter & Terblanche, 2009). Because commercially-reared adults are kept in the dark after eclosion in the SIT facility, treatments were conducted overnight for 16 h (17:00–9:00) to simulate the facility's current handling and transportation protocols and procedures.

On the morning after treatment, both the treated and control groups were marked with 0.5 g of differently coloured fluorescent micronized dust (DayGlo Color Corp., Cleveland, Ohio, USA) to allow distinction between treated and control moths, after which they were kept at 25±1 °C for 60 min to recover. Treatment colours were randomised between groups (i.e. colours were randomly assigned to either group) on each experimental day to eliminate any influence of dye colour on recapture rate. After recovery, the flight performance of moths was assessed in the laboratory or field, as described in the sections below. Scoring of recaptures was done using a UV light in a dark room at the laboratory to differentiate between the coloured markings.

3.2.2.1. Laboratory flight performance

For laboratory tests, a total of 720 moths (360 males and 360 females) were separated from the XSIT colony. Of these, 180 males and 180 females were subjected to the cold-treatment described in section 3.2.2 and equal numbers were kept as a control.

Laboratory flight performance tests were conducted under two different temperature conditions, namely 15 ± 1 °C (cold) and 30 ± 1 °C (warm), from September 2015 to June 2016. The sexes were tested separately, and the control and treated groups of each sex were tested together under each temperature condition. For each test, 30 *T. leucotreta* adults of the same sex from the control and cold-treated groups, respectively (i.e. 30 from the control and 30 from the cold-treated group) were placed in two separate Petri dishes (diameter of 45 mm, height of 15 mm). These Petri dishes were placed on a pedestal in a round water basin ($r = 410$ mm) in a dark, temperature-controlled room ($3 \times 4 \times 1.8$ m) set to either 15 or 30 °C. The two test temperature conditions were simulated via an air-conditioning system in the room, with the air temperature monitored with a digital thermometer (Fluke II, Fluke Corporation, Everett, Washington, USA) connected to a type T thermocouple (36 standard wire gauge) and recorded at an interval of 1 Hz during all experiments. A blue fluorescent light (12 W Hand Scanner UV Light with UV Filter, Equip, Johannesburg, South Africa) was placed 2 m away from the basin to attract the moths to fly over the water obstacle. Moths were left to fly for 2 h, after which each individual was categorised according to its dispersal ability, namely 'sustained flyer' (those that flew from the Petri dish to the light), 'partial flyer' (those that landed in the water) and 'non-disperser' (those that remained at the release site). As mentioned above, the control and cold-treated moths were distinguished based on dye markings. Each test under each temperature condition for each sex was replicated at least three times. No moths were re-used between tests.

3.2.2.2. Field flight performance

Field release–recapture experiments were performed in a citrus orchard 5 km from the rearing facility in Citrusdal. Thirty yellow delta traps containing sex pheromone lures ((E)-8-, (Z)-8- and (E)-7-dodecenyl acetate; CHEMPAC® false codling moth, Paarl, South Africa) and sticky cards (CHEMPAC) for recapture were set up. The traps were placed in three groups of 10 rows of orange trees each with three rows of trees between each group. The traps were placed 6 m apart in the tenth tree from the border of each row, on the eastern side of the tree to distribute the sex pheromones into the orchard. The traps were suspended 1.5 m above the ground in the outer foliage of the trees. Leaves and twigs around the traps were removed to ensure unobstructed air flow and unhindered access for moths.

Upon eclosion, 600 *T. leucotreta* male moths per replicate were separated from the XSIT colony and divided equally between two Petri dishes (diameter of 70 mm). All moths were irradiated and thereafter 300 were cold-treated overnight, as described in section 3.2.2, while the remaining 300 were kept as a control. Moths were marked as described in section 3.2.2. These moths were transported to the test orchard in the separate Petri dishes stored in an insulated box at 25 °C. Although *T. leucotreta* adults are nocturnal, moths were evenly

released by hand (approximately 10 cold-treated and 10 control moths per row) at 10:00 to simulate commercial SIT releases for all 30 rows, from a position of 20 m north of the traps and at the end of each row.

The traps were left undisturbed for three days, after which the sticky floors were collected and the number of fluorescent *T. leucotreta* individuals per trap was counted. New sticky floors and pheromone lures were placed in the traps at the beginning of each trial. This was repeated over two seasons, namely seven times in early summer (18 September to 30 October 2015) and seven times in winter (3 June to 16 August 2016), to ensure that successful release–recapture trials were performed at both cooler and warmer temperatures. An electronic temperature data logger, set to log every hour (accuracy: ± 0.1 °C) (101A, MadgeTech), was placed centrally between the branches of a tree in the orchard to monitor variations in air temperature. Additional data on the climate and environmental conditions associated with the orchard (Table 3.1) were obtained from the Citrusdal North weather station (Agricultural Research Council) approximately 5 km away from the test orchard for three nights and days after release. The total number of released moths for the trial was 9000.

Table 3.1. Additional climatic data and environmental conditions measured associated with orchard conditions for 72 hours after release.

Parameter	Measurement duration per day	Unit
Average maximum night temperature	18:00–06:00	°C
Absolute maximum night temperature		
Average minimum night temperature		
Absolute minimum night temperature		
Mean night temperature		
Cumulative number of hours below 6 °C		
Cumulative number of hours below 10 °C		
Average maximum relative humidity	3 days	%
Average minimum relative humidity		%
Average total evaporation		mm
Average Wind speed		m/s
Total rainfall		mm
Average total radiation		MJ/m ²

3.2.3. Statistical analyses

3.2.3.1. Laboratory assays

For the laboratory assays, we determined which of the four independent variables (sex, treatment, test temperature and biological replicate) played a role in the flight performance of *T. leucotreta* and whether any interactions between these variables existed. The statistically

significant effect of the independent variables on the dependent variable was tested against the dependent variable (dispersal), containing specific categories, namely non-flyers, partial flyers and sustained flyers, while non-flyers was used as a baseline category. Analyses were conducted using R software (v. 3.0.3; R Foundation for Statistical Computing, 2008, Vienna, Austria; packages ‘nnet’ and ‘mlogit’). Data were analysed as the total recaptures for each of the 3 replicates under each temperature condition for each sex and treatment group. Differences in flying performance between sustained, partial and non-flyers and the difference between the treatment and control group were determined using a nominal multinomial model, also known as a multinomial logit model. This model was preferred over the ordinal multinomial regression, as the latter did not hold the proportional odds assumption. Furthermore, the nominal multinomial model explains the relative likelihood of being in one category versus being in the reference category (the log ratio of the variables) using a linear combination of predictor variables. Consequently, the probability of each outcome is expressed as a nonlinear function of the predictor variables. In this study, a distributional assumption was made in terms of the dependent variables. Subsequently, the mean dispersal was linked to a linear function of the predictors through a generalised logit link function (Field *et al.*, 2012; Qian *et al.*, 2012).

3.2.3.2. Field assays

The effect of cold or control treatments on the field flight performance of male moths was investigated to determine the importance and role of each variable (Table 3.1) in terms of the dispersal ability of *T. leucotreta*. Analyses were conducted using R software v. 3.0.3 (packages ‘nnet’ and ‘randomForest’) and Figures were drawn in Statistica (v. 13.3; Statsoft Inc., Tulsa, Oklahoma, USA). Because environmental factors could be closely correlated, random forest analyses were conducted to determine which independent environmental factor contributed the most to the flying ability of *T. leucotreta* adults (Chen & Ishwaran, 2012; Liaw & Wiener, 2002). Using a stepwise ascending variable strategy, the average decline in accuracy of each variable was determined with an iterative algorithm that fits a different regression model at each node and over each iteration. The greater the decline due to the permutation of a single variable was, the greater the importance of this variable was relative to the other tested variables (Genuer *et al.*, 2010). The most important independent environmental variable identified in the random forest analyses (average maximum night temperature) was then run in two separate models to determine the effects of (i) average maximum night temperature and treatment groups and (ii) the interaction with average maximum night temperature. Generalised linear models (negative binomial regression) were run using R to assess the main effects and interactions. Recapture count (x) was used as the dependent variable, expressed as a fraction:

$$x = (\text{number of moths recaptured}) / (\text{number of moths released})$$

From these two models, the best fit model with the lowest Akaike information criterion value (estimator of relative quality of statistical models for a given data set) was chosen. Data were analysed as the total recaptures for both cold treated and control group at different average maximum night temperatures. The data distribution, degrees of freedom and residual deviance were examined and confirmed so that model assumptions were not violated.

3.3. Results

3.3.1. Cold treatment effects on flight activity in the laboratory

The flying ability of *T. leucotreta* in the laboratory was affected by the interaction between the treatment group and test temperature, as well as that between treatment, sex, test temperature and biological replicate. The interaction between treatment and test temperature was highly significant for both partial ($Z = 4.642; P < 0.01; df = 1$) and sustained flyers ($Z = 4.931; P < 0.01; df = 1$) (Table 3.2), whereas the biological replicate showed interactions between treatment ($Z = -2.526; P < 0.05; df = 1$) and sex ($Z = 2.561; P < 0.01; df = 1$) for partial flyers compared to non-dispersers. Treatment had a significant effect on sustained flyers ($Z = -2.057; P < 0.05; df = 1$). The effect of sex was significant for both partial ($Z = -2.5; P < 0.05; df = 1$) and sustained flyers ($Z = -2.721; P < 0.01; df = 1$), whereas the effect of test temperature was significant between non-dispersers and partial flyers ($Z = -1.840; P < 0.1; df = 1$). There was no significant interaction between treatment and sex, and test temperature and sex on the flight performance of adult *T. leucotreta*, whereas biological replicate had no effect on the flying ability of *T. leucotreta* adults (Table 3.2).

Table 3.2. Summarised output of laboratory results of adult *Thaumatotibia leucotreta*. A multinomial model was used to test the effects of cold temperature treatment, test temperature sex and replicate on the flight performance of adult moths. SE: standard error of the mean. Text in bold indicates significant relationships.

Effect	Partial flyers				Sustained flyers			
	Coefficient	SE	Z-value	P-value	Coefficient	SE	Z-value	P-value
Treatment	0.236	0.579	0.406	0.684	-1.882	0.914	-2.057	<0.05
Sex	-1.532	0.612	-2.500	<0.05	-2.622	0.963	-2.721	<0.01
Test temperature	-1.173	0.637	-1.840	<0.1	0.637	0.818	0.777	0.436
Replicate	-0.228	0.238	-0.955	0.774	0.149	0.287	0.517	0.604
Treatment: Sex	0.274	0.433	0.632	0.527	0.192	0.605	0.316	0.751
Treatment: Test temperature	2.002	0.431	4.642	<0.01	3.268	0.662	4.931	<0.01
Sex: Test temperature	0.214	0.432	0.494	0.621	1.177	0.773	1.521	0.12
Treatment: Replicate	-0.672	0.266	-2.526	<0.05	-0.088	0.334	-0.264	0.791
Sex: Replicate	0.666	0.259	2.561	<0.01	0.186	0.339	0.548	0.583
Test temperature: Replicate	-0.072	0.267	-0.268	0.788	-0.613	0.369	-1.660	<0.1

Cold-treated males were more likely to fly under cooler laboratory conditions than were the control males (21 vs. 3%) (Figure. 3.1). Under cold conditions, cold-treated males were also more prone to leave the Petri dish than the control males. More than 80% of the control moths did not fly from the Petri dish. When cold-treated males were released under warm conditions, they were less prone to fly, whereas nearly 40% of control moths successfully flew and another 20% were partial flyers (Figure. 3.1). Similar effects were seen in females (Figure. 3.1), although they were generally less prone to fly than were males, regardless of the temperature, whereas cold-treated females showed a greater attempt to fly under cold conditions, with up to 30% being partial flyers.

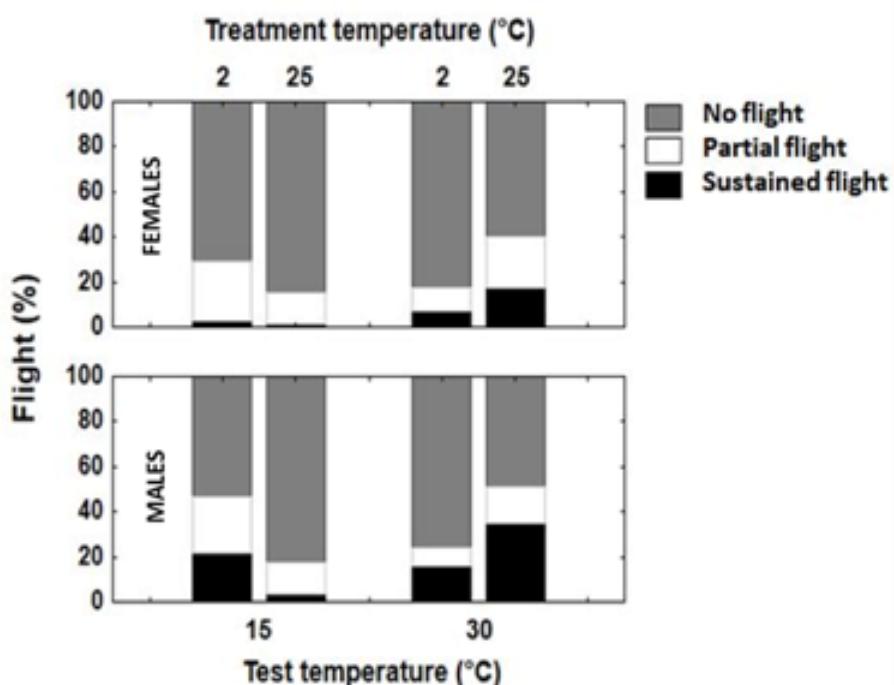


Figure. 3.1. Results of laboratory flight performance assays of cold-treated (2 °C) and control (25 °C) *Thaumatomibia leucotreta* female (F) and male (M) adults as measured at different test temperatures. Flight performance was measured by three possible responses: successful flight (sustained flight), landing in the surrounding water (partial flight) and non-dispersal (no flight).

3.3.2. Effects of cold temperature treatment on field recapture rates

The influence of different environmental parameters on recapture rates is shown in Figure. 3.2. The flight performance of *T. leucotreta* males in terms of recapture rates in the field was significantly influenced by the interaction between treatment and average maximum night temperature ($Z = 2.534, P < 0.05$; $df = 28$) and the effect of treatment ($Z = -2.584, P < 0.01$; $df = 28$) as a single factor (Table 3.3).

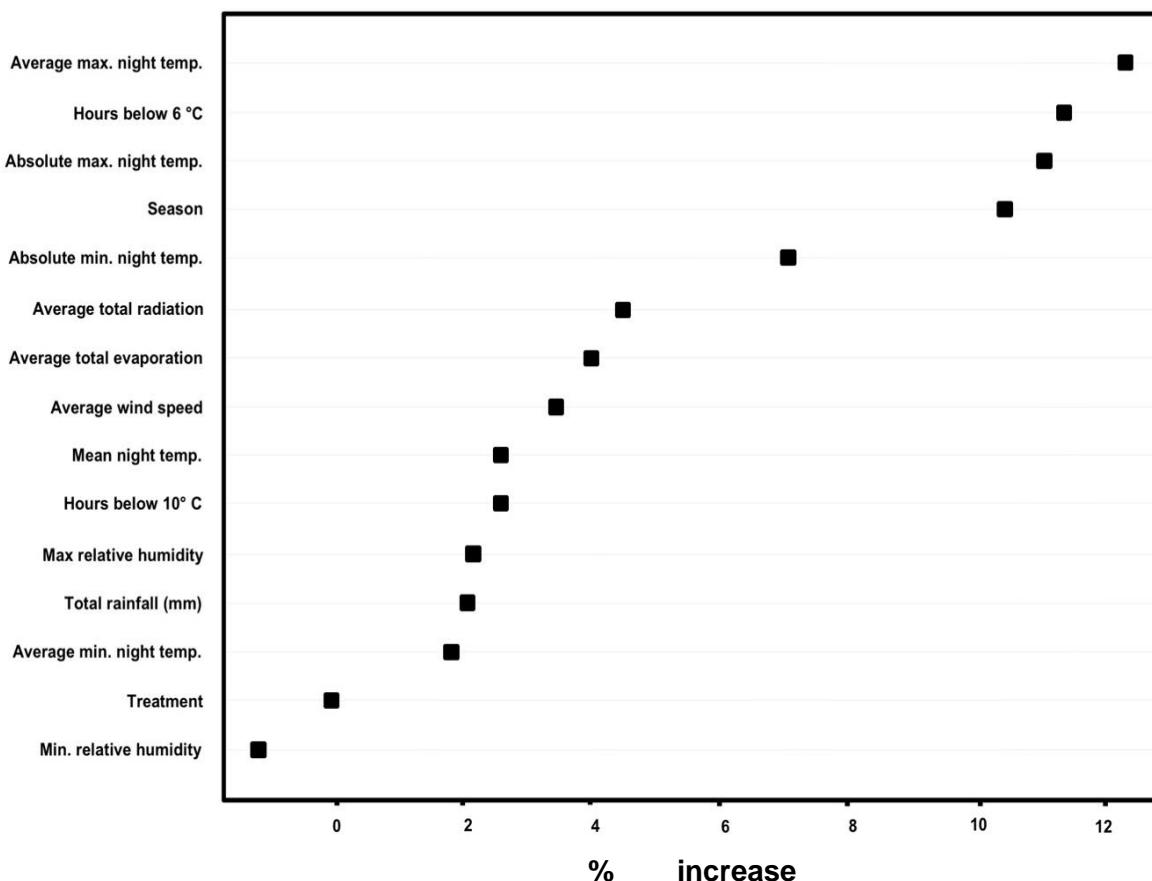


Figure. 3.2. Results from the random forest analyses, displaying the effect of the different independent variables contributing to the flight performance of adult *Thaumatomibia leucotreta* scored as a percentage increase in the model's mean-squared error (MSE). The higher the value (MSE), the more important the variable.

Table 3.3. Summarised results of a general linear model assessing flight performance (in terms of recapture rates) of adult *Thaumatomibia leucotreta* in field tests with average maximum night temperature, treatment and their interaction. SE: standard error of the mean. Text in bold indicate significant relationships. Degrees of freedom = 28.

	Estimate ± SE	Z-value	P-value
Intercept	5.245 ± 1.446	3.626	<0.001
Average maximum night temperature	-0.066 ± 0.075	-0.883	0.377
Treatment	-2.325 ± 0.899	-2.584	<0.01
Average maximum night temperature: Treatment	0.117 ± 0.464	2.534	<0.05

Clear differences were observed between recapture rates of cold-treated and control moths over three days (Figure. 3.3A). The number of cold-treated males recaptured was higher than that of control moths at average ambient night temperatures below 17 °C. As the average maximum night temperature decreased, the number of treated and control males captured decreased, but recapture rates of the latter were less than those of the cold-treated group

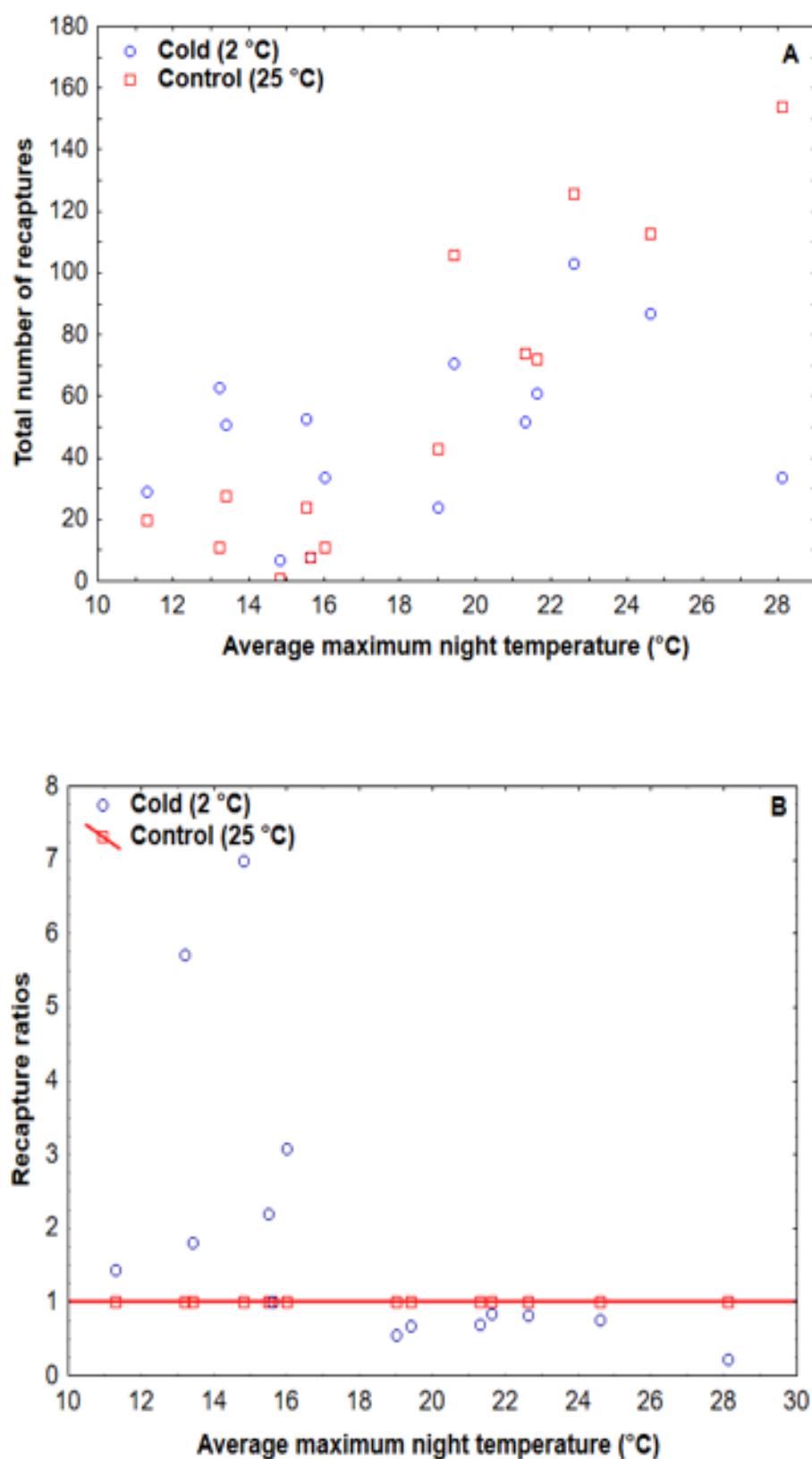


Figure 3.3. Summarised results of field recapture rates (number of male moths) of *Thaumatotibia leucotreta* to estimate flight performance. Each data point represents the moths recaptured on a specific release date. Recapture rates of moths are shown in relation to average maximum night temperatures either as absolute numbers (A) or as a ratio of the control moths (B).

(Figure. 3.3A). For temperatures above 17 °C, cold-treated males had a poorer recapture rate than the control moths did.

Therefore, most of the data points below 17 °C representing cold-treated moths were above the control line (Figure. 3.3B). Generally, recapture rates of both groups were better when the average maximum night temperature was above 17 °C. Under these circumstances, control moths performed better than cold-treated moths did. However, during cold spells (temperatures below 17 °C), cold-treated males were recaptured in greater numbers than were the control moths. At 17 °C, there was no significant difference between recapture rates of cold-treated and control moths, whereas at 15 °C an increase of nearly 50% in the number of cold-treated moths comparing to the control was observed. At 10 °C, no control moths were caught, whereas 29 treated moths were recaptured. Recapture rates of cold-treated moths therefore increased as the ambient temperature decreased. In contrast, the recapture rates of cold-treated moths at warm ambient night temperatures were poorest, with 29% fewer males caught at 20 °C and 53% fewer at 30°C than the control.

3.4. Discussion

Our results reveal that plastic physiological responses in adult *T. leucotreta* elicited during the cold treatment increased both their laboratory and field flight performance under cooler thermal conditions. The effects of cold treatment were more pronounced in the laboratory, whereas these effects were weaker, but nonetheless significant, in the field, probably due to greater variability in factors influencing flight. The laboratory assays showed that almost 20% more cold-treated adult males than control moths took flight. Few moths were prone to take flight during the laboratory trials, but the overall tendency to fly was greater in the field. This is because only 40–50% of control moths, even at warm temperatures, attempted flight in the laboratory. In addition, each flight assay in the laboratory only allowed the moths 2 h to fly from the Petri dish. We suggest that, apart from the shorter exposure time, the reason for the lower flight performance in the laboratory may be that the fluorescent light was not as powerful an attractant as either real or artificial pheromones would be in the field. Moreover, in the field, there may also be important navigational or dispersal cues from horizon lines, polarised light, wind and pheromones, which may promote dispersal over laboratory conditions (Cardé & Willis, 2008; Drake, 1983).

As in the laboratory assays, adult moth recapture rates from our field assays showed that cold-treated males had a greater affinity for flying under cold conditions than control males did, with twice as many recaptures of the former at cooler temperatures (below 17 °C). This

suggests that the efficacy of the SIT programme could be increased by treating adults below their CT_{min} for release during the winter or cold spells. Although a greater recapture rate does not necessarily prove improved efficacy of an SIT programme, it is considered an essential marker of field flight performance and is routinely assessed in SIT programmes (see discussion in Chidawanyika & Terblanche, 2011). Furthermore, our results demonstrate the potential for thermal treatments to offset poor low-temperature performance of mass-reared moths and form a baseline for moving towards demonstrating field SIT efficacy. Further work is necessary to validate other mating and fitness parameters in the field, such as competitive mating events and reduction of the wild moth population under similar ambient conditions. Given our results, however, it can be argued that cold treatment provides mass-reared *T. leucotreta* with a significant advantage to better perform under cooler field temperatures and that this is a worthwhile advantage to pursue. Although this might complicate logistical planning for SIT programmes, such treatments could be easily incorporated during normal handling and transportation procedures.

Our results provide laboratory and field support for the beneficial acclimation hypothesis. In all cases, *T. leucotreta* performed best in environments simulating pre-release conditions (i.e. low temperatures) and poorer in environments to which they had not previously been exposed (Chidawanyika & Terblanche, 2011; Kristensen *et al.*, 2008). This is clear when the advantage (in terms of flight performance) of cold-treated moths released at low temperatures in the field is considered, calculated as a ratio compared to the performance of control moths (Figure. 3.3B). The benefits include better recapture rates and enhanced dispersal performance under cold conditions (5–17 °C). However, when cold-treated males were released at warmer ambient temperatures (17–30 °C), a decline in recapture rates was observed.

Similar effects were found in *C. pomonella* when approximately four times more treated than control moths were recaptured under cooler ambient conditions if they had been acclimated at a low temperature in a laboratory prior to release. However, these improvements in the flight performance of the cold-acclimated moths came at the cost of poorer performance in warmer environments (Chidawanyika & Terblanche, 2011; Judd *et al.*, 2004; Mateev *et al.*, 2017). A different study on the flight performance of *C. pomonella* concluded that fluctuating rearing temperatures increased flying ability of adult moths at cooler temperatures compared to moths reared at a constant temperature. Moreover, the recapture rates of diapaused moths were even higher but with a significant decrease in fecundity (Chidawanyika & Terblanche, 2011; Bloem *et al.*, 1998).

Although improved recapture rates of mass-reared *T. leucotreta* during the warmer part of the season were recorded when adults were not subjected to any cold temperature protocols, this

is not a viable option, as the moths will mate and injure themselves before being released into the field (Boersma & Carpenter, 2016). Moreover, such moths do not perform well under cold ambient conditions, reducing the window for efficient recaptures in the winter (Stotter & Terblanche, 2009). Similar outcomes have been observed in *C. pomonella*. Compared to control adults, adults cold-treated prior to release used less energy while flying with increased distance flown (Judd *et al.*, 2004; Mateev *et al.*, 2017).

Boersma *et al.* (2018) found a difference between male and female performance (cold tolerance, fecundity and longevity) in *T. leucotreta* under different acclimation and treatment temperatures. Females demonstrated a greater response to environmental variation than did males and therefore exhibited a greater capacity for phenotypic plasticity in terms of CT_{min}, longevity and fecundity. However, this was not true for flight performance in this study, as males were more prone to fly than were females under different environmental conditions. Nevertheless, this does not mean that females are less effective in an SIT programme under cooler climatic conditions, as they may still contribute to the programme's efficacy in attracting wild adult males.

The cellular and physiological mechanisms underlying the responses to different environmental conditions in *T. leucotreta* remain unclear. It can be argued that hormesis (the process whereby a beneficial effect results from exposure to low measures of stress) through cold treatment as a mild stressor may mitigate any detrimental effects of radiation that potentially reduce moth fitness. Cold-treating moths after radiation may decrease indirect radiation damage or result in improved damage repair mechanisms upon rewarming, while the application of anaesthesia with the use of carbon dioxide or nitrogen could prevent injury (López-Martínez & Hahn, 2012; López-Martínez *et al.*, 2014). The use of anoxia (nitrogen) while sterilising Lepidoptera has been shown to benefit flight performance in the cactus moth, *Cactoblastis cactorum* (Berg) (López-Martínez *et al.*, 2014). Further work on this topic needs to be done, as the cold treatment of *T. leucotreta* males after radiation has led to poorer recapture rates under warm environmental conditions, resulting in shorter flying distances compared to those of males not subjected to cold treatment (Boersma & Carpenter, 2016).

Finally, our study demonstrated how possible plastic physiological responses to thermal treatment leads to altered laboratory and field flight performance in *T. leucotreta*. Increased recapture rates of sterile adults of up to 50% during the winter months will most probably benefit SIT programmes in terms of the population growth of wild *T. leucotreta* in the summer. Better recapture rates in cooler months as a result of improved flight performance of mass-reared, sterile, cold-treated *T. leucotreta* could lead to a progressive decline of the wild population, with each season starting with a smaller wild population, thereby increasing the

overall efficacy of the SIT programme. This study provides evidence that temperature treatment in the adult stage of mass-reared *T. leucotreta* could potentially improve the dispersal ability of sterile moths in SIT programmes, mitigating the reduced efficacy of such programmes in cooler months.

3.5. References

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CHAPTER 4. GENERAL DISCUSSION AND CONCLUSION

The results of this thesis provide information on how thermal history affects the physiology of adult *Thaumatomibia leucotreta*. It is well documented that temperature, in particular thermal history, has an influence on the performance of insect species. Temperature also forms the basis of insect rearing and its effects significantly impact the ecological role such insects. Therefore, understanding the effect of temperature, particularly thermal history, will not only help understanding of the challenges faced in an insect-rearing facility but also provide reasons for insects under-performance in spite of supposed optimum rearing conditions. Thermal conditions have been shown to have a marked impact on the life traits and behaviour of mass-reared insects, such as cold tolerance, fecundity, longevity and field performance, as outlined in Chapter 1 and discussed in Chapters 2 and 3.

A species may have numerous vital traits that help it to tolerate unfavourable environments in order to survive or adapt, although the response to such environments, including acclimation and cold-hardening, depends on factors such as thermal history, ontogeny, ageing, sex and experimental design, as discussed in Chapter 1 (Bale, 1996; Boersma *et al.*, 2018; Marshall & Sinclair, 2012). These factors form an important baseline for understanding why the outcome of plastic responses such as acclimation or cold-hardening may vary, not only between species in the wild but also between individuals in a mass-rearing facility (Boersma & Carpenter, 2016; Chidawanyika & Terblanche, 2011; Terblanche *et al.*, 2008; Terblanche, 2014; Terblanche *et al.*, 2017). While plastic responses may help populations to survive unfavourable conditions, it may also enhance the performance of individuals under such conditions, as discussed in Chapters 2 and 3. This may include several effects that impact the physiology of an insect in numerous ways, e.g. acute and reversible states of coma or long-term consequences under cold conditions (Overgaard & MacMillan, 2017).

Multiple physiological responses to different temperatures by *T. leucotreta* have been tested in previous research (Boardman *et al.*, 2012; Boardman *et al.*, 2013; Stotter & Terblanche, 2009; Terblanche *et al.*, 2017). However, most of this research was done in the context of post-harvest pest management and was aimed at the larval stage of the species. Although challenges in the broader context of rearing and field performance of mass-reared insects have been studied and described (Sørensen *et al.*, 2012; Terblanche, 2014), little is known about the effect of thermal history on adult mass-reared *T. leucotreta* performance. In this study, the effect of thermal history on adult *T. leucotreta*, in the context of pre-harvest pest

management through mass-reared insects, was examined. Novel findings include the effect of acclimation in combination with sex in terms of cold tolerance, fecundity and longevity. Moreover, the effect of cold-hardening on the flight performance of adult *T. leucotreta* was tested for the first time, with practical value added to the studied pest management programme (Chapters 2 and 3).

4.1. Effects of acclimation and cold-hardening

Thermal conditions were found to influence certain important physiological characteristics, including cold tolerance, fecundity and longevity, of *T. leucotreta* (Chapter 2). Phenotypic plasticity also provided an improved cold-tolerance response, leading to better flight performance under adverse conditions (Chapter 3).

Several different temperature regimes were tested to assess the effect of different environmental conditions on sterile *T. leucotreta* under both laboratory (cold tolerance, fecundity and longevity in Chapter 2) and field conditions (flight performance in Chapter 3). By studying comparable laboratory and field conditions in the latter chapter, an increase in moth recaptures under adverse conditions was observed after pre-release cold-treatment, indicating the extent to which phenotypic plasticity supported improved performance under cold environmental conditions (Chapter 3).

Thermal history plays a significant role in the life cycle of *T. leucotreta*, suggesting that field performance is dependent upon both the environment and physiology (Chapters 2 and 3). Little difference was observed at the lower critical thermal minimum at a controlled cooling rate for different acclimation and temperature treatments on adults, indicating a lack of response to rapid cold-hardening of *T. leucotreta* adults (Chapter 2). Similar observations were recorded for the effect of a short (2 h) temperature treatment and acclimation as single parameters on either the fecundity or longevity of adult *T. leucotreta* (Chapter 2).

However, the effect of acclimation in combination with other factors (temperature treatment and sex) was much more prominent, with significant interactions and effects on both fecundity and longevity, depending on the moth sex combinations. This indicated a complex relationship between thermal history of larval development and subsequent adult thermal conditions (Chapter 2). Plastic responses through acclimation and cold-hardening were also clearly observed in the increased moth flight ability under adverse conditions (Chapter 3) when adults were subjected to longer temperature treatments (16 h).

A significant result involved sex-dependent responses, where all the traits tested (cold tolerance, fecundity, longevity and flight) showed a difference in performance between males

and females. Similar effects have been found in other insect species (Esterhuizen *et al.*, 2014; Fischer & Fiedler, 2000; Johnstone *et al.*, 2017; Rako & Hoffmann, 2006; Roux *et al.*, 2010). Understanding the role of both male and females in a sterile insect technique (SIT) programme provides insight into how sex-dependent responses may influence pest management.

4.2. Implications for sterile insect technique programmes

In SIT programmes, it is vital to understand the influence of thermal history and its contribution to insect tolerance, which can be useful for improving the efficacy of the programme (Rozsypal *et al.*, 2013; Sørensen *et al.*, 2012). Results obtained in this study provide new knowledge regarding these effects and this can be used to boost the efficacy of SIT programmes by producing more field-competitive insects.

Field competitiveness of sterile insects has a direct influence on the success of a sterile release programme. This includes traits such as fecundity, longevity and flight performance. Understanding the influence of rearing and handling temperature during the life of mass-reared insects, including factors such as an insect's critical thermal minimum limitations, is vital for understanding their behaviour after release into the wild (Boersma & Carpenter, 2016; Terblanche *et al.*, 2017). Factors such as the insect's ability to adapt and disperse are crucial for planning and managing an effective SIT programme. Furthermore, the use of certain temperatures may contribute to more competitive organisms under certain conditions due to cost and benefit trade-offs (Chidawanyika & Terblanche, 2011).

A large part of the establishment of a successful SIT programme depends upon the production of high-quality insects capable of optimal field competitiveness. Factors that influence the quality of sterile adults should be taken into account during the optimisation of the programme (Calkins & Parker, 2005; Chidawanyika & Terblanche, 2011; Simmons *et al.*, 2010). These include nutrition, rearing, handling and packaging conditions (Boersma & Carpenter, 2016; Yuval *et al.*, 2007) and irradiation optimisation (Carpenter *et al.*, 2010). Multiple stressors are often experienced by insects in a rearing facility. The response or effects of these stressors may share a similar mechanism (cross-tolerance) with plastic responses. The interaction of acclimation and temperature treatments during normal handling and rearing procedures and their effect on production and field performance are a classic example of this and should be investigated, as the combination of these factors could influence a series of plastic responses affecting the competitiveness of sterile adults (López-Martínez *et al.*, 2014; Mudavanhu *et al.*, 2014; Sinclair *et al.*, 2013; Terblanche *et al.*, 2017).

Current rearing practices in the studied *T. leucotreta* facility include both male and female moths, as no viable commercial process is available to separate the sexes. As males are only

partially sterile after irradiation, the programme relies on the efficacy of the sterile females, which are released with the males on a weekly basis (Hofmeyr *et al.*, 2015). However, it is important to note that the offspring of these partially sterile males in the field results in sterile progeny, known as F1 sterility (Hofmeyr *et al.*, 2015). Because the F1 generation was not subjected to any temperature treatment, it may be speculated that their competitiveness is equal to those of the wild moths. However, the phenotypic effects gained by plastic physiological adjustments from their parents due to rearing and handling conditions might affect their field performance (Lee *et al.*, 1987).

Results detailed in Chapter 2 indicate that acclimation and temperature treatment had the greatest effects on the fecundity of females. It was evident that, in the case of flight performance, males outperformed females (Chapter 3). Moreover, the effects acclimation and cold treatment on treated *T. leucotreta* adult males in terms of fecundity and longevity were far less than those on females, demonstrating that females are more sensitive to thermal history than males (Chapter 2). This influences adults' competitiveness in the field.

Similar effects were observed between acclimated males and females in terms of longevity. Again, the combination of acclimation and sex influences the longevity of especially females. Females lived longer when acclimated at 25 °C, whereas their lifespan was shortened when acclimated at 15 °C and then at 25 °C. The effect of acclimation in males was not as obvious as that in females. This suggests that the effect of plastic responses after acclimation took place over a longer period, which may contribute to a more competitive SIT programme when utilised appropriately. It can be argued that acclimating *T. leucotreta* at colder temperatures could prolong their longevity in cooler months. However, this might not be a viable option from a cost perspective, as colder temperatures during rearing will prolong the total rearing process significantly. On the other hand, the fact that sterile females live longer in the field could increase the efficacy of the programme, as they may continue to mate with wild males, despite the reduction in sterile male numbers.

Because females respond more acutely to thermal acclimation than males do, they could play a much more important role in the field than initially thought. In addition, the difference in flight performance between male and female adults in the laboratory assays could be explained by the fact that female adult moths do not fly great distances (Stotter *et al.*, 2014). Because males follow the pheromone clue provided by females, the efficacy of the sterile release programme in colder months of the season may be increased. In turn, this might decrease the current frequency of releases, as sterile females might still give adequate control in reaching the aims of the SIT programme in colder months, decreasing the cost of the programme. Treating a

cohort and ensuring that sterile females are present in the field to mate with wild males for longer periods should be considered.

The third factor affecting competitiveness, namely flight ability, was tested after adults had received temperature treatment. This study showed that temperature pre-treatments of mass-reared *T. leucotreta* can be beneficial for improving the efficacy of SIT programmes. For example, pre-release cold-treatment enhanced flight performance of adult moths on cold winter days (16 h at 2 °C resulted in a 37% increase in recaptures at 15 °C). However, additional research on hardening treatments followed by field releases, as well as performance assays (including flight, mating and longevity, e.g. Chidawanyika & Terblanche, 2011) are necessary prior to commercial adoption of these practices. Similarly, repeating hardening exposures on eggs and pupae to investigate whether such treatments can improve adult performance might be another worthwhile area of research. While rearing and handling protocols could negatively affect the competitiveness of an SIT programme, the use of other handling methods, such as anoxia, could be considered, increasing the effectiveness of sterile insects in cases where cold handling has a detrimental effect on flight ability (López-Martínez & Hahn, 2012).

The effect of temperature treatment (cold-hardening) on other field traits, such as fecundity and longevity, were not tested in the field, which may result in trade-offs, for example, when *Drosophila melanogaster* (Diptera) was exposed to sub-optimal temperatures, detrimental effects were observed on fecundity on the one hand and improved longevity on the other (Hoffmann *et al.*, 2003). Such trade-offs might be problematic for the *T. leucotreta* sterile release programme, as decreased moth longevity during the programme would require more frequent releases, which increases costs, and a reduction in fecundity could lead to poor competitiveness of sterile adults. Because the effect of thermal history on different species and their reactions are not identical, all costs and benefits of plastic physiologic changes should first be verified before changes are made to a pest management programme, as this might affect field performance of mass-reared insects.

4.3. Future work

The findings of this study have helped to identify directions for future research, which are discussed in the following sections.

4.3.1. Acclimation and test temperatures

Acclimation and test temperatures used in this study to determine the effect of certain traits on competitiveness included moderate temperatures of between 15 and 25 °C (Chapter 2).

Although the use of acclimation temperatures was dictated by current rearing protocols, more extreme test temperatures over different time frames should be explored to determine any plastic responses in terms of cold hardiness, fecundity, longevity and flight performance (Lee *et al.*, 1987; Liefting *et al.*, 2009). Fluctuating rearing temperatures, compared to constant low temperatures, have been shown to improve the survival of false codling moth, although this is not the case for constant moderate temperatures (Boardman *et al.*, 2013). The use of moderate fluctuating thermal regime could be further explored with reference to the trade-off between an prolonged rearing cycle and field competitiveness.

4.3.2. Effects of cold-treatment on other traits

The SIT is an area-wide approach in an integrated pest management system (Vreysen *et al.*, 2006). Although the benefits of cold-treatment in an SIT programme to enhance flight ability were successfully demonstrated in both the laboratory and field, the effects of such treatments on other traits, such as fecundity and longevity, need to be tested on an area-wide basis. Another factor to consider is the effect of temperature treatment on the stock or breeding population. By treating the mating adults and egg-laying females, physiological plastic responses, such as increased flight ability or fecundity, could enhance the competitiveness of the SIT programme and should be investigated (Lee *et al.*, 1987).

4.3.3. Effect of temperature treatment on F1 generation

Knowledge regarding the effect of temperature treatment on the F1 generation is lacking. No research to date has examined the effects of temperature treatment or acclimation of the parents on the F1 generation. Such effects may play a significant role in manipulating the field performance of sterile insects, as temperature treatments may affect subsequent generations (Lee *et al.*, 1987). The opposite may also be true in that temperature protocols used during rearing, handling and transportation might have a negative impact on the F1 generation.

4.3.4. Enhancing the sterile insect technique

Although the SIT has proven to be an efficient way of controlling pests (Krafsur, 1998; Lindquist *et al.*, 1992; Simmons *et al.*, 2010), there is renewed interest in enhancing this method or developing other biological methods in conjunction with the SIT. An example of this is the male annihilation technique. This technique has been used to suppress *Bactrocera* pest species as part of an integrated pest management approach (Ndlela *et al.*, 2016). The integration of these methods should be investigated as part of an integrated pest management system.

4.3.5. Other approaches

Other novel approaches to controlling pest populations involve the use of maternally transmitted *Wolbachia* bacteria as a biological intervention and for population suppression against various pest insect species, including mosquitos species and other insect taxa, such as the African army worm (*Spodoptera exempta*) (Lepidoptera), coconut beetle (*Brontispa longissima*) (Coleoptera) and Mediterranean fruit fly (*Ceratitis capitata*) (Diptera) (Ahmed *et al.*, 2015; Bull & Turelli, 2013; Zabalou *et al.*, 2009). One approach involves the suppression of the host population by extensive releases of males unsuited for mating with wild females due to cytoplasmic incompatibility. Another intervention comprises the alteration of the insect population with *Wolbachia* that spreads through the frequency-dependent fitness advantage of *Wolbachia*-infected females (reviewed in Bull & Turelli, 2013). Combining the SIT with *Wolbachia* symbiosis has been proposed as potential control of various pests such as *C. capitata* and *Drosophila suzukii* (Diptera) (Nikolouli *et al.*, 2018). *Wolbachia* has further been proved to be present in at least 300 different lepidopteran species (Ahmed *et al.*, 2015). While *Wolbachia* compatibility has not been tested in *T. leucotreta*, infection incidence is likely to be high (Ahmed *et al.*, 2015) and therefore may play a significant part in integrated pest management programmes in the future, as it could improve the efficacy of pest management programmes.

4.4. Conclusion

This study adds to the existing knowledge base on *T. leucotreta* thermal responses and assists in the understanding of this species' physiology by demonstrating how rearing conditions in conjunction with field temperature may impact the efficacy of sterile release programmes. It was found that rearing temperature may have a positive or a negative impact on various fitness traits of sterile moths. The study also demonstrated costs and benefits of acclimation on the performance of *T. leucotreta*, which have been documented for *D. melanogaster* (Kristensen *et al.*, 2008) and *C. pomonella* (Chidawanyika & Terblanche, 2011). Although only two temperatures were tested (Chapter 3), this study demonstrated the possibility that beneficial acclimation might have contributed to the results obtained in this study (Leroi *et al.*, 1994), which may aid future SIT programmes for the following reasons:

- Benefits gained from acclimation during development are expressed in adult *T. leucotreta* moths. This could be applied to SIT programmes in terms of modified acclimation conditions as part of rearing protocols.
- Adult *T. leucotreta* performed best in environments simulating pre-release conditions and poorest in environments to which they had not previously been exposed (Chidawanyika &

Terblanche, 2011; Kristensen *et al.*, 2008). These benefits could be used to decrease the costs of SIT programmes, as the use of fewer moths might achieve current efficacy levels. Improved longevity would require fewer releases and increased fecundity would elevate the frequency of successful mating with wild adults.

- The study demonstrated the potential viability of thermal acclimation for potentially boosting the efficacy of the SIT programme for *T. leucotreta* control under cooler spring and autumn conditions, where poor sterile moth performance has been reported (Stotter & Terblanche, 2009).

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