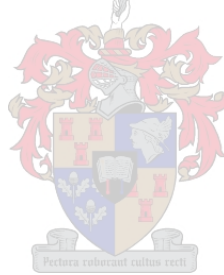


Thermal tolerance of *Cydia pomonella* (Lepidoptera: Tortricidae) under ecologically relevant conditions

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree

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Abstract

Ambient temperature plays a key role in insect-physiology, -population dynamics and ultimately -geographic distribution. Here, I investigate the survival of codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), which is a pest of economic importance in pome fruit production, to a wide range of temperature treatments. In this thesis, I first explore how temperature affects the survival and limits to activity of codling moth and secondly investigate if thermal acclimation can improve field performance of moths used in sterile insect technique control programmes under ecologically relevant conditions. First, I found that absolute temperature as well as the duration of temperature exposure significantly affects adult *C. pomonella* survival. Lethal temperatures, explored between -20 °C to -5 °C and 32 °C to 47 °C over a range of durations, showed that 50% of the adult *C. pomonella* population killed at -12 °C and at 44 °C after 2 hrs for each treatment. At high temperatures a pre-treatment at 37 °C for 1 hr dramatically improved survival at 43 °C for 2 hrs from 20% to 90% ($p < 0.0001$). Furthermore, high temperature pre-treatments (37 °C for 1 hr) significantly improved low temperature survival at -9 °C for 2 hrs. In sum, my results suggest pronounced plasticity of acute high temperature tolerance in adult *C. pomonella*, but limited acute low temperature responses. Secondly, low-temperature acclimated laboratory-reared moths were recaptured in significantly higher numbers (d.f. = 2, $\chi^2 = 53.13$ $p < 0.001$), by sex pheromone traps, under cooler conditions in the wild relative to warm-acclimated or non-acclimated moths. However, these improvements in low temperature performance in cold-acclimated moths came at a cost to performance under warmer conditions in the wild. This novel study demonstrates the importance of thermal history on *C. pomonella* survival and clear costs and benefits of thermal acclimation on field and laboratory performance, and thus, the potential utility of thermal pre-treatments for improved efficacy in the sterile insect technique programme for *C. pomonella* control under cooler, springtime conditions. Finally, on a global scale, this study highlights that low and high temperatures could play a role in CM adult survival through direct mortality and thus, may influence, or have influenced in the pest, population dynamics.

Opsomming

Temperatuur speel 'n belangrike rol in die fisiologie, populasiedinamika en geografiese verspreiding van insekte. In hierdie tesis ondersoek ek die rol van 'n wye reeks temperature op die oorlewing van kodlingmot *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), 'n sagtevrug pes-spesie van ekonomiese belang. Ek ondersoek hoofsaaklik die effek van temperatuur op die fisiologie en fiksheid van kodlingmot, asook die mate waartoe termiese akklimasie ('n mate van aanpassing) die veldgedrag van die steriele insek beheer-metode (SIT), d.m.v. kodlingot, in relevante omgewingstemperature kan verbeter. Ek het (i) gevind dat die temperatuur en duur van die temperatuur toediening 'n betekenisvolle toename in volwasse *C. pomonella* oorlewing tot gevolg het. In die deel van die studie is temperature tussen -20 °C en -5 °C and tussen 32 °C en 47 °C ondersoek oor 'n reeks van 0.5, 1, 2, 3 en 4 ure van duur. In kort lei -12 °C en 44 °C vir 2 uur onderskeidelik tot die uitsterf van 50% van die volwasse *C. pomonella* populasie. Indien die motte vooraf gehou is by 37 °C vir ongeveer 1 uur, is oorlewing by 43 °C vir 2 ure betekenisvol verbeter van 20% tot 90% ($p < 0.0001$). Hoër temperatuur vooraf-blootstellings (akklimasie), by 37 °C vir 1 uur, het daartoe gelei dat lae temperatuur lae-temperatuur-oorlewings by -9 °C vir 2 ure betekenisvol verbeter het. Oor die algemeen het die resultate gedui dat hoër akute temperatuurstoleransie in *C. pomonella* bestaan, maar beperkte akute lae-temperatuur reaksies bestaan. Verder het lae-temperatuur akklimasie (laboratorium geteelde) motte 'n betekenisvolle hoër getal hervangste deur geslagsferomone in koeler omgewings opgelewer (v.i. = 2, $\chi^2 = 53.13$, $p < 0.001$) in vergelyking met warmer-temperatuur geakklimatiseerder motte. Hierdie verbeteringe in lae-temperatuur reaksies vanaf lae-temperatuur akklimasie groepe is teen 'n koste teen warmer reaksie-toestande in die natuur geï. Hierdie eersdaagse studie demonstreer die belang van historiese temperatuur op die oorlewing van *C. pomonella*. Die kostes- en voordele van termiese akklimasie op veld- en laboratoriumpopulasie reaksies en die potensiële gebruik daarvan in die verbetering van steriele insek tegniek programme, onder koeler omstandighede, is uitgelig. Laastens, beklemtoon hierdie studie die belangrikheid van temperatuur as bepalende faktor van kodlingmot-oorlewing en die invloed daarvan op die vrugte-pes populasiedinamika.

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Table of Contents

Declaration	ii
Abstract	iii
Opsomming	iv
Acknowledgements	v
Table of Contents	vi
Chapter 1 General introduction.....	1
1.1 History, geography and occurrence	2
1.2 Biology, phenology and life-cycle of <i>C. pomonella</i>	3
1.3 Damage and pest status of codling moth	6
1.4 Current control methods for codling moth.....	8
1.4.1 Chemical control.....	8
1.4.2 Biological control.....	8
1.4.3 Mating disruption.....	9
1.4.4 Sterile Insect Technique	10
1.5 Temperature biology and population dynamics of <i>C. pomonella</i>	11
1.5.1 Physiological responses of CM to temperature	12
1.5.2 Evolutionary responses of CM to temperature	14
1.6 Phenotypic plasticity	22
1.7 Insect thermal biology.....	23
1.7.1 Lethal, Sub-lethal temperatures and insect thermal performance curves.....	23
1.7.2 Acute exposure effects: patterns, mechanisms and potential influencing factors	27
1.8 Quality control in SIT: laboratory cultures versus field performance	28
1.9 Goals of this research.....	31
References.....	32

Chapter 2 Rapid thermal responses and thermal tolerance in adult codling moth <i>Cydia pomonella</i> (Lepidoptera: Tortricidae)	42
2.1 Introduction.....	43
2.1 Methods.....	46
2.1.1 <i>Insect culture</i>	46
2.2.2 <i>Lethal temperature assays</i>	47
2.2.3 <i>Rapid thermal responses</i>	47
2.2.4 <i>Effects of ramping rates on critical thermal limits</i>	48
2.2.5 <i>Statistical analyses</i>	49
2.3 Results.....	50
2.3.1 <i>Lethal temperature assays</i>	50
2.3.2 <i>Effect of ramping rates on critical thermal limits</i>	52
2.3.3 <i>Rapid thermal responses</i>	55
2.4 Discussion.....	60
2.4.1 <i>Lethal temperatures</i>	61
2.4.2 <i>Rapid thermal responses</i>	63
2.4.3 <i>Conclusions</i>	65
References.....	67

Chapter 3 Costs and benefits of thermal acclimation for sterile insect release programmes in codling moth, <i>Cydia pomonella</i> (Lepidoptera: Tortricidae): physiological plasticity can be used to enhance pest control	72
3.1 Introduction.....	73
3.2 Material and methods.....	75
3.2.1 <i>Moth rearing conditions</i>	75
3.2.2 <i>Thermal acclimation effects on critical thermal limits</i>	75
3.2.3 <i>Developmental thermal acclimation effects on activity of CM</i>	76
3.2.4 <i>Thermal acclimation effects on field release-recapture rates</i>	77
3.2.5 <i>Statistical analyses</i>	77
3.3 Results.....	78
3.3.1 <i>Laboratory assays of critical thermal limits and temperature dependence of activity</i>	80

3.3.2 <i>Field performance trials</i>	82
3.4 Discussion	86
References	88
Chapter 4 Conclusions	96
References	104
Appendix 1 Photos from field release and recapture trials.....	105

Chapter 1

General Introduction

1.1 History, geography and occurrence

Codling moth (CM) is the primary pest of economic importance in pome fruit production in South Africa's prime growing region, the Western Cape (Giliomee and Riedl, 1998; Pringle et al., 2003) and worldwide (Dorn et al., 1999; Kührt et al., 2006b). It is native to Asia but primarily occupies Middle Europe, the Mediterranean, Minor and Central Asia. *Cydia pomonella* has established itself in Europe, Northern and southern Africa including a number of islands such as Madeira, Canary Islands and Mauritius (Wearing et al., 2001). Moreover, Pakistan, China, America, South Australia and New Zealand, have all been invaded by CM. In Russia, CM is distributed throughout all of the European parts except for the north, in Ural, southern Siberia and the Far East (Amur Region, Khabarovsk and Primorskii Territories) (Wearing et al., 2001) (See Fig. 1.1)



Areas with confirmed *Cydia pomonella* infestation in the world

Figure 1.1 World distribution of *Cydia pomonella*

In South Africa, first infestations of CM were recorded in the Western Cape in 1898 in three farms near Stellenbosch (Anneck and Moran, 1982). Codling moth has demonstrated that it has the potential to establish in most climates where apples, *Malus domestica*, and

pears, *Pyrus communis*, are grown with the exception perhaps of Japan's pome fruit growing regions (Wearing et al., 2001). Codling moth was probably spread across the world in the nineteenth century by transport of infested fruits due to lack of firm quarantine regulations and seedlings coming from infested areas for planting in other areas (Wearing et al., 2001).

1.2 Biology, phenology and life-cycle of *C. pomonella*

Overwintering in CM occurs as diapausing fifth instar larvae spun in cocoons located under or in crevices and cracks of bark on host trees. This pest species has the potential to remain in diapause over two winters (Yothers and Carlson, 1941; Garlick, 1948; Wearing et al., 2001). In the Western Cape of South Africa, a maximum of two to four generations of CM in a season have been documented (Nel and Addison, 1993; Pringle et al., 2003).

Larval diapause is terminated by optimal chilling and long photoperiods (Riedl, 1983; Wearing et al., 2001). The sex ratio of adults is approximately 1:1 and sexual reproduction is obligatory with both sexes having the ability to mate more than once (Wearing et al., 2001). Mating of the adults can occur as early as twelve hours after emergence (Gerhring and Madsen, 1963) with most female sexual activity occurring within four days of eclosion (Knight, 2007). Oviposition commences a day after mating (Gehring and Madsen, 1963) with egg production per female varying with individual and season ranging from 0 to 284 eggs but the general average oviposition per female is 50-100 eggs per individual female per life cycle (Geier, 1963; Wearing and Ferguson, 1971). Fruit odour stimulates oviposition (Wearing and Hutchins, 1973) on twigs, leaves, and the fruits of the host tree (Wearing et al., 1973; Jackson, 1979).

The fruit odour also acts as an attractant to the first instar larvae after eclosion (Sutherland et al., 1974) which will enter the fruit through the calyx, preferring the ripe side of the fruit (Wearing et al., 2001). Upon entry to the fruit, CM larvae form a spiral gallery underneath the exocarp before moulting and beginning radial penetration to the endocarp to feed on the seeds (Wearing et al., 2001). Rate of development in CM increases significantly when larvae feed on seed and larvae about to diapause feed longer than the non-diapausing ones (Putman, 1963; Wearing et al., 2001). Rearing of larvae on apple leaves achieved limited success (Hall, 1934; Wearing et al., 2001) with those reaching maturity on a leaf diet failing to produce eggs (Herriot and Waddell, 1942). Codling moth larvae develop through five instars (L1 to L5) within the fruit, although some of the larvae may move to other fruits

before looking for a cocooning site as fully developed L5 instars (Fig. 1.2) (Wearing et al., 2001). Larvae will therefore be in a diapause or non-diapause condition depending on the temperature and photoperiod experienced (i.e. time of year and location/latitude) (Wearing et al., 2001). Adult CM are mottled gray in colour with alternating bands of gray and white on the wings with a tip of copper/bronze on each forewing (Fig. 1.2).

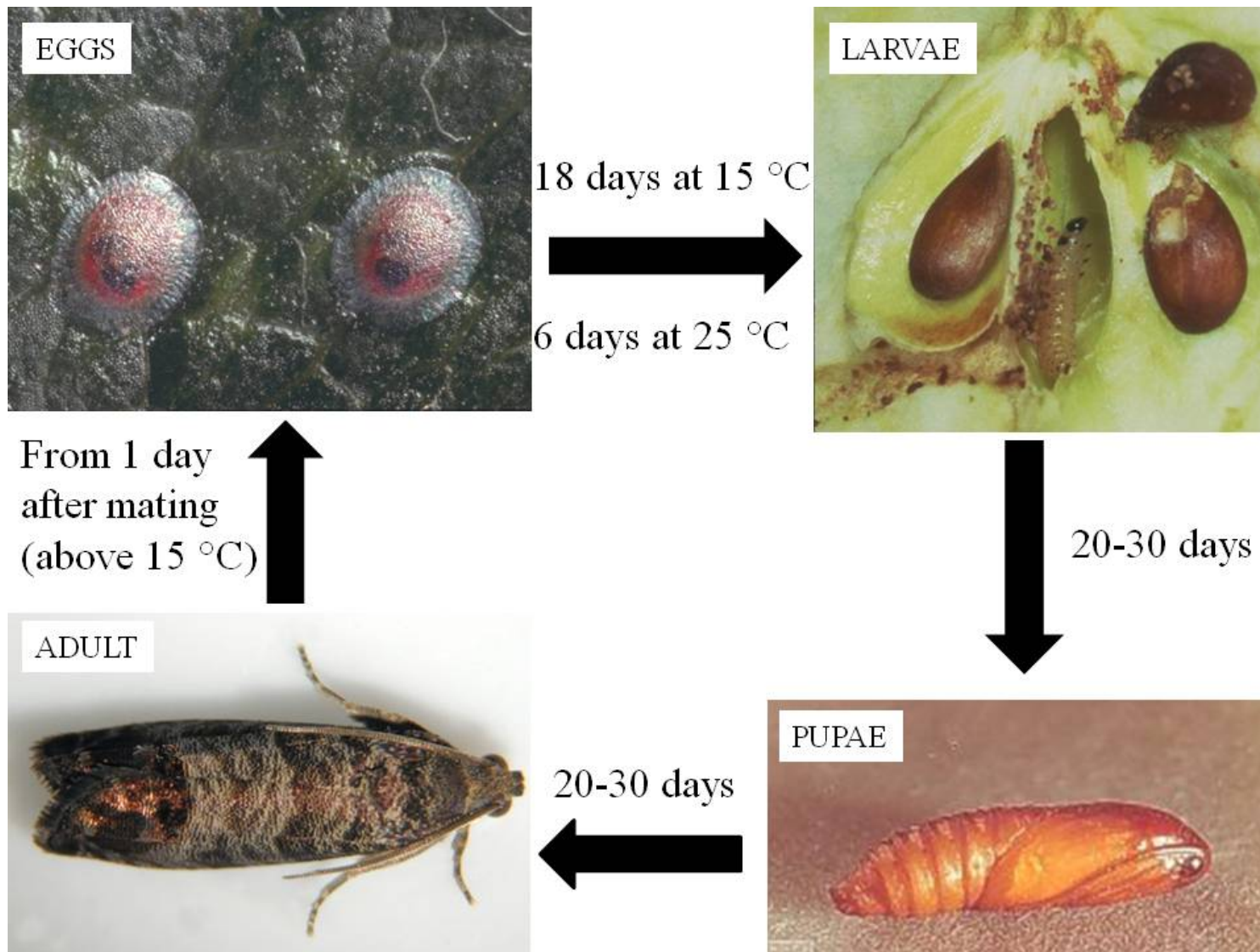


Figure 1.2 Life cycle of *Cydia pomonella*

1.3 Damage and pest status of codling moth

Codling moth is a cosmopolitan insect preferring apples as the host (Azizyan et al., 2002), with the addition of other pome fruits such as pear and quince, although CM is capable of thriving relatively well on some stone fruit such as plum, apricot, peach and walnut (Thaler et al., 2008). The potential for crop loss due to CM infestations makes *C. pomonella* the pest of most economic importance in pome fruits (Azizyan et al., 2002). In South Africa for example, uncontrolled CM has the capacity of infesting up to 80% of an apple crop (Giliomee and Riedl, 1998; Pringle et al., 2003; Bell and McGeoch, 1996).

The larval stage of CM is what causes the primary damage to commercial fruits (Wearing et al., 2001). Female moths lay eggs singly on or near the fruits (Wood, 1965). This increases the chance of larvae survival after hatching because the larvae will quickly enter the fruits, hence obtaining protection from predators and parasitoids during development (Wearing et al., 2001). Larvae make fruits unmarketable by causing surface scarification or burrowing deep into the fruit to feed on the seed whilst depositing frass on the entry hole as shown in Fig. 1.3.

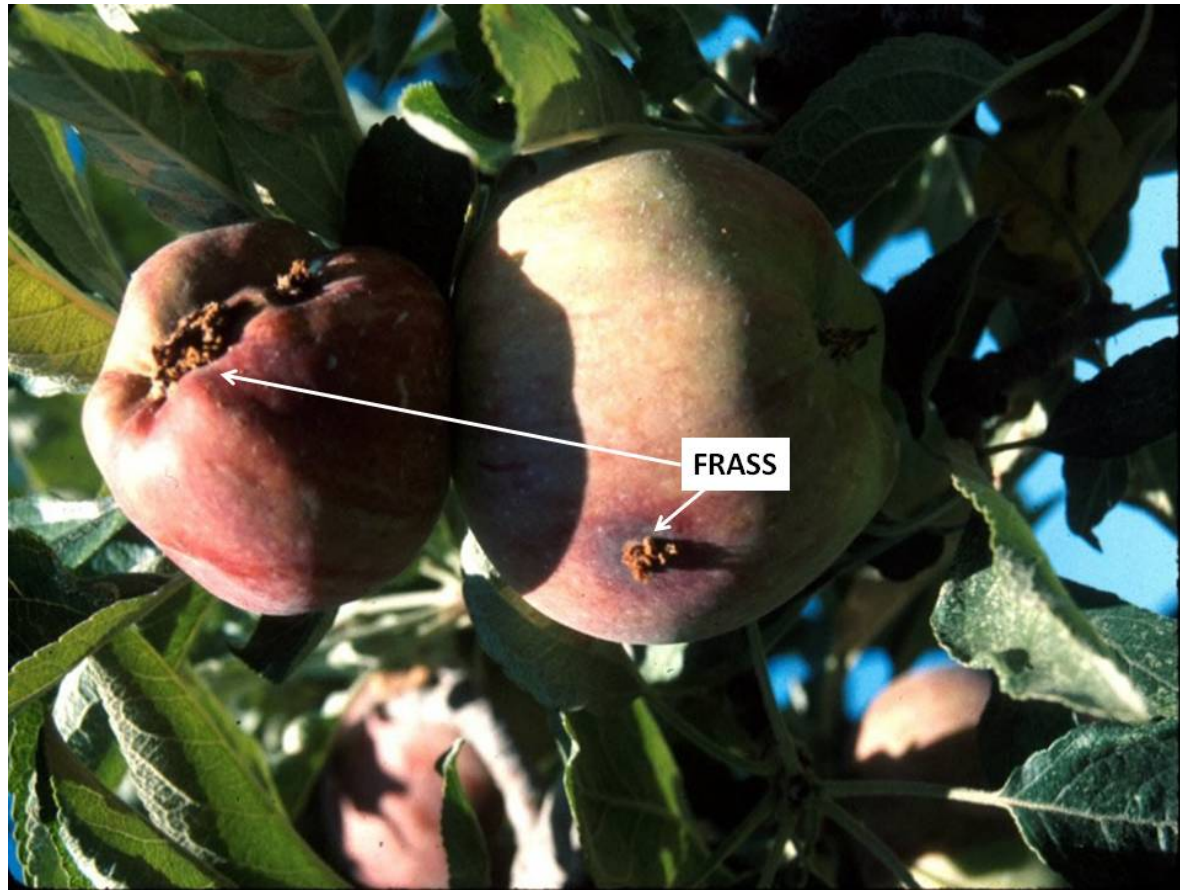


Figure 1.3 Typical *Cydia pomonella* damage on apples showing frass at the entry hole

1.4 Current control methods for codling moth

1.4.1 Chemical control

Suppression of CM can be achieved through cultural, chemical and biological control methods with varying degrees of success. Since the 1960s, chemical control of CM has been based on the use of broad-spectrum insecticides, such as organophosphates, carbamates, and to a limited extent, synthetic pyrethroids (Malik et al., 2002). More than 70% of the insecticide sprayed in apple orchards worldwide is applied to combat CM populations (Malik et al., 2002, Franck et al., 2005). These highly toxic products have provided very effective control in well organised IPM control of CM and other pests (Malik et al., 2002) but they have had the disadvantage of wider toxicity to non target organisms, mainly natural enemies. Furthermore, chemical control has resulted in the development of insecticide resistance as is reported by Malzieux et al., (1995) and Speich, (1996) for CM against organophosphate. The resistance could be attributed to the continual use of the same insecticide or improper use of the insecticide (Malik et al., 2002). In addition, global market demands have forced control methods to explore alternatives to chemical control as chemical control poses environmental and health risk. Even with costly chemical control programmes, CM remains a pest of economic importance (Addison, 2005).

1.4.2 Biological control

The augmentation of natural enemies of CM has been useful in biological tactics against the species, in particular, during the egg and larval life-stage (Falcon and Huber, 1991). Some of the biocontrol agents which have been known to be effective against CM include birds, insect predators, spiders, parasitoids, protozoa, bacteria, fungi and viruses (Falcon and Huber, 1991). The *Trichogramma* species (Hymenoptera: Trichogrammatoidea), used as an egg parasitoid, has been the most important biological agent (Mansfield and Mills, 2002; Hassan, 1989; Makee, 2006). The efficacy of this parasitoid depends on the distance between the parasitoid to host, temperature and density per parasitoid over a number of moths (Kutsryavtseva and Teshler, 1994). Highest parasitisation (i.e. efficacy) occurs at 21-23 °C with a ratio of parasitoid to host as 10:1 (Malik et al., 2002). Control of viable eggs with *T. platneri* has also been reported by Zhang and Cossentine, (1995). However effective use of these parasitoids is subject to several confounding factors. Timing, temperature regimes and

population density all can have a significant effect on CM control (Zhang and Cossentine, 1995).

Another biological agent that has been used against CM is known as *Cydia pomonella* granulovirus (CpGV) (Lacey et al., 2008). It is of highly virulent (Tanada and Hess, 1991; Federici, 1997) microbial agent which is host specific and thus provides an effective environmentally friendly way of controlling CM in IPM systems (Gröner, 1986; 1990; Lacey et al., 2008). Optimal efficacy of CpGV is achieved when it infects or parasitizes the larval life-stage of CM (Lacey and Shapiro-Ilan, 2008), hence, timing of CpGV application is essential as it should coincide with egg hatching to achieve maximum control. In Europe, Huber and Dickler (1977) demonstrated that CpGv gives adequate protection when compared with traditional insecticide programmes. Despite all the advantages, control using CpGv remains a challenge. First, CpGv is highly sensitive to ultraviolet (UV) radiation and is susceptible to deleterious portions of the UV-B range 280-320nm (Arthurs et al., 2006). Second, CpGv lacks the desired persistence in the field (Lacey et al., 2008). In addition, CpGv specificity can be a disadvantage since chemicals might still be needed to control secondary pests.

In South Africa, as is the case in other parts of the world, efforts are also currently underway to control CM using entomopathogenic nematodes (EPN) (de Waal, 2008; Lacey and Shapiro-Ilan, 2008). Pome fruit orchards are sprayed with EPN to target *C. pomonella* larvae (Lacey and Unruh, 1998; de Waal, 2008) under favourable soil moisture conditions. Nematodes are particularly effective at controlling CM as they are able to penetrate the cryptic habitats (Lacey and Unruh, 1998) occupied by *C. pomonella* larvae which can otherwise be inaccessible by chemical control. The use of EPN has been highly appreciated for not causing harm to the environment, users or consumers, natural enemies and for being compatible with other methods of control.

1.4.3 Mating disruption

Mating disruption is an alternative to insecticides and a relatively new way of combating CM. Mating disruption mainly relies on a synthetic pheromone called codlemone (Calkins and Faust, 2003) which is applied in large quantities in the orchard thereby creating a false pheromone trail. In consequence, males fail to locate and mate with calling females, thereby reducing population sizes indirectly. The application of the pheromone ranges from

hand held dispensers to spraying depending on the size of the canopy (Grant et al., 2003). The advantages of mating disruption are the same as those of other biological control methods. However mating disruption does not completely avoid mating in CM as some males can still locate calling females by chance. Mating disruption has also been shown to be less effective in mountainous regions which may provide physical barriers that are quite common in South Africa (Pringle et al., 2003).

1.4.4 Sterile Insect Technique

Sterile insect technique (SIT) has been used against a wide range of pests including Tephritids, Coleoptera and Lepidoptera (Tyson et al., 2008; Klassen and Curtis, 2005). Past experience indicates that SIT is mainly effective when used as part of an appropriate area-wide integrated pest management (Tyson et al., 2008) and has the potential to lower costs to the environment and pest management in general when compared to other current conventional methods (Klassen and Curtis, 2005). The SIT has been applied in an attempt to eradicate CM in areas like western Canada and in the United States of America (Bloem et al., 2004; Bloem et al., 1998) and here in South Africa, efforts are currently underway in the Elgin area of the Western Cape Province. The method was developed by Knippling, (1955) and has made it possible to control insect pests such as screwworm, fruit flies and tsetse flies (Klassen and Curtis, 2005).

Sterile insect technique depends greatly on the production of good quality sterile male insects that are released in high numbers into target wild populations (Calkins and Parker, 2005; Judd and Gardiner, 2005). In short, released moths should be of good quality to be able to compete successfully with their wild counterparts (Koyama et al., 2004). Consequently, mass-reared sterilised CM males must be able to behave as closely as possible that to their wild counterparts. Any departure from the “wild” behaviour, or lack of field competitiveness could therefore cause the failure of an SIT programme (Terblanche et al., 2007).

Other factors which can be manipulated to improve quality of the released moths include irradiation optimisation (Parker and Mehta, 2007; Judd and Gardiner, 2006), rearing temperature and nutrition (Chang et al., 2001; Niyazi et al., 2004; Yuval et al., 2007). In this respect, irradiation doses greatly affect the quality of reared insects. Thus, in choosing the optimum dose for sterilising insects, a balance needs to be reached between the levels of sterility and maintaining competitiveness (Toledo et al., 2004). This is because extremely

high dosage cause “greater” sterility but compromise insect’s competitiveness, on the other hand, extremely low dosage causes “lower” sterility but insects will be more competitive. Low radiation in CM results in absolute sterility in females and partial sterility in males (Bloem, et al., 1999b; Bloem, et al., 2004). It is therefore important to minimise the somatic effects induced by radiation during the radiation process (Bakri et al., 2005) so as to maintain the competitiveness required in CM. Unfortunately, it appears many of the current operational programs applying the SIT settle for high doses and are not achieving an appropriate balance (Parker and Mehta, 2007).

Judd and Gardiner, (2006) explored comparisons between wild and mass-reared moths using pheromone response, flight activity and recapture as the parameters in field and flight tunnel experiments and reported that there was no evidence of mass reared moths being less responsive to pheromone at low temperature than the wild moths. At 15 °C, no wild moths were caught and only 58% of the released moths were caught at 25 °C as compared to 85% catch made at 25 °C for the mass-reared moths. However, Judd and Gardiner, (2006) failed to demonstrate any effect of mass-rearing on temperature thresholds for pheromone mediated flight. This would have been useful in explaining the differences in wild and mass-reared CM to pheromones under same temperature regimes.

1.5 Temperature biology and population dynamics of *C. pomonella*

Insects are sensitive to temperature owing to their ectothermic nature (i.e. body temperatures closely approximate ambient temperatures) and extreme thermal conditions can therefore be detrimental to their fitness (Chown and Nicolson, 2004; Angilletta, 2009). Insects can respond to temperature variation using i) behaviour (short time-scales, hours to days), ii) physiological compensation (e.g. acclimatization/diapause) (short to intermediate time-scales, hours to days, within or between generations) iii) physiological adaptations which evolve over longer timescales (weeks to years and between generations) (Angilletta, 2009).

Temperature largely determines the phenology of CM (Rock and Shaffer, 1983, Wearing et al., 2001) by influencing developmental rates (i.e. life stage duration). The rate of development can be estimated using a degree-day model which relates CM physiological development to mean prevailing temperatures (Pitcairn et al., 1992; Howell and Neven, 2000). The model is based on a positive linear relationship between temperature and rate of development. Low and high temperature development responses of CM are different as

shown by Saethre and Hofsvang, (2002). The basal temperature for CM egg development is around 10 °C Saethre and Hofsvang, (2002), whilst egg mortality increased as temperature increased from 14.8 °C, and at 34.4 °C egg mortality was 99.5%; larval response to temperature indicated that physiological development was optimal at 25.5 °C, significantly shorter at 29.6 °C (Howell and Neven, 2000).

In their assays, Howell and Neven (2000) found that pupal physiological development time was affected at 14.8 °C which reduced emergence (15% of larvae went into diapause state) and 35 °C where it was deleterious resulting in 100% mortality. Mortality at temperatures above 34 °C only occurred for those moths reared at constant temperature, whereas moths reared at fluctuating temperatures (as in the wild) greatly reduced CM mortality. Most research indicates that the lower developmental threshold for CM is 10 °C (Saethre and Hofsvang, 2002; Howell and Neven, 2000; Riedl and Croft, 1978) and physiological time needed to complete the life cycle of the moth from black head stage of eggs to adult eclosion was on average 550 degree days (DD) (Saethre and Hofsvang, 2002).

Codling moth has been known to exhibit cryptic basking in its larval stage (Kührt et al., 2005). They feed preferably on the warmer side of the fruit, consequently increasing their body temperature (T_b) and thereby accelerating their development (Kührt et al., 2006b). This positive thermal response only disappears when the larvae leaves the fruit in search of overwintering sites. Short-range migration in adults is determined by a number of factors including biotic cues (Hern and Dorn, 1999; 2002; Vallat and Dorn, 2005) and sex pheromones influencing males (Witzgall et al., 1999). The influence of temperature on flight, mating and fecundity has been well documented (Putman, 1963; Hagley, 1976; Saethre and Hofsvang, 2002).

However, a gap in knowledge still exists regarding thermal tolerance of CM in the wild and whether it shows hardening or acclimation responses. Studies on CM thermal tolerance have been widely explored on the larvae mainly with respect to post-harvest sterilization of CM infested fruit as summarised in Table 1.1, 1.2 and 1.3. However, larval response to temperature may vary significantly from adults (Bowler and Terblanche, 2008).

1.5.1 Physiological responses of CM to temperature

The physiology of insects is highly responsive to environmental temperature due to their ectothermic nature. Understanding CM thermal physiological responses is crucial for

post harvest control techniques and control in the field as thermal physiological data may be used in phenological models. Growth and development for CM lies in the range of 10 to 30 °C (Rock and Shaffer, 1983; Neven, 2000; Riedl, 1983; Glenn, 1922). The developmental temperature threshold for development of all three immature stages is 10 °C (Riedl, 1983) although, for pupal development, it may be slightly higher (Glenn, 1922). Growth rate increases linearly with increase in temperature; but decrease sharply as the temperature approaches the critical maximum temperature. Glenn, (1922) hypothesised that this critical maxima is life stage dependant; 31 °C for eggs, 29 °C for larvae and 30 °C for the pupae. Mortality increases at temperatures exceeding these critical maxima. Temperatures below 10 °C result in low or zero development but are not lethal unless freezing occurs (Riedl, 1983).

Effects of temperature on respiration rates of CM larvae were explored by Neven, (1998b). Respiration increases in response to increasing temperature up to a critical upper limit and then decreased thereafter (Neven, 2000). Moths seemed to recover after peak respiration; however, mortality occurred soon after temperature began to drop even if thermal conditions returned to optimum. This indicates systemic cell death (Neven, 1998b).

Most insects experience seasonality in their life cycles. The diapause stage affects the seasonal orientation of the entire life cycle. Consequently the environmental pressures exerted on other active stages of the life cycle depend directly or indirectly on the stage at which diapause occurs (Masaki, 1967). Diapause is a neuro-hormonally mediated, dynamic state of low metabolic activity where reduced morphogenesis, increased resistance to environmental extremes and altered or reduced behavioural activity occurs (Danks, 2002; Denlinger, 2002). It is a genetically determined stage of metamorphosis and is species specific, usually in response to a number of environmental stimuli that precede unfavourable conditions resulting in suppressed metabolic activity.

Areas inhabited by CM are characterized by definite seasonality with cool or cold winters followed by variably long periods of warm weather allowing one to several generations per year (Riedl, 1983). In addition to climatic patterns is the cyclic seasonality of the food source (as fruits only appear during specific periods in a year). Synchronisation of CM life cycle with seasonal rhythms and food availability is mandatory for this pest to be successful.

Several mechanisms of diapause induction have been reported in CM (Dickson, 1949; Headlee, 1931; Garlick, 1948; Ivanchich-Gambaro, 1958). Dickson (1949) reported that photoperiodic reaction to decreasing day length induced diapause in CM. Most larvae have a facultative diapause but some are univoltine even under favourable, diapause-averting, long day conditions (Riedl, 1983). Headlee (1931) had earlier on reported that diapause is induced in response to temperatures below 15 °C in the summer months. Garlick (1948) on the other hand, implicated nutritional factors as the cues for diapause induction. This was further supported by Ivanchich-Gambaro (1958) who argued that nutritional factors related to ripeness of fruit induce diapause, however not as a single factor, but as an interaction with photoperiod. All the factors discussed affect diapause in CM but they seem to only act in modifying the photoperiodic reaction (Riedl, 1983).

Termination of diapause in CM must occur at the correct time to allow synchronisation of adult emergence with fruit development. Diapause termination in CM can be achieved by chilling, long photoperiod or by a combination of both (Riedl, 1983). Under short-day conditions diapause can be terminated by temperatures between 0 and 10 °C (Sheldova, 1967). Peterson and Harmer (1968) reported that chilling at 4 to 5 °C for 20 days was required to terminate diapause and this duration can increase to more than 50 days at 4 to 7 °C (Cisneros, 1971). Long photoperiod alone can also terminate diapause in CM, but only at high temperatures (Russ, 1966; Sheldova, 1967). However, with long photoperiod and no chilling adult emergence took longer and was erratic (Cisneros, 1971). Short pre-chilling before exposure to long photoperiod and high temperatures shortened the emergence period or time needed to terminate diapause and increase the percentage of adult emergence (Widbolz and Riggenschach, 1969; Cisneros, 1971). Short photoperiods maintain diapause regardless of temperature (Russ, 1966). Codling moth has the ability to remain in diapause for two years and this may be seen as a protection against years of scarce fruit supply (Yothers and Carlson, 1941).

1.5.2 Evolutionary responses of CM to temperature

Populations faced with a plethora of environmental changes must adapt behaviourally or physiologically, shift their range or possibly face extinction (Williams et al., 2008; Calosi et al., 2008; Balanya et al., 2006; Bradshaw and Holzapfel, 2001). Adaptation may occur in two forms namely: i) phenotypic plasticity at the individual level or ii) transition of genetic composition of populations through natural selection, a change that favours the “fitter”

genotypes at the expense of “weaker” ones (Frankham and Kingsolver, 2004). However, the extent of current and future changes can be subject to temporal and spatial variation consequently becoming important for the potential ecological and evolutionary responses of organisms to environmental change. The time scales for environmental changes relative to the generation period of a population determine whether the changes (together with potential evolutionary response) are gradual or abrupt (Frankham and Kingsolver, 2004). This can be very important to pests like CM that have multiple generations (Pringle et al., 2003) within a year as they experience changes like global warming as a gradual process over scores of generations (Frankham and Kingsolver, 2004). On the other hand, some organisms can experience the same climatic event, within a single generation, resulting in differences in evolutionary potential and selection intensity which can cause a different genetic response to the selection.

Codling moth diapause initiation, for example, is triggered by optimal photoperiod (Riedl and Croft, 1978). However, inter-population variation in CM was reported in diapause traits (Riedl, 1983). Genetic analysis of CM indicates that different critical day length initiate diapause in different geographic populations (Riedl and Croft, 1978; Frankham and Kingsolver, 2004). This may be an indication that CM can rapidly adapt to local climate conditions over multiple generations.

Table 1.1 Summary of past research on low-temperature treatments of codling moth

Trait investigated	Life-stage	Source for insects	Rearing temperature (°C)	Mean trait	Reference
Low temperature responses					
Supercooling point Survival Trehalose accumulation ^a	Larvae	Field	N/A	SCP: -13.4 °C (summer); -22.0 °C (winter) Survival: 0% at -20 °C for 24 hrs; 77% at 5 °C for 1 mth; 8% at -15 °C for 24hrs (summer)	Khani et al., 2007
Cold hardiness (freezing, melting and thermal hysteresis points). Haemolymph polyhydroxy alcohol levels ^a	Diapause larvae	and Laboratory and field	Diapausing larvae 18 °C. Non diapausing larvae 25 °C	Melting point for CM haemolymph: for induced diapause -0.56 °C and for 0.93 °C natural diapause. Freezing point for CM haemolymph: for induced diapause -1.18 °C and -1.07 °C for natural diapause. Thermal Hysteresis points: for induced diapause -0.17 °C and -0.14 for natural diapause	Neven, 1999

^aData not repeated in this table for the sake of brevity

Table1. 2 Summary of past research on high-temperature treatments of codling moth

Trait investigated	Life-stage	Source for insects	Rearing temperature (°C)	Mean trait	Reference
High temperature responses					
Mortality ^a	Larvae	Laboratory	N/A	18 °C/min: 100% after 50 min at 46 °C; 30% after 25 min at 46 °C; 100% after 25 min at 48 °C; 45% after 5 min at 48 °C; 100% after 5min at 50 °C; 100% after 2.5 min at 52 °C	Wang et al., 2002
Mortality, HSP70 accumulation ^a	Larvae	laboratory	27 °C	85% mortality at 50 °C after 2 hrs at 35 °C 100% mortality at 50 °C after zero hrs at 35 °C	Yin et al., 2006
Mortality	Larvae 5 th instar	Laboratory	N/A	95% mortality at 4, 6 and 8 °C/hr increase to 42, 44 and 46 °C. The slower the rate the more lethal.	Neven, 1998a

^aData not repeated in this table for the sake of brevity

Table 1.2 Cont.

Trait investigated	Life-stage	Source insects	for	Rearing temperature (°C)	Mean trait	Reference
Mortality	Larvae	laboratory		N/A	100% mortality for 45 °C for 45min and 47 °C for 25min at low oxygen levels and not increased carbon dioxide levels	Neven, 2005
Behaviour (cryptic basking)	Larvae	laboratory		Day: 24 °C. Night: 18 °C	74% fed on the warmer side of the fruit. 24% (Of the 74%) fed exclusively on the radiated hemisphere 8% fed exclusively on the cooler side	Kührt et al., 2005
Survival Fecundity ^a Viability of eggs	Eggs and larvae	laboratory		N/A	1 °C increase between 45 °C and 51 °C resulted in lower LT50 and less time to reach 100% mortality 20min resulted in fewer eggs per female and lower egg viability later when developed into adults. 0% histological abnormalities were found for testes of males for 5th instars exposed to 46 °C for 20min	Yokoyama et al., 1991

^aData not repeated in this table for the sake of brevity

Table 1.3 Summary of past research on combined high and low-temperature treatments of codling moth

Trait investigated	Life-stage	Source for insects	Rearing temperature (°C)	Mean trait	Reference
Combined heat and cold responses					
Mortality	Larvae	Laboratory	N/A	Mortality of larvae increased with intensity of heat treatment and duration of cold when larvae were exposed to 43, 44 or 45 °C for 30min followed by cold storage of 5 or 0 °C for up to 28dys	Neven, 1994
Mortality	Larvae	Laboratory	N/A	100% Mortality for 46 °C for 8hrs followed by 28 days at 0 °C	Neven and Rehfield, 1995

Cold hardiness adaptations in CM were explored by Neven (1999) (Table 1.1). In her study of diapausing CM reared at 18 °C and non diapausing CM at 25 °C, Neven (1999) reported that diapause induction had no significant effect on the supercooling points of the body and haemolymph of CM. Studies of adult CM thermoregulation by Kührt et al. (2006a) showed that unmated females and males prefer to rest at the low-temperature ends of temperature gradients between 15 and 32 °C. A striking contrast was shown in their study of ovipositing females which tended to deposit high proportion of eggs at high temperatures (Table 1.2). The difference in thermal preference of the pre-mating and ovipositing CM may reflect an adaptation to different selection pressures from the thermal environments. Unmated moths may benefit from low temperatures by a longer lifespan whilst high temperatures in oviposition sites will favour faster development of eggs (Kührt et al., 2006). Thermoregulation however only helps an insect in maintenance of stable thermal ranges of body temperature (T_b) (Kührt et al., 2005) either above or below the prevailing ambient temperature (Heinrich, 1980; Heinrich and Heinrich, 1983). Arthropods have developed other mechanisms to control their T_b independently of passive thermoregulation processes such as radiation, convection and evaporation (Heinrich and Heinrich, 1983; Kührt et al., 2005). For example, insects can modify T_b through behavioural methods such as microhabitat selection which is the most common and effective way (May, 1979; Kührt et al., 2006). Insects can benefit from short-term selection of thermally favourable microclimates especially of sunny or shaded substrates (May, 1979) and this temperature selection behaviour has been widely reported in a number of species including Lepidoptera (e.g. Clench, 1966; Kingsolver and Moffat, 1982; Kingsolver, 1985; Kührt et al., 2006b; reviewed in Heinrich 1980; Chown and Nicolson, 2004). However knowledge gaps still exist on how adult CM will perform in rapid changing temperature environments.

Insects can also overcome the effects of climatic extremes via physiological acclimation (a form of phenotypic plasticity) (Chown and Nicolson, 2004). In other words, some time at a particular set of environmental temperatures allow insects to adjust their biochemistry and physiology within a single generation to better cope with those particular conditions. However, acclimation to a particular temperature, for example, cold acclimation might decrease performance under high temperatures suggesting trade-offs in performance. This has recently been demonstrated in *Drosophila melanogaster* by Kristensen et al. (2008).

In the study by Kristensen et al. (2008) flies cold-acclimated in the laboratory had higher recapture rates at bait stations in the field under cold conditions, while warm-acclimated flies had much lower probability of recapture. By contrast, cold-acclimated flies were seldom recaptured under warm conditions in the field yet warm-acclimated flies were often caught. Furthermore, laboratory assays showed no advantage of acclimation on high temperature survival although low temperature survival did respond to acclimation, indicating that laboratory assays of performance and survival may be insufficient to assay field performance accurately. There is therefore a considerable need to explore these physiological changes and assess insect fitness in field conditions to make such data ecologically relevant because this information will be important for efficacy of programmes such as the S.I.T, which depend on the released male moths' performance. The ability of an insect to survive different temperature regimes may depend on its ecological and evolutionary history making species coming from tropical conditions respond differently to those from the temperate environment when compared under similar thermal conditions (Chown and Terblanche, 2007; Bowler and Terblanche, 2008). Thus the complex interactions of these factors including, severity of exposure (the longer the exposure the more lethal), determine an insect's thermal tolerance (Hoffmann et al., 2003).

It is equally important to understand the acclimatory capacity of thermal tolerance (Calosi et al., 2008; Chown and Nicolson, 2004) as estimating population and ecosystem level effects of climate change without considering such factors (i.e. based only on large scale numbers) may result in erroneous predictions (Helmuth et al., 2005; Calosi et al., 2008; Portner and Knust, 2007). One of the mechanisms that enable insects to withstand harsh conditions is rapid cold hardening (Lee et al., 1987) which can be defined as the rapid improvement in survival of extreme thermal conditions after pre-treatment of one to two hours to a prior sub-lethal temperature exposure. In *Drosophila melanogaster* such hardening has been known to improve survival by above 80% (Jensen et al., 2007). Some insects are actually able to survive freezing temperatures by the ability to supercool thereby avoiding ice formation in their cells (Sømme and Zachariassen, 1981) showing that insects do differ in the way they survive extremes from inducible tolerance/hardening to physiological adaptation thereby enabling them to maintain or even increase their mating and feeding patterns and thus perpetuate their populations. The same is also true for high temperature as reported by Scott et al., (1997). For example, a brief exposure to sub lethal high temperatures resulted in better survival of *Trichogramma carverae* in high temperatures. However some insects still do not

exhibit such mechanisms as is the case with *Glossina pallidipes* which does not have inducible cold tolerance at short periods (Terblanche et al., 2008) but does however exhibit seasonal adjustments in low temperature (Terblanche et al., 2006). Some insects do not show any rapid adjustments to thermal stress (Chown and Terblanche, 2007) hence it is a fallacy to assume that all insects have the same rapid response to temperature and have the same regulatory and adaptive means for compensating physiologically for temperature variation. Thus there is the need to understand the variation in thermal tolerance of insect pests. Studying the ability of the insects to rapidly tolerate various thermal conditions is useful since the data generated will be useful in area wide management through bioclimatic and phenology modelling (e.g. Kührt et al., 2006b). It will be difficult to predict pest abundance and geographic distribution without the knowledge of the species thermal tolerance. Earlier thermal assays on CM mainly focused on a suitable post harvest technique (e.g. Yokoyama et al., 1991; Neven and Rehfield-Ray, 2006). In conclusion, one can argue that the temperature biology of CM is not fully documented despite the fact that environmental temperature influences behaviour, activity, energetics, reproductive performance and survival. Hence it is the goal of this project to provide an empirical framework useful in the prediction of temperature-dependent population dynamics at short and intermediate time-scales.

1.6 Phenotypic plasticity

Phenotypic plasticity can be defined as “the ability of an organism to react to an environmental input with a change in form, state, movement or rate of activity” (West-Eberhard, 2003). In essence, it is the capacity of a genotype to exhibit a range of phenotypes in response to environmental variation (Fordyce, 2006). Phenotypic plasticity is an important attribute of an organism’s fitness in response to heterogeneous or changing environments, thereby contributing to phenotypic diversity observed in nature (Scheiner, 1993; Via and Lande, 1985; Price et al., 2003; Fordyce, 2006). The mean and variance of a phenotype within a population can be affected by plasticity. For example, a shift in the average phenotype of a population can occur when all individuals respond to an environmental cue. Likewise the variance observed for a trait can be reduced by a mere response among individuals of a population (Fordyce, 2006). Ultimately the influence of plasticity on the mean and variance of a population’s phenotype will be influenced by the time scale over which the plasticity is expressed and the time scale over which a plastic response is expressed can be acute as is the physiological and behavioural response of some animals (West-Eberhard, 2003). The plastic

responses can be comparatively slow and vary in their permanency (Fordyce, 2006) since some plastic responses such as behaviour are quickly reversible whereas some responses can be developmentally fixed (Greene, 1989; Fordyce, 2006; Terblanche and Chown, 2006).

Cross-generational effects are phenotypic modifications transmitted by parents to offspring (Crill et al., 1996; Hazell et al., 2010) with the environment in which the parents live having no genetic influence on the phenotypes of their offspring (Gilchrist and Huey, 2001). These parental effects are important from an evolutionary perspective as they may influence short-term responses to selection (Falconer, 1989; Kirkpatrick and Lande, 1989; Riska, 1989; Gilchrist and Huey, 2001) and are potentially adaptive (Mousseau and Dingle, 1991; Rossiter, 1996; Fox et al., 1997). Parental thermal environment has been shown to have diverse effects on subsequent offspring in *D. melanogaster* (Huey et al., 1995; Crill et al., 1996) and aphids (Hazell et al., 2010). Parents from warm environments tend to have fitter offspring in the same hot environment (Gilchrist and Huey, 2001). Within-generation effects might also involve developmental switches which allow plastic responses and irreversible phenotypic change in response to environmental conditions during a critical stage in development (Chown and Nicolson, 2004).

1.7 Insect thermal biology

1.7.1 Lethal, Sub-lethal temperatures and insect thermal performance curves

As mentioned previously, temperature has an effect on most physiological processes in insects (Chown and Nicolson, 2004; Cossins and Bowler, 1987). Extreme temperature influences the physiological processes in organisms by affecting the ability of the living organisms to perform normal, life-sustaining functions (see Fig. 1.3 and 1.4) and can be lethal (Cossins and Bowler, 1987). However, lethal temperatures are a function of both the severity and the duration of exposure (Cossins and Bowler, 1987). The optimal temperature range for different organisms varies among species, possibly owing to evolutionary thermal history. Thus, polar species are generally warm sensitive but more cold tolerant and tropical species are cold sensitive whilst being more high-temperature tolerant (Addo-Bediako et al., 2000). However insects may exhibit capacity adaptations by the modification of their response to temperature effects (Chown and Nicolson, 2004). Insect response to temperature may be summarized in two ways using Vannier's (1994) thermobiological scale as shown in (Fig. 1.4) and the performance curve (Fig. 1.5) (Angilletta et al., 2002, Chown and Nicolson,

2004). The thermobiological scale is convenient when resistance responses are considered and the thermal performance curve is useful when explaining capacity responses of the insect (Chown and Terblanche, 2007).

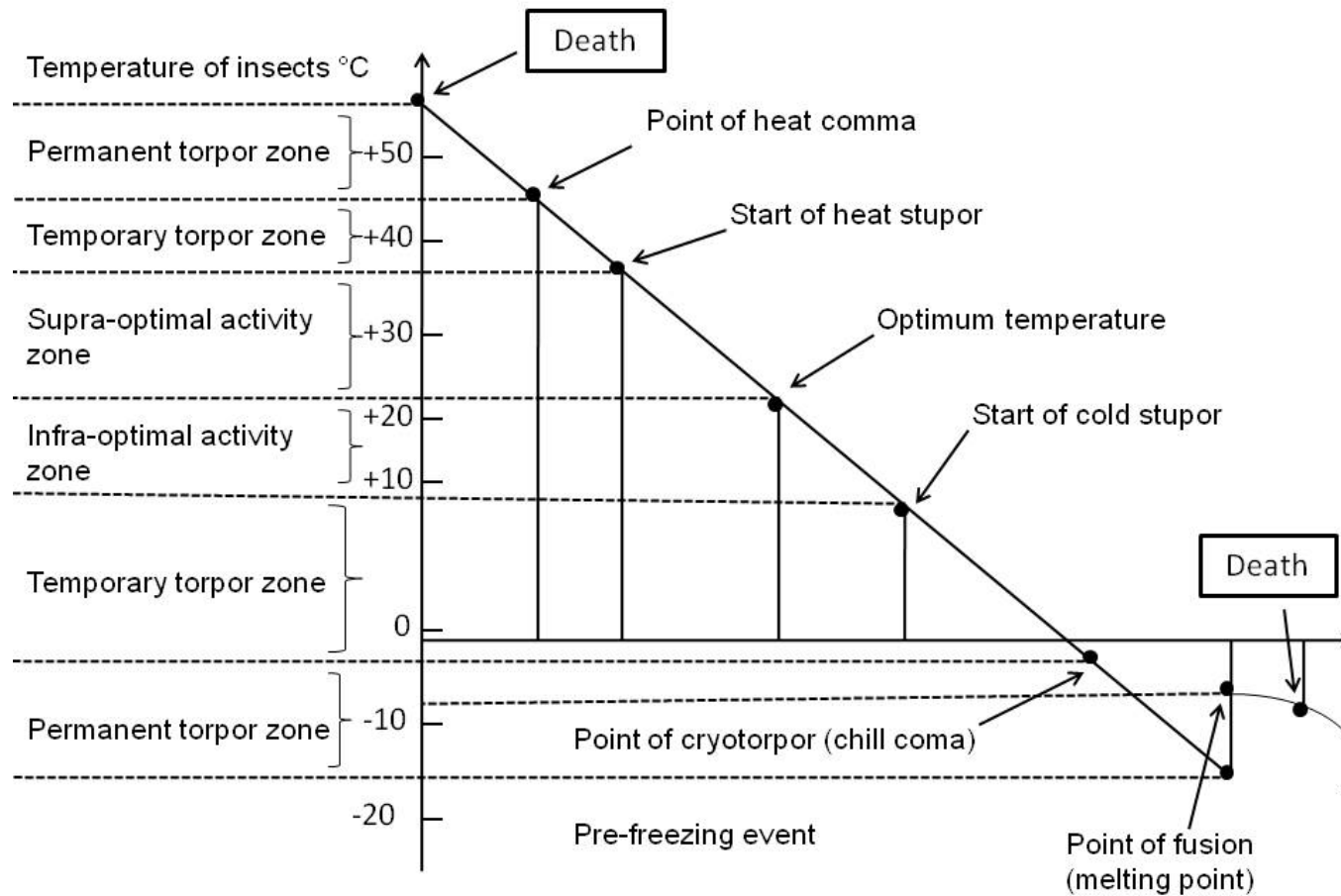


Figure 1.4 The thermobiological scale redrawn from Vannier (1994).

In the middle of Fig. 1.4 is the optimum temperature for an insect's survival, growth and evolutionary fitness. At temperatures higher than optimum, insects enter the supra-optimal activity zone before experiencing temporary or permanent torpor and finally death. At temperatures lower than optimum, there is the infra-optimal activity zone followed by the temporary torpor zone at more extreme temperatures. This zone is followed by permanent torpor, possible chill coma, and depending on the insect's freeze tolerance strategy, i) freezing but survival ii) freezing and death iii) death and then freezing (reviewed in Sinclair et al., 2003). Variation in temperature towards the extremes thus results first in knockdown or torpor then in prolonged coma, and eventually in irreversible trauma, with concomitant cell and tissue level damage, and death (Chown and Nicolson, 2004). It is worth noting insects tend to show a wider range of responses to sub lethal (non freezing and sub-zero temperatures) and potential lethal temperatures at low temperatures than is the case with high temperatures (e.g. Addo-Bediako et al., 2000).

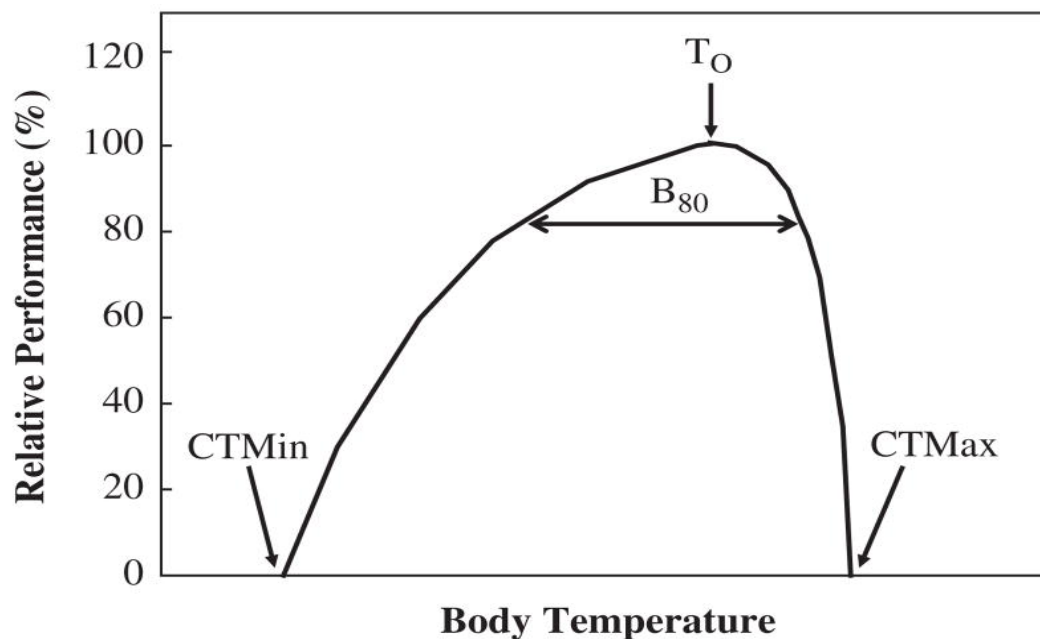


Figure 1.5 The generalised thermal performance curve for insects showing the optimum temperature (T_o), performance breadth (B_{80}) and critical thermal maxima and minima (CT_{max} and CT_{min}) (Redrawn from Angilletta et al., 2002).

Maximum performance (e.g. locomotion, development, nutrient digestion) of the insect (in Fig. 1.5) occurs at the optimal body temperature and thermal performance breadth is the range of T_b that allows a certain level of performance (Huey and Stevenson, 1979; Chown and Nicolson, 2004). The performance curve may however be shifted by acclimation or evolutionary adaptation (e.g. Gilchrist et al., 1997; Deere and Chown, 2006; Angilletta et al., 2002) resulting in changes in position and shape. Thus, thermal adaptations can assist insects in achieving higher evolutionary fitness by for example, prolonging flight activity in otherwise thermally unfavourable conditions or improve flight performance in favourable conditions. The thermal performance curve can be applied to any quantitative trait such as egg production, development rate and metabolic efficiency (Denlinger and Yocum, 1998). It is also important to note that there is a steeper drop in performance at temperatures above the optimum than at the lower temperatures, indicating faster reduction in performance at high temperatures above the optimum temperature (see discussion in Martin and Huey, 2008).

1.7.2 Acute exposure effects: patterns, mechanisms and potential influencing factors

The way insects are exposed to their thermal environment varies spatially and temporally. In cold environments for example, exposure ranges from long periods with relatively mild temperatures to acute exposures with much lower temperatures (Sinclair and Roberts, 2005). Thus, acute exposure is direct injury caused by brief exposures of great intensity (Sinclair and Roberts, 2005). Acute exposure may thus occur for cold (but non freezing) and hot temperatures, causing cold shock (Rajamohan and Sinclair, 2008) and heat shock (Chown and Nicolson, 2004) respectively. Various organisms show that mild thermal stress can increase hardiness of the organism to similar extreme conditions (Bowler, 2005). Short-duration exposure to sub-lethal temperatures is often referred to as hardening (Loeschcke and Sørensen, 2005); this will be true for both the high and low temperatures. Insects increase their cold tolerance over short exposure through rapid cold hardening (RCH) which is an improvement in survival in response to a brief exposure to sub-lethal low temperatures (Lee et al., 1987). Mechanisms underlying RCH in insects include increased glycerol concentration within the haemolymph as in *Sarcophaga crassipalpis* (Chen et al., 1987) or changes in membrane phospholipid composition (e.g. Overgaard et al., 2006). In contrast, high temperatures in most insects induce the synthesis of heat-shock proteins (Feder and Hofmann, 1999) which act as molecular chaperons, minimizing the aggregation of foreign proteins thereby avoiding thermal injury (Kelty and Lee, 2001). Pre-exposure to a

sub-lethal high temperature is called heat hardening (Chen et al., 1990; Yocum and Denlinger, 1992) and this hardening results in the organisms acquiring heat shock resistance for a prolonged period of time (Loeschcke et al., 1994). Acute cold injury therefore results in membrane phase transition (Rajamohan and Sinclair, 2008) whereas acute heat injury also results in disruption of membrane function especially synaptic membranes (Cossins and Bowler, 1987; Chown and Terblanche, 2007), changes in cell conditions such as pH, protein denaturation, and DNA lesions (Somero, 1995; Feder and Hofmann, 1999) ultimately affecting development, muscle contraction and several other essential processes (Denlinger and Yocum, 1998; Chown and Nicolson, 2004).

1.8 Quality control in SIT: laboratory cultures versus field performance

Quality of mass-reared insects can be described as a measure of how well those insects function in their intended role Heuttel (1976), or how effectively they interact with the target population (Bloem et al. 2004). The need for such quality moths has been reported by many authors working on CM (Bloem et al., 2004; Hutt, 1979; Judd et al., 2006; Judd and Gardiner, 2006). Wide interest in moth quality has given rise to a number of opinions as to its causes, and suggestions on how it might be improved have been diverse (see Table 1.4) (reviewed in Calkins and Parker, 2005; Simmons et al., 2010). Generally, however, issues of quality control in pest management focus largely on improving the numbers of insects reared, rather than focusing on insect performance in the field. Field performance has however been the focus of extensive investigation from evolutionary biology research where performance is typically interpreted as Darwinian ‘fitness’ (Gilchrist and Huey, 2001; Kristensen et al., 2008).

Table 1.4 Summary of studies done on laboratory populations of *Cydia pomonella* to improve moth quality

Treatment/trait	Life-stage treated	Targeted trait for improvement	Result	Reference
Incorporation of diapause in rearing ^a	Diapause	<i>mating competitiveness</i>	↑ <i>improved mating competitiveness</i>	Bloem et al., 1997 Judd et al., 2006
Lower dosage of gamma radiation ^a	Adults	<i>flight activity, pheromone response</i>	100Gy ↑ <i>male response to calling females</i> 250Gy ≈ <i>on trap catches</i>	Bloem et al., 1999a; 1999 b; 2001 Judd and Gardiner, 2006
Combination of diapause and irradiated moths ^a	Diapause/adults	<i>field competitiveness</i>	150Gy and diapause ↑ <i>mating competitiveness</i>	Bloem et al., 2004
Fluctuating temperatures ^a	Through development	<i>Recapture rate</i>	≈ <i>in recaptures of the 1st generation</i> ↑ <i>in recaptures</i>	Hutt, 1979

^aData not repeated for the sake of brevity

↑ = improved

≈ = no difference

Laboratory experiments thus need to be confirmed through further investigations from field experiments to help clarify some of the factors that can be involved in constraints on fitness or performance (Blows, 1993; Jenkins et al., 1997; Magiafoglou and Hoffmann, 2003). One of the confounding factors constantly facing insect laboratory populations is harsh/extreme climatic conditions due to the sudden change from standard controlled conditions to highly variable natural environment.

Living organisms however, can escape effects of climatic extremes by physiological acclimation thereby allowing them to survive and reproduce in otherwise harsh conditions. However the acclimation to one extreme might actually decrease performance in a different set of conditions (Kristensen et al., 2008). For example, laboratory populations exposed to various thermal regimes can show fitness trade-offs across environments because populations perform relatively better in the environment where they evolved (Lenski and Bennet, 1993; Partridge et al., 1995). Field release studies of *Drosophila* undertaken by Kristensen et al., (2008) revealed the costs of cold acclimation that were never detected by standard laboratory assays. The selection for traits closely associated with fitness may also help identify constraints to evolutionary change (Magiafoglou and Hoffmann, 2003). However, inferences on natural systems based on laboratory selection responses can be misleading if the selections and treatments are not ecologically relevant, which is also true if genetic variances of the laboratory populations are not reflective of those in nature (Harshman and Hoffmann, 2000; Hoffmann et al., 2001; Scheiner, 1993). In sum, laboratory reared insects may exhibit differences in biological or behavioural traits (Huettel, 1976) from their wild counterparts as differences may arise due to different adaptive responses to artificial and natural field conditions. There is therefore a need to define how physiological responses to temperature in CM might impact on their fitness and performance in the wild as laboratory experiments do not incorporate all aspects of fitness (Kingsolver, 1999).

1.9 Goals of this research

The goals of this project are two-fold:

1. To explore rapid thermal responses and thermal tolerance in adult *C. pomonella* (Chapter 2).
2. To explore the costs and benefits of thermal acclimation for field performance of *C. pomonella* in sterile insect release programmes (Chapter 3).

Specifically, in Chapter 2, my objectives are as follows:

- to determine the range of time-temperature combinations which may be lethal at short time-scales
- to examine a range of conditions which might induce rapid cold- or rapid heat-hardening responses
- to assess cross-tolerance to temperature by assessing survival at low temperatures and their responses to a brief high temperature pre-treatment and *vice versa*
- to explore critical thermal limits to activity at high and low temperatures and their plasticity thereof

In Chapter 3, my specific objectives are as follows:

- to investigate trade-offs in thermal performance using laboratory and field experiments
- to explore the costs and benefits of manipulating thermal environments during CM rearing for field activity in sterile insect release.

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Chapter 2

Rapid thermal responses and thermal tolerance in adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae)*

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2.1 Introduction

Temperature plays a key role in the life of insects. Over longer periods, temperature influences seasonality and evolutionary responses of insects (Bale, 2002; Lee and Denlinger, 2010; Chown and Nicolson, 2004). However, the ability of diel temperature fluctuations to affect activity and survival at short-time scales is also of critical importance. Indeed, insect responses to temperature extremes over short periods may be an important driver of population dynamics and, consequently, species' abundance and geographic distribution over longer timescales (reviewed in Bale, 2002; Chown and Terblanche, 2007; Lee and Denlinger, 2010; Hoffmann, 2010). Insect responses to temperature extremes may also be essential in phenological or distribution modelling of climate change impacts (e.g. Estay et al., 2009; Lima et al., 2009; Hazell et al., 2010a; reviewed in Bale, 2002). In addition, most current control methods used in quarantine and post-harvest pest control involve some form of temperature treatment (Neven and Hansen, 2010). However, insect survival in variable thermal environments has been known to be influenced by a host of factors including the rate of temperature change (Powell and Bale, 2006; Terblanche et al., 2007; Mitchell and Hoffmann, 2010), thermal history (or acclimation/acclimatization) (Nyamukondiwa and Terblanche, 2010; Hoffmann et al., 2005; Hazell et al., 2010b), and pre-exposure to sub-lethal environments enabling them to survive otherwise lethal ambient temperatures (T_a) (e.g. Powell and Bale, 2005; Loeschcke and Hoffmann, 2007; Slabber and Chown, 2005). Such plasticity of thermal tolerance may make some quarantine protocols less effective in controlling pests if the protocol itself results in enhanced thermal tolerance (Stotter and Terblanche, 2009; and see discussions in Denlinger and Lee, 2010) or, on the other hand, may be manipulated to enhance temperature-dependent performance and survival and perhaps also benefit control programmes (Bloem et al., 2006; Chidawanyika and Terblanche, *in press*).

When threatened by temperature extremes, insects employ a range of mechanisms to adjust their body temperature (T_b), or the extremes they can withstand, using either physiological or behavioural mechanisms or some combination of both. For example, an insect experiencing adverse high T_a can lower its T_b by avoidance of sunny hot spots and vice versa (e.g. Kührt et al., 2006; Huey and Pascual, 2009). However, behavioural adjustments only act as the first line of defence against sub-optimal T_a and depend to a large degree on

microsite opportunities in their habitat (Kührt et al., 2006). If unfavourable T_a persist, physiological mechanisms may become critical to ensure survival. Examples of such physiological adjustments include alteration of thermal tolerance at daily (e.g. Sinclair et al., 2003; Overgaard and Sørensen, 2008) or seasonal (e.g. Khani et al., 2007; Khani and Moharrimipour, 2010) time-scales.

Temperatures lethal to insects are a function of both the magnitude of the temperature variation and the duration of exposure (Chown and Nicolson, 2004; Angilletta, 2009; Denlinger and Lee, 2010). However, phenotypic plasticity of thermal tolerance means that insects can modify the time-temperature phase space, thereby promoting survival. Induction of such plastic responses can be achieved after pre-exposure to sub-lethal temperatures or perhaps also in anticipation of extremes (discussed in Chown and Terblanche, 2007), enabling insects to survive what would otherwise be lethal conditions. However, insects unable to increase thermal tolerance through rapid plastic responses may have even greater mortality during the subsequent exposure (e.g. Terblanche et al., 2008). Acute pre-exposures altering thermal tolerance have been referred to as ‘hardening’ responses and sometimes also as cold or heat ‘shock’ (Bowler, 2005; Sinclair and Roberts, 2005; Loeschcke and Sørensen, 2005). Here, I use ‘hardening’ to refer to the physiological responses which are of primary interest. Rapid cold-hardening (RCH) or rapid heat-hardening (RHH) not only helps to improve survival in lethal conditions but can also help organisms to continue performing routine activities, such as mating and feeding, despite adverse conditions (e.g. Fasolo and Krebs, 2004) and can thereby increase fitness (e.g. Powell and Bale, 2005 and see discussions in Lee and Denlinger, 2010).

Mechanisms of injury caused by extreme temperatures in insects vary from cellular to tissue levels and a range of biochemical responses are probably significant to counter potentially deleterious effects. In freeze intolerant insects, low temperature injury is largely regulated by a depression of supercooling (freezing) point of the body, typically involving polyhydric alcohols (polyols) and sugars which act as cryoprotectants. Also of importance to survival in these insects is removal of potential nucleating agents through, for example, cessation of feeding (Bale, 2002). However, some freeze intolerant insects die at temperatures well above their supercooling point and this type of injury is thought to be related to neuromuscular damage at the tissue level, while at the cellular level injury is attributed to membrane phase transitions, thermoelastic stress and damage to essential proteins (Lee and

Denlinger, 1991, 2010; Bale, 2002; Chown and Nicolson, 2004). In freeze tolerant insects, low temperature injury may be associated with extracellular ice formation or the re-establishment of ion homeostasis after thawing and, consequently, much attention has been given to ice nucleating agents, antifreeze proteins and cryoprotective sugars and polyols (e.g. Košťál et al., 2007; Duman et al., 2004). High temperatures result in the disruption of membrane function, DNA lesions, changes in cell microenvironment, and protein denaturation which can restrict enzyme-catalysed reactions (Chown and Nicolson, 2004). Insects under high temperature stress may produce heat-shock proteins (Hsps) (e.g. McMillan et al., 2005) which act as molecular chaperones protecting other cellular proteins and conserving key enzyme function. Heat shock proteins have also been implicated in low temperature tolerance (e.g. Rinehart et al., 2007) although the role of Hsps over short time-scales (i.e. hardening responses) are more contentious (Sinclair and Roberts, 2005; Chown and Nicolson, 2004). Over brief periods of low temperature exposure, membrane phospholipid composition may also be radically altered to enhance low temperature tolerance (e.g. Overgaard et al., 2006; but see MacMillan et al., 2009). Insects exposed to low temperatures over longer periods, e.g. during initiation of overwintering or entering diapause, may increase the synthesis of various polyols or sugars which function as cryoprotectants and can lower the risk of freezing. For example, glycerol or sorbitol concentration can be elevated during overwintering and is associated with a decrease in supercooling point (Minder et al., 1984; Khani et al., 2007).

In this study, I investigate how various acute temperature changes affect the activity limits and survival of 1-2 day old adult codling moth, *Cydia pomonella* (Lepidoptera Tortricidae), a polyphagous pest of global agricultural importance (Barnes, 1991; Dorn et al., 1999). Most work to date investigating thermal tolerance of *C. pomonella* has focused on larvae for post-harvest control and fruit disinfestation (e.g. Neven and Rehfield, 2006; Neven and Hansen, 2010) or overwintering physiology (Khani et al., 2007; Khani and Moharramipour, 2010). When other life-stages of *C. pomonella* have been investigated, these have typically employed extreme, fairly ecologically unrealistic thermal conditions which may nevertheless allow comparison among life-stages (e.g. Wang et al., 2004). Here I specifically focus on adult thermal biology as this is the life-stage responsible for reproduction and probably most dispersal in natural and agricultural conditions (Barnes, 1991; Timm et al., 2010). In addition, it is the life-stage used for the sterile insect technique (SIT) control programme (Botto and Glaz, 2010; Vreysen et al., 2010). The SIT programme for *C.*

pomonella usually includes cooling moths for a brief period for ease of handling and to avoid excess damage during transportation, prior to release in the wild (Carpenter et al., 2010; Simmons et al., 2010). However, it is unclear how such chilling, or indeed, short term temperature fluctuations more generally, may influence adult *C. pomonella* activity and survival upon release in orchards (but see Bloem et al., 2006). It is therefore important to understand how thermal history might influence performance and survival as this could help improve quarantine or post-harvest control procedures, or alternatively, may be used to enhance SIT efficacy (Bloem et al., 2006; Simmons et al., 2010; Chidawanyika and Terblanche, *in press*).

The objectives of this study were several-fold. First, I determined the range of time-temperature combinations which may be lethal at short time-scales to give insight into their impact on population dynamics and fitness of the species (Gilchrist and Huey, 2001; Loeschcke and Hoffmann, 2007). Second, I examined a range of conditions which might induce rapid cold- or rapid heat-hardening responses and thus investigated short-term, plastic responses of survival at extreme temperatures. Third, I assessed cross-tolerance to temperature by assessing survival at low temperatures and their responses to a brief high temperature pre-treatment and *vice versa* (i.e. responses of high temperature survival after low temperature pre-treatment). Finally, I measured critical thermal limits to activity at high and low temperatures and assessed their plasticity by varying the rates of temperature change in these dynamic assays. These results are discussed in the context of local agroecosystem microclimate recordings and survival of adult *C. pomonella*.

2.1 Methods

2.1.1 Insect culture

The *C. pomonella* culture used for our experiments was originally established in 2004 at the Deciduous Fruit Producer's Trust (DFPT) Stellenbosch rearing facility. Rearing was done on a diet described by Guennelon et al. (1981) on trays of food medium for developing larvae. Pupae were held in darkened cardboard boxes (800 mm³) for adult eclosion in the laboratory under (12:12) (L: D) photoperiod in air-conditioned, insulated rooms at 25±1 °C. On emergence, all adult moths had access to 50% sugar/water solution until they were used in thermal tolerance assays as 24 to 48 hr old adults. This age class was used as it represents the moths typically used for SIT release. Despite the access to the sugar/water solution, I could

not distinguish between individual moths that fed from those that did not. Hence, feeding status was not strictly controlled for in these trials but I maintained high sample sizes to randomize these effects across treatments.

2.2.2 *Lethal temperature assays*

Programmable water baths (GP200-R4, Grant Instruments, UK) were used to measure thermal tolerance using a direct plunge protocol (as in e.g. Sinclair et al., 2006; Terblanche et al., 2008) to determine both the upper lethal temperature (ULT) and lower lethal temperature (LLT) for a range of times (from 0.5 to 4 hrs). A mixture of propylene glycol and water (1:1 ratio) was used to enable water baths to operate at sub-zero temperatures without freezing. Live 1-2 day old adult insects were placed in 60ml polypropylene vials ($n=10$ in each vial x 5 vials) for each temperature/time treatment until a range of 0-100% mortality was covered. Relative humidity (RH) in the ULT experiments was controlled and maintained at >80% RH using strips of filter paper moistened with drops of distilled water suspended from the perforated lids of the vials to avoid desiccation-related mortality. However, care was taken to ensure that there was no free water in the vials during experiments to avoid accidentally drowning the insects. Temperatures in water baths were verified using NIST certified thermometers before each treatment (as in e.g. Stotter and Terblanche, 2009). Vials containing the post-assay *C. pomonella* were placed in a 25 ± 1 °C climate chamber for 24 hrs after which survival was recorded. Previous studies have shown that water baths and small vials such as those used here are generally rapidly in thermal equilibrium (e.g. Stotter and Terblanche, 2009). Moreover, in small insects especially, insect body temperature almost always equals air temperature since they are ectothermic and small in size (Terblanche et al., 2007). For the purposes of this study, survival was defined as coordinated muscle response to stimuli such as gentle prodding, or normal behaviours such as feeding, flying or mating.

2.2.3 *Rapid thermal responses*

Rapid cold-hardening and rapid heat-hardening experiments were performed using established protocols (e.g. Terblanche et al., 2008; Stotter and Terblanche, 2009; Sinclair and Chown, 2003). In most cases, I used a discriminating temperature at which 25% survival was estimated in the LLT and ULT experiments. The magnitude of pre-treatment temperature and duration of exposure was varied using different temperature, plunge, gap and ramping rate treatments to allow for potential Heat shock protein (Hsp) responses (for rationale see e.g. Sinclair and Chown, 2003; Stotter and Terblanche, 2009) (Fig. 2.1). In all cases, control

groups were included in each daily assay were performed in order to eliminate any bias related to handling stress or cohort effects. To investigate the mechanism used by *C. pomonella* to improve survival after low or high pre-treatment, I also undertook cross-tolerance experiments (e.g. MacMillan et al., 2009). In brief, moths were pre-treated at non-lethal low temperatures and then mortality was assayed at lethal high temperatures, or *vice versa*, before their survival was scored after 24 hrs recovery at 25 °C.

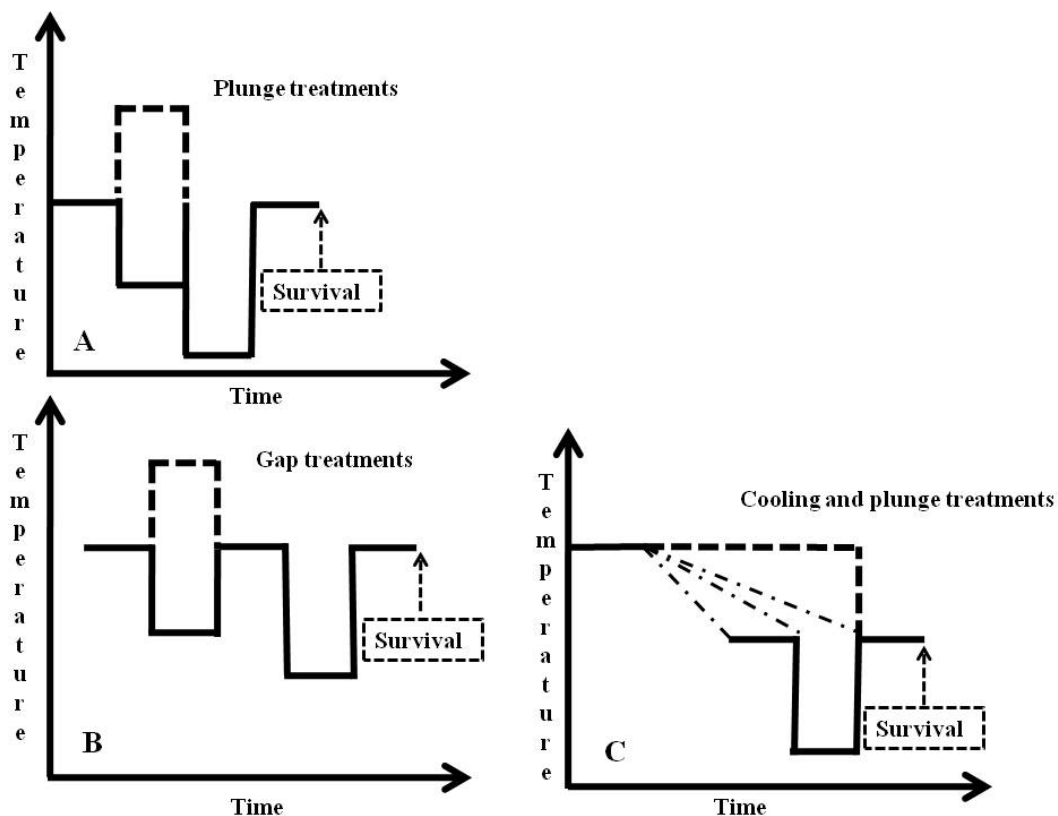


Figure 2.1 Schematic diagram of experimental protocols for plunge (A), gap (B) and combination of cooling and plunge (C) treatments that were followed to elicit hardening responses in adult *Cydia pomonella*

2.2.4 Effects of ramping rates on critical thermal limits

We assessed CTLs under a range of heating and cooling rates to determine if RCH and RHH responses may be elicited by different rates (e.g. Powell and Bale, 2006; Overgaard et

al., 2006), and to assess ecologically-relevant CTLs. In these experiments, moths were individually placed in insulated double-jacketed series of chambers ('organ pipes') connected to a programmable waterbath and subjected to different constant rates of heating or cooling (0.06, 0.12 and 0.25 °C min⁻¹ starting from 25 °C to determine their critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) respectively (see e.g. Nyamukondiwa and Terblanche, 2010; Mitchell and Hoffmann, 2010). A mixture of water and glycol solution (1:1 ratio) was used in the waterbath to enable sub-zero operation. Upon placement in the 'organ pipes', moths were given 10 minutes to equilibrate at 25 °C before temperature ramping started and their CT_{max} or CT_{min} was measured. A copper-constantan (Type T, 36 SWG) thermocouple connected to a digital thermometer (Fluke 53/54II, Fluke Cooperation South Africa; accuracy 0.01 °C) was inserted into the control chamber to detect and verify organ pipe chamber temperatures. I assumed that body temperature of the moths was in equilibrium with the chamber temperature (for justification, see Terblanche et al., 2007). The CT_{min} and CT_{max} were defined as the temperature at which a moth lost coordinated muscle function or experienced onset of muscle spasms, respectively (e.g. Nyamukondiwa and Terblanche, 2010). Moths were never removed from the organ pipes to assess behaviour. Because age can have a major effect on thermal tolerance of insects (Bowler and Terblanche, 2008; Nyamukondiwa and Terblanche, 2009) it was strictly controlled in all assays. For CTL assays, 1-2 day old moths which had access to sugar and water solution (50:50 ratio) were used in all experiments. However, gender was not taken into consideration as preliminary assays showed no effect on *C. pomonella* CTLs. Each individual moth was treated as a replicate and twenty individuals were used per ramping rate for all CT_{min} and CT_{max} experiments. Individuals used in CT_{min} assays were not re-used for CT_{max} assays and were discarded.

2.2.5 Statistical analyses

Temperature-time interaction effects on survival (number of moths alive / total moths exposed as the dependent variable) for both lower and upper lethal limits was analysed using non-linear models (*proc probit*) in SAS 9.1 (SAS Institute Inc. Cary, USA). Tests for significance of temperature, duration of exposure and their interactions were undertaken using generalized linear models (Wald χ^2 test) with a single degree-of-freedom approach, corrected for overdispersion and assuming a bimodal distribution. A wafer-estimation approach in Statistica 8.0 (Statsoft, Oklahoma, USA) was used to generate surface plots of time and temperature effects on survival (as in e.g. Stotter and Terblanche, 2009).

Generalized linear models (GLZ), performed in SAS 9.1 (*proc genmod*) were used to assess the effects of hardening pre-treatments on the survival of *C. pomonella*. A binomial distribution was assumed for survival data with a logit link function and correction for overdispersion. In addition, all comparisons of pre-treatments were made relative to the corresponding replicated control groups. Analyses of high and low pre-treatments were also performed separately to avoid biased outcomes of statistical tests due to pooling of test groups. Similar GLZ analyses were also used to test the significance of hardening pre-treatments on the cross-tolerance of *C. pomonella*. Statistically homogenous groups were identified using overlap in 95 % confidence limits.

The effects of ramping rates on critical thermal limits were compared using One-Way ANOVAs in STATISTICA 9 (Statsoft, Tulsa, OK, USA). In this instance, the categorical predictor was the ramping rate (0.06, 0.12 or 0.25 °C min⁻¹) and the dependent variable was either CTmin or CTmax. Key assumptions of ANOVA were checked and were met for homogeneity of variance and normality of data distributions. Tukey's HSD *post hoc* tests were used to identify statistically homogenous groups at $\alpha = 0.05$.

2.3 Results

2.3.1 Lethal temperature assays

The temperatures and times to which *C. pomonella* were exposed significantly affected their survival at either high or low temperature (Table 2. 1). An increase in intensity of heating or chilling temperatures resulted in increased mortality (Fig. 2.2 and Fig. 2.3). Similarly, an increase in the duration of exposure at any given temperature resulted in a reduction in *C. pomonella* survival (Fig. 2.2 and Fig. 2.3). The interaction of temperature and the duration of exposure was highly significant resulting in shorter periods of time required to inflict 100% mortality at extremely severe low or high temperatures, suggesting limited plasticity of survival in these trials (Table 2.1; Fig.2.2 and Fig. 2.3).

Table 2.1 Summary of probit non-linear analyses results of the effects of temperature and duration of exposure on the survival of *Cydia pomonella*. Tests of significance were done using generalized linear models (GLZ) (Type III) analyses assuming a logit link function in SAS and correcting for overdispersion. Five replicates of ten individuals were used for both low (n=1700 moths, n=170 replicates) and high (n=2000 moths, n=200 replicates) temperature treatments. Lethal temperatures ranged from -20 to -5 °C and 32 to 47 °C for 0.5 to 4 hrs treatments (see Fig. 2.2 and Fig.2.3).

Parameter	d.f.	Estimate ± S.E.	Wald χ^2	p
<i>Lower lethal temperature</i>				
Intercept	1	23.0510±2.8417	65.80	<0.0001
Time	1	3.8732±0.5852	43.80	<0.0001
Temperature	1	1.9595±0.2363	68.75	<0.0001
Time x Temperature	1	0.0485±0.0485	49.01	<0.0001
<i>Upper lethal temperature</i>				
Intercept	1	35.6723±2.9514	146.08	<0.0001
Time	1	3.4180±0.7700	19.71	<0.0001
Temperature	1	0.8428±0.0690	149.34	<0.0001
Time x Temperature	1	0.0834±0.0179	21.80	<0.0001

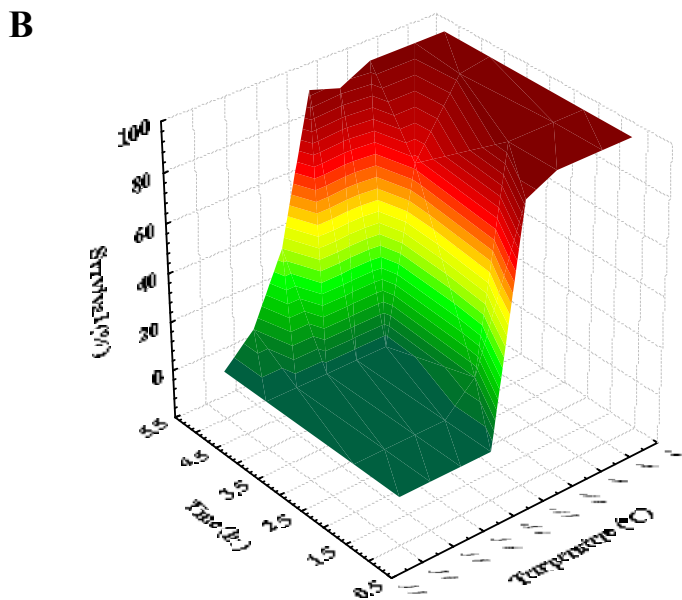
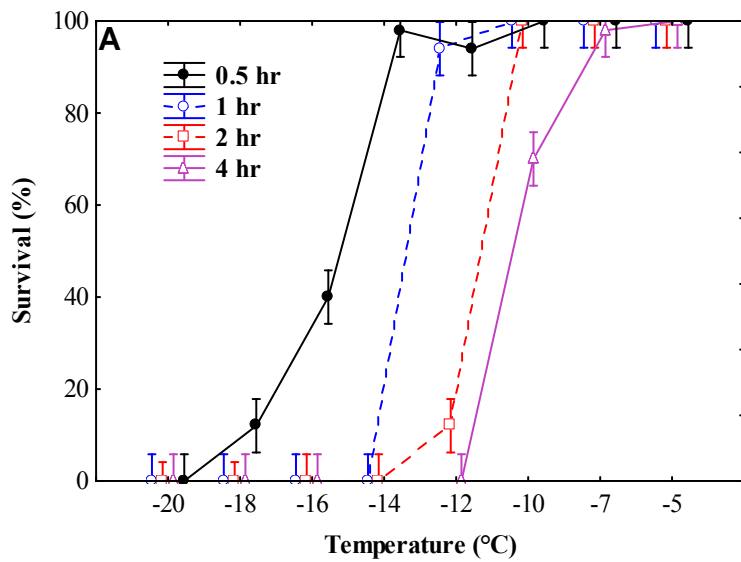
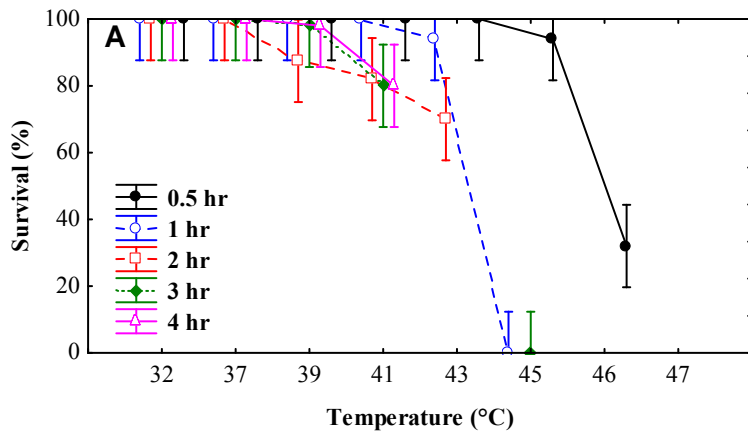


Figure 2.2 Mean survival (\pm 95% confidence limits (CL)) of *Cydia pomonella* under different low temperatures during different durations of exposure (A). Three-dimensional surface plot of the relationship between survival of *Cydia pomonella*, low temperature and time fitted using the wafer estimation method in Statistica (B). Data points for the figures represent averages of 5 replicates of $n=10$ individual moths per replicate per treatment.



B

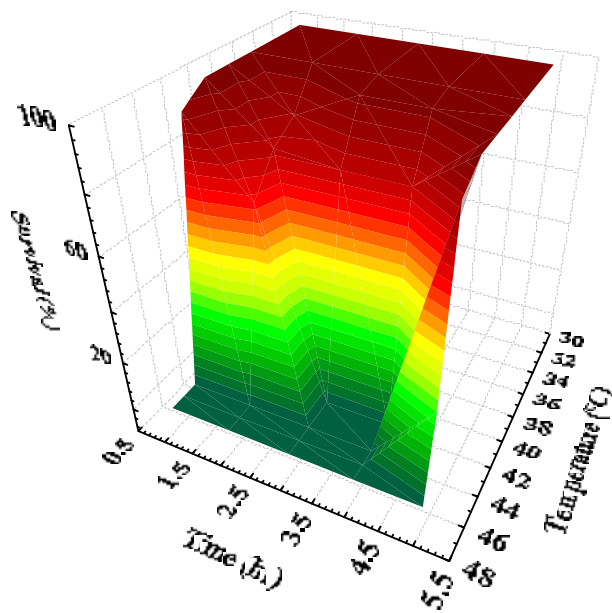


Figure 2.3 Mean survival (\pm 95% CL) of *Cydia pomonella* under different high temperatures during different durations of exposure (A). Three-dimensional surface plot (Wafer fit method) of the relationship between *Cydia pomonella* high temperature survival and time (B). Data points for the figures represent averages of 5 replicates of $n=10$ individual moths per treatment.

2.3.2 Effect of ramping rates on critical thermal limits

Critical thermal minima were not significantly affected by variation in cooling rates ($F_{2,57} = 2.322$, $p = 0.107$) (Fig. 2.4A). By contrast, CTmax was significantly affected by variation in heating rates ($F_{2,57} = 19.28$, $p < 0.0001$). However, mean CTmax for moths exposed to heating rates of 0.12 and 0.25 °C min⁻¹ were statistically homogenous while 0.06 min⁻¹ yielded higher CTmax (Fig. 2.4B).

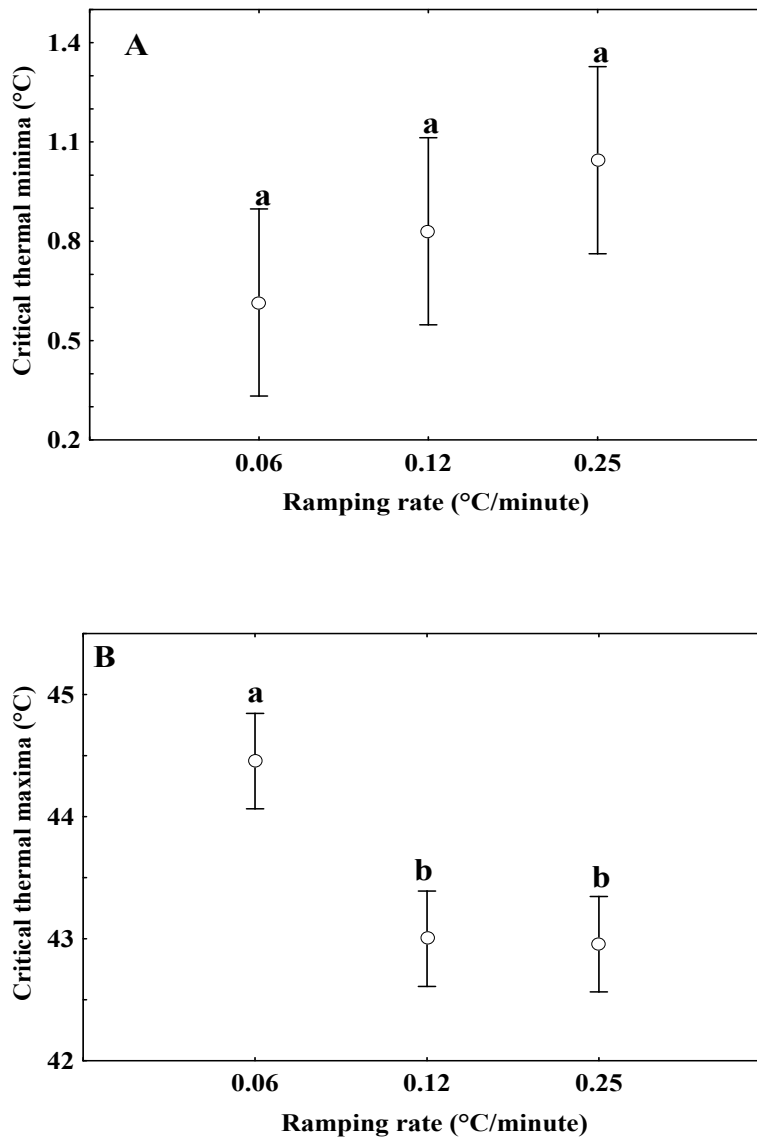


Figure 2.4 Effects of cooling and heating rates on (A) critical thermal minima and (B) critical thermal maxima of adult *Cydia pomonella*. Data points represent means of $n=20$ for each treatment of mixed gender. Error bars represent 95% CLs.

2.3.3 Rapid thermal responses

Low temperature pre-treatment of -7, 0 or 5 °C for 1 hr significantly reduced survival of *C. pomonella* relative to the control group (Table 2.2; Fig. 2.5A). Adult moths that were pre-treated at 5 °C for 1 or 2 hrs and given 1 hr (gap treatment) at 25 °C significantly increased survival at -9 °C for 2 hrs (Table 2.2; Fig. 2.5A). Survival of adult moths pre-treated at 5 °C for 2 hrs and exposed to -10 °C for 2 hrs using direct plunge protocols was not significantly different from controls (Table 2.2; Fig. 2.5A). A slow cooling protocol, at a rate of 0.01 °Cmin⁻¹ from 25 °C to 5 °C and then holding moths at this temperature for 1 hr significantly improved survival when assayed at -10 °C for 2 hrs (Table 2.2.) However, cooling rates of 0.1 and 0.5 °C min⁻¹ did not significantly improve *C. pomonella* survival at -10 °C for 2 hrs (Table 2.2).

Adult *C. pomonella* pre-treated at 35 °C for 1 hr did not show significant improvements in survival after a direct exposure to 43 °C for 2 hrs (Table 2.3; Fig. 2.5B). However, there was a dramatic improvement in survival in moths exposed to 37 °C for 1 hr followed by a 1 hr gap at 25 °C and then assayed for survival at 43 °C for 2 hrs (Table 2.3; Fig. 2.5B). One or 2 hr pre-treatment at 32 °C, with or without a gap period at 25 °C, did not improve survival at 45 °C for 2 hrs (Fig. 2.5B).

Cross tolerance experiments showed that adult *C. pomonella* can improve survival at low temperatures after high temperature pre-treatment, but not vice versa. A pre-treatment at 37 °C for 1 hr improved *C. pomonella* survival of 2 hrs at -9 °C (Table 2.4, Fig. 2.6A). However, a 2 hr exposure at 5 °C did not improve survival at 45 °C (2 hrs) (Table 2.4, Fig. 2.6B).

Table 2.2 Summarised output of generalized linear models (GLZ) for the effects of low thermal pre-treatments on the survival of *Cydia pomonella*. First, hardening pre-treatment of 0, -7 and 5 °C for 1 hr and plunged immediately thereafter into -12 °C for 2 hrs. Second, moths hardened at 5 °C for 1 and 2 hrs before being plunged into -9 °C for 2 hrs. Third, moths hardened at 5 °C for 2 hrs and given 1 hr at 25 °C (gap treatments) before being plunged into -10 °C for 2 hrs. Lastly, moths at 25 °C were cooled at the rate of 0.01, 0.1 and 0.5 °C min⁻¹ to 5 °C before being plunged into -10 °C (cooling rates and plunge treatment combination).

Treatment	d.f.	Estimate ±S.E.	Wald χ^2	p
Control 1 (25°C/1hr/-12 °C)	1	0.6633±0.1753	14.32	0.0002
0°C/1hr/-12 °C	1	28.6110±135530	0.00	0.9998
-7/1hr/-12 °C	1	28.6110±135530	0.00	0.9998
5°C/1hr/-12 °C	1	28.6110±135530	0.00	0.9998
Sub-treatment effects	4		14.32	0.0063
Control 2/25°C/2hr/-9 °C)	0	0.000±0.000		
5°C/1hr/-9 °C	1	0.7331±0.2953	6.16	0.0131
5°C/2hrs/-9 °C	1	1.9357±0.4016	23.23	<0.001
Sub-treatment effects	2		25.48	<0.001
Control 3	0	0.000±0.000		
5°C/2hrs/-10 °C	1	0.4833±0.3722	1.69	0.1941
Sub-treatment effects	1		1.69	0.1941
Control 4	1	0.4953±0.4001	1.53	0.2158
0.01 °C min ⁻¹	1	1.5964±0.4548	12.32	0.0004
0.1 °C min ⁻¹	1	0.6554±0.4001	2.68	0.1014
Control 5	0	0.000±0.000		
0.5 °C min ⁻¹	1	0.9659±0.4202	5.28	0.0215
Sub-treatment effects	4		36.70	<0.0001
All treatment effects	14		122.56	<0.0001

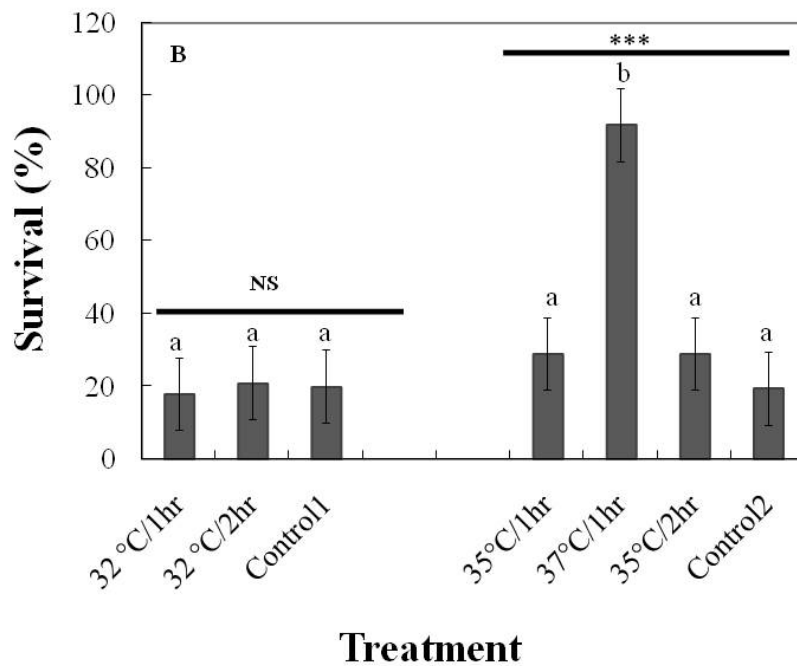
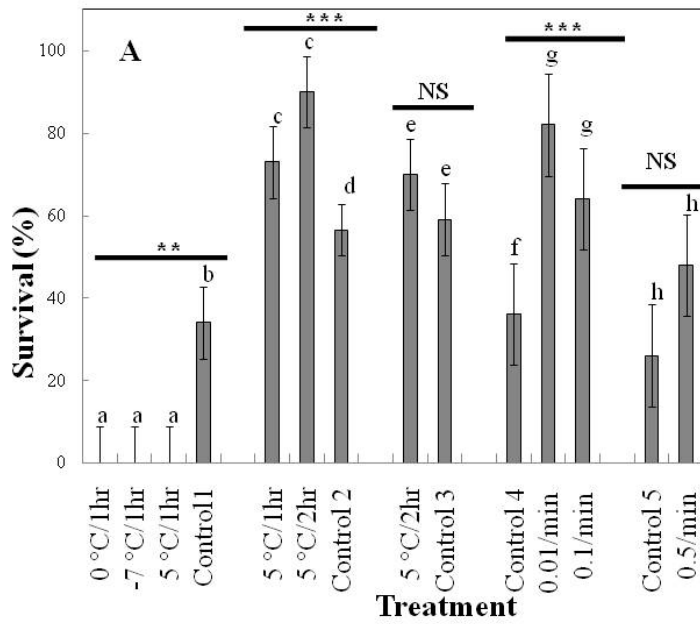


Figure 2.5 Mean survival of *Cydia pomonella* at -9, -10 and -12 °C for 2 hrs (A) and at 43 and 45 °C for 2 hrs (B) after receiving a range of pre-treatments. Detailed statistical results are given in Table 2.2. (***: $p < 0.001$) (**: $p < 0.005$) (NS: non-significant). Data points for the figures represent means of $n=50$ per treatment. Error bars represent 95% confidence intervals.

Table 2.3 Summarised output of generalized linear models (GLZ) for the effects of high temperature pre-treatments on the survival of *C. pomonella* at 43 and 45 °C for 2 hrs. Pre-treatments were done at 35 °C for 1 hr or 2 hrs and 37 °C for 1 hr before being exposed to 43 °C test temperature. Similarly, moths which were exposed at 45 °C test temperature were pre-treated at 32 °C for 1 or 2 hrs.

Treatment	d.f.	Estimate ±S.E.	Wald χ^2	p
Control 1 25 °C 1hr/43 °C	1	0.0000±0.0000		
35 °C/1hr/43 °C	1	0.5225±0.3493	10.88	0.1347
37 °C/1hr/43 °C	1	3.8602±0.5044	58.56	<0.0001
35 °C/2hr/43 °C	1	0.4734±0.3515	1.81	0.1781
Sub-treatment effects	3		59.30	<0.0001
Control 1 25 °C 2hr/45 °C	1	0.0000±0.0000		
32 °C/1hr/45 °C	1	0.1301±0.2831	0.21	0.6460
32 °C/2hr/45 °C	1	0.0614±0.2749	0.05	0.8233
Sub-treatment effects	2		0.48	0.7869
<i>All treatment effects</i>	6		85.29	<0.0001

Table 2.4 Summarised output of results of the cross-tolerance experiments in adult *Cydia pomonella*. Generalized linear models (GLZ) were used to test for the effects of high and low temperature pre-treatments on the survival of *C. pomonella* at low and high temperatures, respectively. Adult moths were pre-treated at 37 °C for 1 hr and given 1 hr at 25 °C before exposure to -9 °C for 2 hrs. An identical pre-treatment but without the recovery period at 25 °C before plunging into -9 °C was also undertaken. Similarly, two groups of moths were pre-treated at 5 °C for 2 hrs and given a gap at 25 °C before being exposed at 45 °C for 2 hrs. As in the high temperature pre-treatments, one group of moths was denied the gap period at intermediate temperatures and instead were plunged directly into 45 °C for 2 hrs after the 5 °C pre-treatment.

Treatment	d.f.	Estimate ±S.E.	Wald χ^2	p
Control 1 25°C 1hr/-9 °C	1	0.2412±0.2713	0.79	0.3741
37 °C 1hr/1hr gap /-9 °C	1	1.0245±0.3005	11.62	0.0007
37 °C 1hr/ no gap/- 9 °C	0	0.000±0.0000		
<i>Treatment effects</i>	2		19.12	<0.0001
Control 2 25°C 2hr/45 °C	1	0.000±0.1996	0.00	1.000
5 °C 2hr/1 hr gap/45 °C	1	0.0841±0.2005	0.18	0.6750
5 °C 2hrs/no gap/45 °C	0	0.000±0.000		
<i>Treatment effects</i>	2		0.23	0.8897

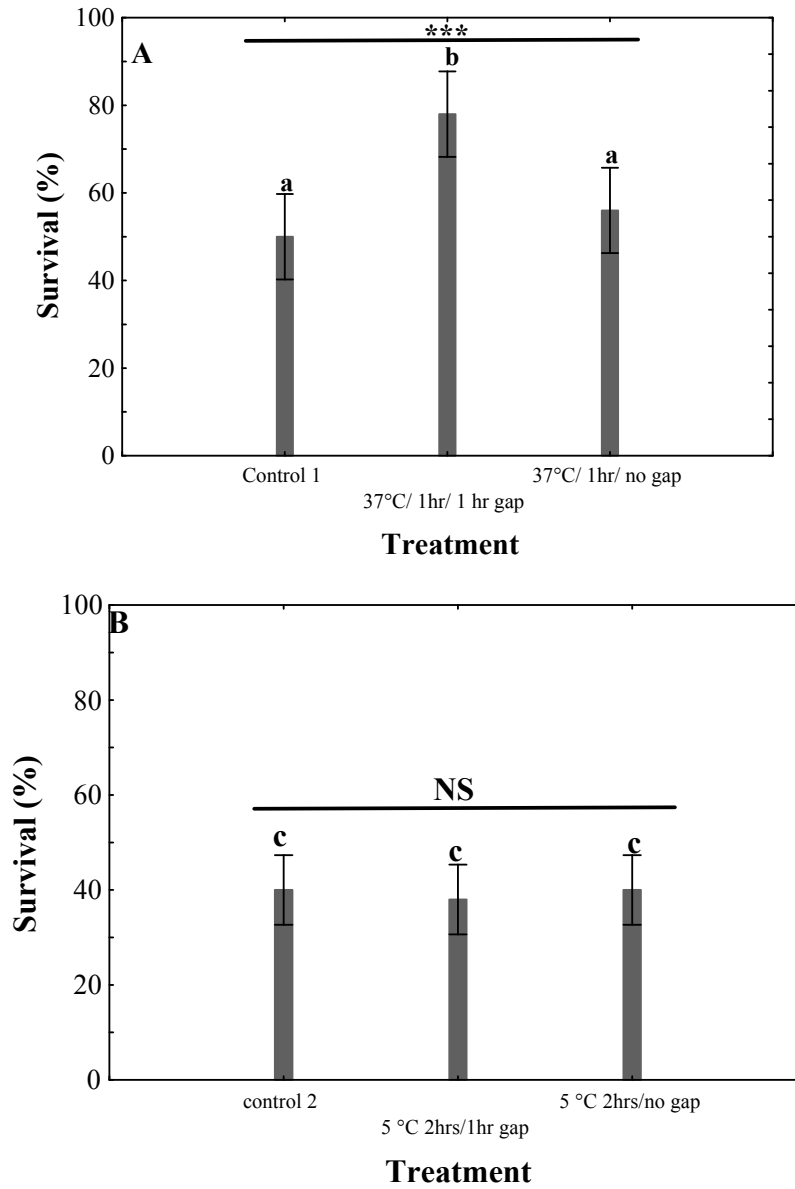


Figure 2.6 Mean survival of adult *Cydia pomonella* at -9 °C for 2 hrs (A) and at 45 °C for 2 hrs (B) after receiving a range of pre-treatments. Detailed statistical results are given in Table 2.4. (***: p<0.001) (NS: non-significant). Data points for the figures represent means of n=50 per treatment. Error bars represent 95% confidence intervals.

2.4 Discussion

2.4.1 Lethal temperatures

The adult life-stage in *C. pomonella* is probably the stage responsible for most dispersal. In addition, it contributes directly to changes in population size through reproduction, and is also the life-stage used for SIT (see Introduction). Yet most work examining thermal tolerance of *C. pomonella* has not explicitly focused on adult thermal biology. It is clear, however, that adult thermal tolerance of *C. pomonella* at daily scales is critical to SIT success (through e.g. minimum temperatures required for flight to high temperatures possibly limiting flight and mating), thermal tolerance can be a crucial aspect of laboratory-reared moth quality (see discussions in Bloem et al., 2006; Stotter and Terblanche, 2009; Chidawanyika and Terblanche, *in press*) and may also determine survival upon release in the wild. The present study therefore investigated the survival of adult *C. pomonella* under varying low or high temperatures of different short durations. These results showed that both the magnitude of temperature variation and duration of exposure were important in determining the survival of *C. pomonella*, as might be expected for insects in general (Chown and Terblanche, 2007). Over a four hour period, for example, 50% of the population would be killed by temperatures of 42 °C or -10 °C. By contrast, temperatures which would be lethal for 50% of the population were 45.5 and -15.0 °C for one hour. Duration of exposure at sub-lethal or lethal low temperatures is of ecological significance as it determines the limits to activity, severity of tissue injury and possibly the time required for recovery or repair of injury from stress (Chown and Nicolson, 2004; Denlinger and Lee, 2010; Hoffmann, 2010).

The absolute temperature tolerance determined in these experiments for adult codling moth raises the issue of their ecological significance. For example, one important question is whether adult *C. pomonella* are likely to die from thermal stress in their natural or agricultural environments. This can be addressed by combining the microclimate data with the thermal tolerance estimates performed in the laboratory. The short-term (~9 months), high frequency microclimate temperature data I recorded in an apple orchard at Welgevallen farm in Stellenbosch (South Africa) ranged from 4.7 to 42.2 °C and suggests that temperatures potentially causing low temperature mortality never occurred, neither did they even approach lethal levels at any duration (Fig. 2.7). By contrast, the high temperatures I recorded fell within the range of lethal high temperatures and also critical thermal maxima (Fig. 2.7). This suggests that high temperatures are more likely to cause mortality as compared to low

temperatures for the months of October through to early June, particularly for this South African location, and encompasses the period of peak moth activity and abundance (Pringle et al., 2003). Over longer periods (~3 years), absolute minima and maxima recorded in this location at nearby weather stations reached 3.1 and 42.8 °C, further substantiating the view that minimum temperatures are not likely to be lethal, although temperature maxima may well be lethal for large portions of the adult population at certain times of the year (summer). However, other geographic locations, particularly in the Northern Hemisphere, may experience low temperatures that are likely to induce CTmin in *C. pomonella* adults, especially in autumn (see Bloem et al., 2006), but lethal low temperatures are unlikely given the timing of peaks in adult trap catches.

Microclimate temperature recordings (Fig. 2.7) had an average (\pm s.d.) heating rate of 0.04 ± 0.01 °C min⁻¹ and average cooling rate of 0.03 ± 0.01 °C min⁻¹ calculated over 12 days. This suggests that the slowest rate of temperature change used to estimate CTLs in the laboratory would probably be best for approximating thermal limits to activity under field conditions. The highest temperature experienced in Stellenbosch over the 6 month recording was 42.2 °C, whereas the CTmax recorded in the laboratory assays was 44.5 ± 0.01 °C at 0.06 °C min⁻¹. The lowest temperature recorded over this period was 4.7 °C which was 4.1 °C higher than the CTmin determined in the laboratory (0.6 ± 0.01 °C at 0.06 °C min⁻¹). This suggests that temperatures eliciting CTmin and CTmax are not frequently encountered in this site over the period recorded and that *C. pomonella* probably has a greater thermal tolerance and activity range than typically experienced in this habitat.

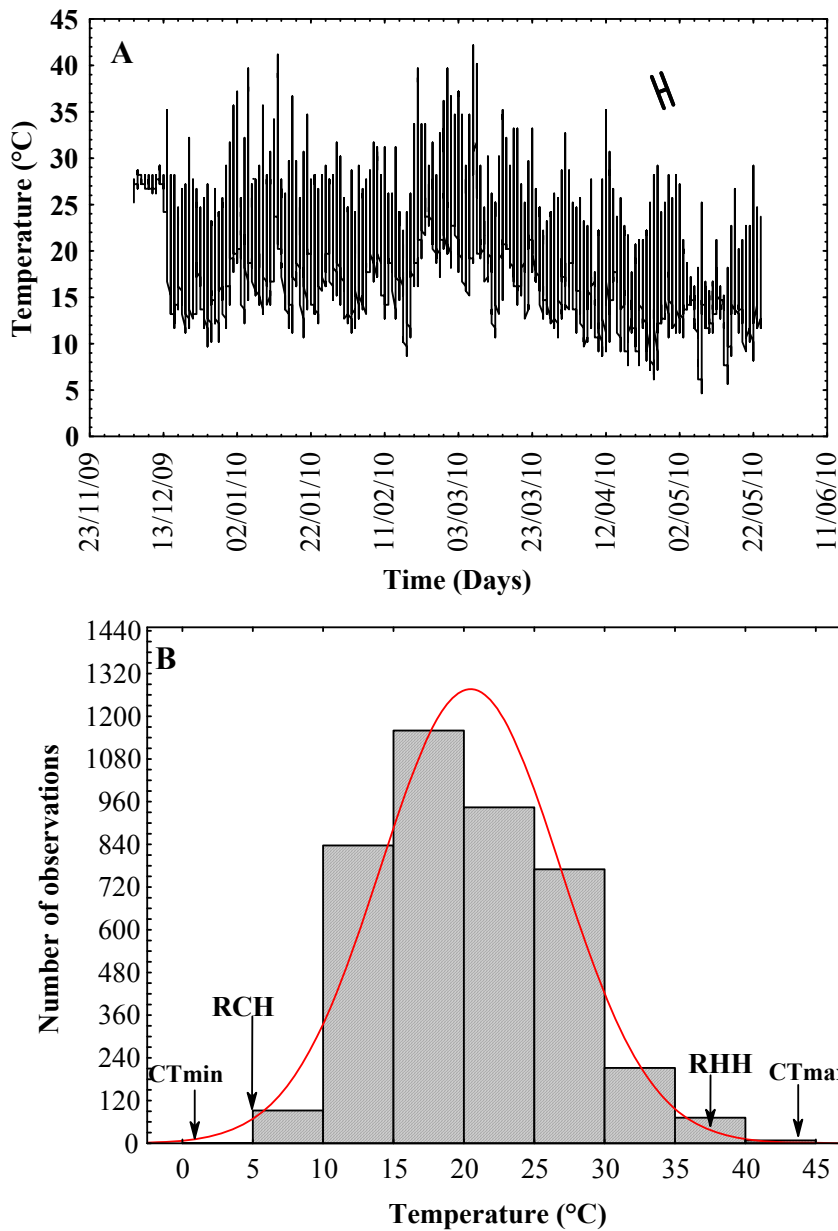


Figure 2.7 (A) Microclimate temperature recordings by Thermocron iButtons (Model DS 1920; Dallas Semiconductors, Dallas, Texas) (0.5 °C precision; 1 hour sampling frequency) located at ground level, mid and canopy level of apple tree in an orchard at Welgevallen farm, Stellenbosch, South Africa (33°56'884"S, 18°52'373"E) which hosts *Cydia pomonella*. Sampling was done for 8 months November 2009 – June 2010). Heating and cooling rates were calculated using Expedata software, version 1.1.25 (Sable Systems, Las Vegas, Nevada). (B) Frequency distribution of the recorded temperatures over the same period. Arrows indicate critical temperatures for rapid cold-hardening (RCH), rapid heat-hardening (RHH), lower critical thermal limits for activity (CTmin), and upper critical thermal limits for activity (CTmax).

2.4.2 Rapid thermal responses

The present work found evidence for both rapid heat-hardening and rapid cold-hardening responses in adult *C. pomonella*. The maximum survival improvement that could be induced at low temperatures was following the slow cooling (0.01 °C/min) protocol, and in that case improved survival from ~35% to 80% (Fig. 2.5A). Using direct plunge protocols, the most survival improved was from 55% to 88% after two hours at 5 °C, with many treatments not improving survival. This suggests restricted responses to low temperature, and when survival responses could be induced, they were of a low magnitude by comparison with other species showing rapid cold-hardening (e.g. fruit flies, Nyamukondiwa et al., 2010; Lee and Denlinger, 2010). Nevertheless, RCH responses in *C. pomonella* were similar to responses documented for some other Lepidoptera species to date (e.g. Larsen and Lee, 1994; Stotter and Terblanche, 2009; Sinclair and Chown, 2003; Kim and Kim, 1997).

At high temperatures, a marked RHH response was detected following 37 °C for 1 hour, improving survival from 20 % to ~90% (Fig. 2.5B). However, several treatments which were explored failed to elicit any RHH response. This is typical of RHH responses of insects more generally. For example, in *D. melanogaster* and some other insects, RHH is limited to only a small range of pre-treatment conditions (discussed in Denlinger et al., 1991; and see e.g. Chen et al., 1991; Overgaard and Sørensen, 2008; Nyamukondiwa et al., 2010). To my knowledge, no studies have explicitly examined the biochemical responses underlying changes in heat tolerance of codling moth. However, it is highly likely that *C. pomonella* employs a heat shock protein responses as is the case in several other insect species (MacMillan et al., 2009; Zi-wen et al., 2009; Kalosaka et al., 2009). Moreover, the results of the cross tolerance experiments further support this possibility since the high temperature pre-treatment improved survival at -9 °C (and see Chen et al., 1991). The brief gap at 25 °C after the pre-treatment seemed to be important, possibly allowing full expression of Hsps. The results of the cross-tolerance experiments for *C. pomonella* are similar to responses documented for *Sarcophaga crassipalpis* flesh flies which improved low temperature survival in response to high temperature pre-treatment but not the opposite way around (Chen et al., 1991; but see also Sinclair and Chown, 2003 for a similar Lepidopteran example).

In the context of field T_a in South Africa, the ecological significance of RCH is questionable given the microclimate recordings. Temperatures eliciting RCH virtually never occurred, although slow cooling conditions may occur more frequently (see discussion of

CTLs). By contrast, 1 hour at 37 °C was recorded relatively frequently (Fig. 2.7B) and thus, RHH responses may play an important role in increasing survival and perhaps also enhancing flight and other behaviours (e.g. mating and feeding) at high temperatures.

Investigation of CTLs and the effects of varying rates of temperature change on CTLs also yielded novel insights into adult codling moth thermal biology. I found that slower rates of cooling did not significantly reduce the CT_{min} of *C. pomonella* as might be expected for a species with a pronounced rapid cold-hardening response (e.g. Chown et al., 2009; Nyamukondiwa and Terblanche, 2010). However, similar CT_{min} recorded across different cooling rates might also be viewed as a form of plasticity since duration of exposure varied among these treatments (see discussions in Terblanche et al., 2007; Chown et al., 2009). Although a trend seems evident in the results perhaps indicating low statistical power (Fig. 2.4A), this result is likely also a consequence of a limited RCH response. Indeed, the lack of a rate effect on CT_{min} contrasts with the survival assay results (Fig. 2.4A). For CT_{max} the rate effect was more marked, with slow heated moths having significantly higher tolerance (by about 1.5 °C) and also reinforces the notion that RHH is probably relatively more important under natural diel fluctuations compared to the RCH response. Indeed, a longer duration during temperature increase allowed moths to better withstand high temperatures, but not particularly well at low temperatures. Moreover, the limited low temperature plasticity of adults, albeit limited, confirms the notion that temperate insects generally show more plasticity than their tropical counterparts since tropical environments are less variable than temperate environments (e.g. Hazell et al., 2010b; reviewed in Chown and Terblanche, 2007). This pattern seems to hold true in comparison between *C. pomonella* and false codling moth, *Thaumatotibia leucotreta* rapid cold-hardening responses. False codling moth is native to tropical regions (Stofberg, 1954; Catling and Aschenborn, 1974) while *C. pomonella* is native to temperate regions (Wearing et al., 2001). Indeed, *C. pomonella* has a more pronounced RCH response, albeit rather limited, than *T. leucotreta* which shows virtually no responses to a range of low or high pre-treatments (Stotter and Terblanche, 2009). However, such a comparison is limited by a lack of information on field T_b for both these nocturnal species, and due consideration of the peak activity times relative to T_a commonly encountered.

2.4.3 Conclusions

This study reports thermal tolerances for adult *C. pomonella* and the plasticity thereof. Thermal fluctuations experienced over diurnal scales likely play a significant role in the

survival of adult *C. pomonella*, especially at high temperatures but probably to a much lesser extent at low temperatures, at least for the geographic region investigated here. At longer timescales, within-generation changes in thermal tolerance have also been demonstrated (Chidawanyika and Terblanche, *in press*), suggesting an important role for thermal history in modifying future tolerance of adults over moderate (sub-lethal) and extreme thermal conditions. Knowledge of the temperatures (and time-temperature combinations) which can elicit RCH or RHH is also important for post-harvest protocols for fruit and disinfestations of harvest bins as some protocols may induce hardening giving *C. pomonella* the capacity to resist potentially lethal thermal treatments, although this would likely be more critical for larvae and pupae in these cases. Nevertheless, apart from a handful of studies (e.g. Wang et al., 2002; 2004) such rapid responses have not been well examined for developing *C. pomonella*. This is particularly important for quarantine treatments of fruits where thermal treatments might impact on fruit quality (Hallman, 2000). However, non-lethal temperature treatments may indeed improve survival of adult *C. pomonella* at lethal temperatures. Hence, post-harvest treatment protocols will need to be mindful of the capacity of *C. pomonella* adults to rapidly cold- and heat-harden as this may influence the efficacy, or time required to achieve efficacy, of a post-harvest treatment.

The present results may also be of importance to *C. pomonella* SIT programmes because it is clear that short-term fluctuations (diel thermal history), rate of temperature change, magnitude and duration of temperature exposure affects survival of the adult moths. The implications of these effects for fitness and performance, and the impact of the observed rapid thermal responses on behaviour, are however not clear and further investigation would be valuable. It would also be useful to know if the thermal tolerances of laboratory-reared moths are comparable to those of the wild moths within the context of laboratory adaptation (see discussions in Stotter and Terblanche, 2009). Although low temperatures seem unlikely to be a direct cause of mortality in this species, low T_a may still contribute to reducing population abundance due to impacts on activity, growth rates, reproduction and fecundity (Bloem et al., 2006; Chidawanyika and Terblanche, *in press*). High temperatures appeared much more likely to influence survival in two main ways. First, high temperatures in some months were similar to those that could induce rapid heat-hardening making it more likely that moth's could tolerate subsequent lethal high temperatures. Second, the high temperatures recorded (Fig. 2.7) were probably high enough to cause direct mortality. Therefore, high temperatures potentially experienced during SIT releases undertaken in mid summer, are

likely to be negatively affected and may limit the wild *C. pomonella* population, although such a speculation requires further information regarding use of microclimates and behavioural thermoregulation. One positive implication of the close relationship between temperature maxima and lethal limits of adult moths is that increased temperatures predicted under climate change scenarios may aid in controlling *C. pomonella* populations. Nevertheless, future work could incorporate the thermal responses reported here into predictive models of *C. pomonella* population dynamics, phenology, and potential agricultural impacts.

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Chapter 3

Costs and benefits of thermal acclimation for codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae): implications for pest control and the sterile insect release programme*

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3.1 Introduction

Sterile Insect Release (SIR) has been used to suppress populations of agricultural insect pests and vectors of human and animal disease with varying levels of success. The SIR method for insect control typically involves sterilisation (mainly through gamma radiation) of mass-reared insects which are released into the wild for mating with their wild counterparts. Thus, the pest population produces non-viable offspring leading to population declines (Vreysen and Robinson, 2010). Apart from financial, social and political issues, field performance of laboratory-reared insects probably remains one of the greatest challenges to SIR success (Enserink, 2007; Terblanche and Chown, 2007; Simmons et al., 2010). Codling moth (CM), *Cydia pomonella* (Lepidoptera: Tortricidae), is a major pest of global pome fruit production with huge economic losses suffered in cases where integrated control strategies are not implemented (Vreysen et al., 2010; Simmons et al., 2010). For CM, in particular, the lack of competitiveness of laboratory moths as compared to their wild counterparts in springtime has been a major setback (Thistlewood and Judd, 2003; Judd et al., 2004; Judd et al., 2006a; b). Emphasis on laboratory-reared moth maintenance and quality has therefore increased in an effort to improve the efficacy of SIR programmes (Calkins and Parker, 2005).

Quality of a laboratory population incorporates aspects of biological, physiological and behavioural factors. This is critical for SIR success as released moths must respond to biotic cues under field conditions and behave accordingly if a beneficial interaction (i.e. mating) is to be achieved. Research focusing on improvement of mass-reared CM quality has ranged from the inclusion of diapause in rearing (Bloem et al., 1997; Judd et al., 2006a), lower dosage of gamma radiation (Bloem et al., 1999a; b; 2001; Judd and Gardiner, 2006) and the combination of both (Bloem et al., 2004). Typically, CM rearing and maintenance in SIR programmes use only constant, optimal temperatures, probably in order to maximise rearing productivity (Bloem et al., 2004) regardless of the environmental conditions moths will be released into. This factor in particular may negatively impact the competitiveness of CM for SIR in the field. By contrast, a less well established method for improving CM for SIR is thermal preconditioning. This idea probably originates from Fay and Meats (1987), who suggested thermal treatment prior to release may be a potential solution to poor low temperature performance in the Queensland fruit fly *Bactrocera tryoni*. However, to our knowledge, no studies have demonstrated the field efficacy of such an approach in any Lepidopteran or agricultural pests.

Physiologists have long hypothesized that acclimation to a particular environment enhances performance in that environment (Angilletta, 2009; Hochachka and Somero, 2002; Prosser, 1986). However, acclimation responses are controversial for a number of reasons, of which two are probably of primary importance to the present study. First, the beneficial acclimation hypothesis, which posits that individuals acclimated to a particular environment perform better than those which have not been given the opportunity to acclimate, has been increasingly questioned and its importance debated (e.g. Leroi et al., 1994; Huey et al., 1999; Wilson and Franklin, 2002; Chown and Terblanche, 2007; Angilletta, 2009). Second, the link between a particular acclimation response and evolutionary fitness in the wild is not well understood (reviewed in Chown and Terblanche, 2007; Ghalambor et al., 2007; Angilletta, 2009). Instead, it may be costly to acclimate in certain environments (Gilchrist and Huey, 2001; Loeschcke and Hoffmann, 2002) which could lead to tradeoffs in thermal performance under different thermal conditions (Angilletta et al., 2002; Kristensen et al., 2008). Recent demonstrations of the costs and benefits of thermal acclimation on field performance in insects suggest that such a method may have practical benefits to the SIR programmes for codling moth, and other insect pests. Using release-recapture in *Drosophila*, for example, it has recently been shown that field performance is traded-off when flies are acclimated to varying thermal regimes (Kristensen et al., 2008). Specifically, cold-acclimated flies were recaptured more than control (non-acclimated) flies suggesting strong benefits for acclimation in the field. In addition, under warmer environmental conditions, warm-acclimated flies were recaptured in higher numbers than control or cold-acclimated flies (and see Loeschcke and Hoffmann, 2007; Kristensen et al., 2007). This clearly indicates an important role for phenotypic plasticity in altering behaviour and field performance (Kristensen et al., 2008). Moreover, these results imply that if similar responses were widespread among other insect taxa it could have practical value in manipulating field performance with potential improved efficacy in an SIR programme.

Here, we report laboratory and field experiments investigating the thermal physiology of performance and activity of codling moth. Specifically, we test the hypothesis that *C. pomonella* have plastic thermal physiology which leads to tradeoffs in performance depending on their immediate thermal environment. Furthermore, we assess whether there are costs and benefits of manipulating thermal environments during CM rearing for field activity in a pest control programme. First, we test if upper and lower critical thermal limits (CT_{max}

and CT_{min} , respectively) for activity are altered in response to adult or developmental rearing temperature. Second, we test if short-term (within-generation) changes in rearing temperatures (4-5 °C above or below growth optima) during larval development significantly improves field performance of adult CM, scored as recapture rates at pheromone traps, under a range of ambient environmental temperatures (T_a). The implications of these results are discussed in the context of *C. pomonella* pest control programmes and plastic thermal responses.

3.2 Material and methods

3.2.1 Moth rearing conditions

The CM culture used for our experiments was first established in 2004. Eggs hatched and developed (from larvae to adult) on a diet described by (Guennelon et al., 1981) in black perspex boxes in the laboratory under 12:12 (L: D) photoperiod in air-conditioned, insulated rooms at 20 ± 1 , 25 ± 1 , or 30 ± 1 °C. On emergence, adult moths were given access to 50% sugar/water solution from eclosion until they were used in thermal tolerance assays as 24 to 48 hr old adults. Gender and irradiation were not taken into consideration during main experiments as preliminary assays comparing critical thermal limits between males and females, and between irradiated or non-irradiated moths showed no significant differences ($p > 0.05$ in all cases). Owing to logistic limitations – specifically, the transportation and time required to move the moths between the laboratory where the acclimations took place, the cobalt radiator where sterilization occurs and the field site where release work was undertaken, - we did not include irradiation treatments since we were initially concerned that any induced acclimation effects may have worn off rather quickly in the adults and so our trials focused mainly on the release recapture effects of temperature in the orchards rather than the impacts of irradiation. In pilot trials undertaken in the laboratory we found that developmental thermal acclimation effects are similar for critical thermal limits when compared between irradiated and non-irradiated adult CM (acclimation x radiation effects: $F_{2, 114} = 0.866$, $p = 0.42$). However, it is clear that some aspects of locomotor performance of irradiated moths are generally poorer than non-irradiated moths (e.g. Judd and Gardiner, 2006) and the implications of irradiation for the field performance therefore requires further validation.

3.2.2 Thermal acclimation effects on critical thermal limits

We used a degree-day model for CM (Pitcairn et al., 1992; Howell and Neven, 2000) to predict the impact of the three treatments on expected time to eclosion. Thus, starting each treatment at a different time-point (coolest treatment first, warmest treatment last) we were able to synchronise the emergence times of the acclimation groups, although all acclimation groups treated the same age 5th instar larvae at initiation. Three groups of *C. pomonella* (n=2000 per group) larvae were exposed to three different ambient temperatures 20±1, 25±1, and 30±1 °C for 6 days (developmental acclimation) before eclosion, upon eclosion transferred to 25 °C, and all three groups had their critical thermal limits assayed thereafter. One group of adults that developed at 25±1 °C was partitioned into cages at 20±1, 25±1, and 30±1 °C for 6 days (adult acclimation) before their critical thermal limits were measured. All the cages in adult acclimation provided moths with access to 50% sugar: water solution.

For the determination of CT_{min} and CT_{max}, a programmable water bath was used for regulation of water/glycol solution (1:1) flowing through an insulated, double-jacketed series of chambers ('organ pipes') following previously established methods (Terblanche et al., 2008). Prior to CT_{min} and CT_{max} determination, moths were chilled at 5 °C for five minutes to restrict movement and allow individual placement into the chambers. Moths located individually into the chambers were given 10 minutes to equilibrate at 25 °C, and then subjected to either controlled heating or cooling at a constant rate of 0.25 °C/minute to determine the upper or lower critical thermal limits to activity (CT_{max} and CT_{min}, respectively). This standard protocol was followed for all critical thermal limit experiments as they are known to be affected by start temperature and rate of temperature change (Terblanche et al., 2007; Chown et al., 2009). A copper-constantan (Type T, 36 SWG) thermocouple attached to a digital thermometer (Fluke 53/54II, Fluke Cooperation South Africa, accuracy 0.01 °C) was inserted into the control chamber to measure and verify chamber temperatures. During ramping protocols, body temperatures of 40-50 mg flies is in equilibrium with chamber temperatures (Terblanche et al., 2007), and we therefore assumed that thermal inertia effects are limited in these similar-sized insects. The CT_{min} and CT_{max} were defined as the temperatures at which the moths lost coordinated muscle function. Each individual moth was treated as a replicate and critical thermal limit experiments were repeated until at least twenty individuals were used per acclimation or experimental treatment group for all CT_{min} and CT_{max} experiments.

3.2.3 Developmental thermal acclimation effects on activity of CM

Using moths developmentally-acclimated as in the critical thermal limits experiments above, we assessed the effects of these acclimations on temperature-dependent activity. A programmable waterbath was used to maintain constant test temperatures of 20, 25 and 30 °C on a custom built thermal arena. Ten insects from a specific acclimation group were placed on marked spots on the thermal arena and given 1 hr before activity was scored as total number of individuals that moved from their start location as a percentage of the total number of moths placed on the arena per trial. Five replications of 10 individuals per acclimation group were done for each test temperature (total n=450). Surface temperature was verified using an infrared thermometer (Fluke 63, Fluke Cooperation South Africa, accuracy 0.01 °C) before and at the end of experiments.

3.2.4 Thermal acclimation effects on field release-recapture rates

Developmental acclimation effects on performance of adult SIR moths was investigated using field release-recapture trials broadly following the methods outlined in Kristensen et al. (2008) but with one distinct difference. Specifically, we used a sex pheromone trapping system compared to their food bait trapping method. Developmental acclimation in these trials differed from the earlier laboratory trials on critical thermal limits and activity, mainly in that moths were acclimated for their entire egg-larval duration owing to logistic constraints on climate chambers. Laboratory-reared codling moth eggs were held until the 5th instar as developmental acclimation at 20±1, 25±1 and 30±1 °C then all groups were transferred to 25°C until adult eclosion. A staggered experimental protocol was used for developmental acclimation based on a day-degree model (Pitcairn et al., 1992; Howell and Neven, 2000) to ensure synchronisation of eclosion of moths from different acclimation temperature regimes. Adult moths from all the acclimation groups were chilled at 5 °C for 5 minutes before being marked by different fluorescent micronized dust (Day GLO Colour Corporation, Cleveland OH). Releases of the different acclimation groups were done simultaneously within 24-48 hrs of eclosion on a total of 11 occasions. Treatment colours (undertaken using the fluorescent powder) were randomized between acclimation groups on each experimental day to eliminate any influence of dye colours on recapture-rates.

All the field release-recapture trials were done in a single Rosemarie cultivar apple orchard at Stellenbosch University Experimental Farm (33°56' S, 18°52' E). The orchard was planted in 1998 with a 4.5 x 1.25m (inter-row x in-row) plant spacing. A total of 8 yellow delta traps

baited with a pheromone lure, CM1X Biolure[®] with E8-E10 dodecadienol (5.25g/kg) (Chempac, Paarl, South Africa) as the active ingredient were used. The traps were hung in a rectangular pattern around a single central release point with three traps (15m apart) in each external row and 2 traps in the middle row (30m apart) with the single release point in the middle. All traps were hung at ~1.8m (Thwaite and Madsen, 1983) oriented along the rows and secured to reduce wind-related movement. This ensured that moths were forced to cross rows, likely by flight, to reach traps.

On each field release day, adult moths from the three different developmental thermal acclimation regimes were taken simultaneously to the field in three large (10L) containers stored within an insulated box. All moths were released at ground-level from the central release point simultaneously. All field releases took place within 0.5 hours of transportation from the laboratory to the field at 12h00 on any given release. Each release therefore involved three groups of moths ($n > 700-1000$ for each thermal acclimation group). Releases were replicated at least five times until at least three successful release-recapture trials had taken place at low, intermediate and high temperatures (thus, the total number of released moths over the entire study was $n \geq 30180$). Weather forecasts (South Africa National Weather Service, www.weathersa.co.za), were used to ensure that moths were not released on days with extremely hot (>38 °C predicted daily maximum) or rainy (>5 mm predicted) weather conditions which are known to influence trap catches (Pitcairn et al., 1990). Environmental microclimate temperatures were recorded at 30 minute intervals using three ThermoChron iButtons (0.5 °C accuracy) (Dallas Semiconductors Model DS1920) located on the ground, mid tree height and upper canopy of the tree during the course of each release-recapture experiment. The traps were then monitored once every day for three days. On each day, Sticky Pads (Chempac, South Africa) were replaced and returned to the laboratory. Scoring recapture numbers was done on the retrieved Sticky Pads with daily CM catches using a UV light in a dark room. We waited several days (typically 4-5 x longer than time to starve or desiccate to death in CM) at the end of a release trial before undertaking another trial to ensure that moths caught in different releases were temporally independent.

3.2.5 Statistical analyses

The effects of either developmental or adult acclimation on upper or lower critical thermal limits were compared using separate One-Way ANOVAs in STATISTICA 9 (Statsoft, Tulsa, OK, USA). The dependent variable was either CT_{\min} or CT_{\max} , while the categorical effect of

acclimation temperature (either adult or developmental) was the independent variable in these analyses. Key assumptions of ANOVA were checked and were met for homogeneity of variance and normality of data distributions. Tukey's HSD *post-hoc* tests were used to identify statistically homogenous groups. Locomotor activity determined in adult CM in the laboratory was compared between different acclimation groups using a generalized linear model (GLZ), assuming a Poisson distribution and an identity link function, in SAS 9.1 (SAS Institute Inc., Cary NC, USA) and correcting for overdispersion. Here, proportion of active moths relative to total moths tested was the dependent variable, while acclimation temperature and test temperature were included in the model as categorical effects.

Field data for laboratory-reared moth recapture rates were regarded as count data and analysed using GLZ which is less sensitive to homogeneity of variance and normality assumptions, as in e.g., ANOVA. Here, using a generalized linear model (GLZ) and assuming a Poisson distribution and an identity link function in SAS statistical software (Proc Genmod), with corrections for overdispersion, we investigated the effect of acclimation temperature (as a categorical variable) on recapture ratios (dependent variable) at varying average field temperatures (as a continuous variable). The Wald χ^2 statistic was used to test for significant differences between acclimation groups. These GLZ analyses were run using absolute moth abundance (summed across all traps on a given day) per acclimation group relative to the total moths released per acclimation group and separately also repeated for proportion of moths recaptured in field trials relative to the intermediate (25 °C) acclimation group. Thus, in the latter analysis, the intermediate (25 °C) group acted as a control for variation in weather or cohort effects among release days and allowed for more standardised comparisons of temperature effects among release days. The rationale for including an intermediate group as a control is discussed in detail in previous studies (e.g. Huey et al., 1999; Sinclair and Chown, 2005; Terblanche and Chown, 2006; Kristensen et al., 2008), and specifically accounts for factors such as ageing, handling stress, or cohort effects between trials, and is also commonly employed in other areas of biological statistics (see Quinn and Keough, 2002).

3.3 Results

3.3.1 Laboratory assays of critical thermal limits and temperature dependence of activity

Both upper and lower critical thermal limits (CT_{max} and CT_{min}) to activity were affected by thermal acclimation occurring either in developing larvae or adult moths ($p < 0.001$ in all cases, Table 3.1, Fig. 3.1). Low temperature acclimation (20 °C) reduced CT_{min} for both developmental and adult acclimation in CM. High temperature (30 °C) acclimation increased CT_{min} and CT_{max} in adult CM. For CT_{min} in either developmental or adult acclimation experiments, all three acclimation groups were statistically heterogeneous, with the rank order $20 < 25 < 30$ °C (Fig. 3.1C, D). By contrast, effects of developmental acclimation on CT_{max} differed when compared with adult acclimation effects and adults appeared less responsive to thermal acclimation for this trait of high temperature tolerance. In the developmental acclimation experiment, 20 °C acclimation resulted in lower CT_{max} compared with 25 and 30 °C acclimation (Fig. 3.1A). In the adult acclimation experiment, however, CT_{max} for 20 and 25 °C acclimation groups were statistically homogeneous with the 30 °C-acclimated moths having a significantly higher CT_{max} (Fig. 3.1B). Most significantly, the developmental acclimation experiments showed that physiological changes in response to the thermal rearing environment carried over to the adult stage and persisted in the age-group of moths which would typically be used for release in SIR programmes.

Laboratory assays of adult CM temperature-dependent activity showed a significant interaction between developmental acclimation and test temperature (GLZ Wald $\chi^2 = 138.54$, d.f.=4, $p < 0.0001$; Fig. 3.2). The effects of acclimation ($\chi^2 = 16.89$, d.f.=2, $p = 0.0002$) and test temperature ($\chi^2 = 7.28$, d.f.=2, $p = 0.262$) however were not significant when pooled across groups. Thus, a higher proportion of cold-acclimated (20 °C) moths were active at 20 °C test temperature than those acclimated at 25 °C (intermediate) and 30 °C (Fig. 3.2). However, at a 30 °C test temperature, a higher proportion of 30 °C-acclimated moths were active and thus, showed locomotor performance, than cold-acclimated moths.

Table 3.1 Results of One-Way ANOVAs showing the effects of developmental and adult acclimation temperature (20, 25, 30 °C) on upper and lower critical thermal limits of adult codling moth. Each ANOVA was run separately and assumptions of homogeneity of variance and data normality were checked and met in all cases. Tukey's HSD *post-hoc* test was used to separate heterogeneous groups.

Effect	SS	d.f.	<i>F</i>	<i>p</i>
<i>CT_{max}</i>				
Adult acclimation	30.52	2, 57	21.78	<0.001
Developmental acclimation	9.9	2, 57	19.2	<0.001
<i>CT_{min}</i>				
Adult acclimation	105.6	2, 57	140.1	<0.001
Developmental acclimation	39.6	2, 57	66.6	<0.001

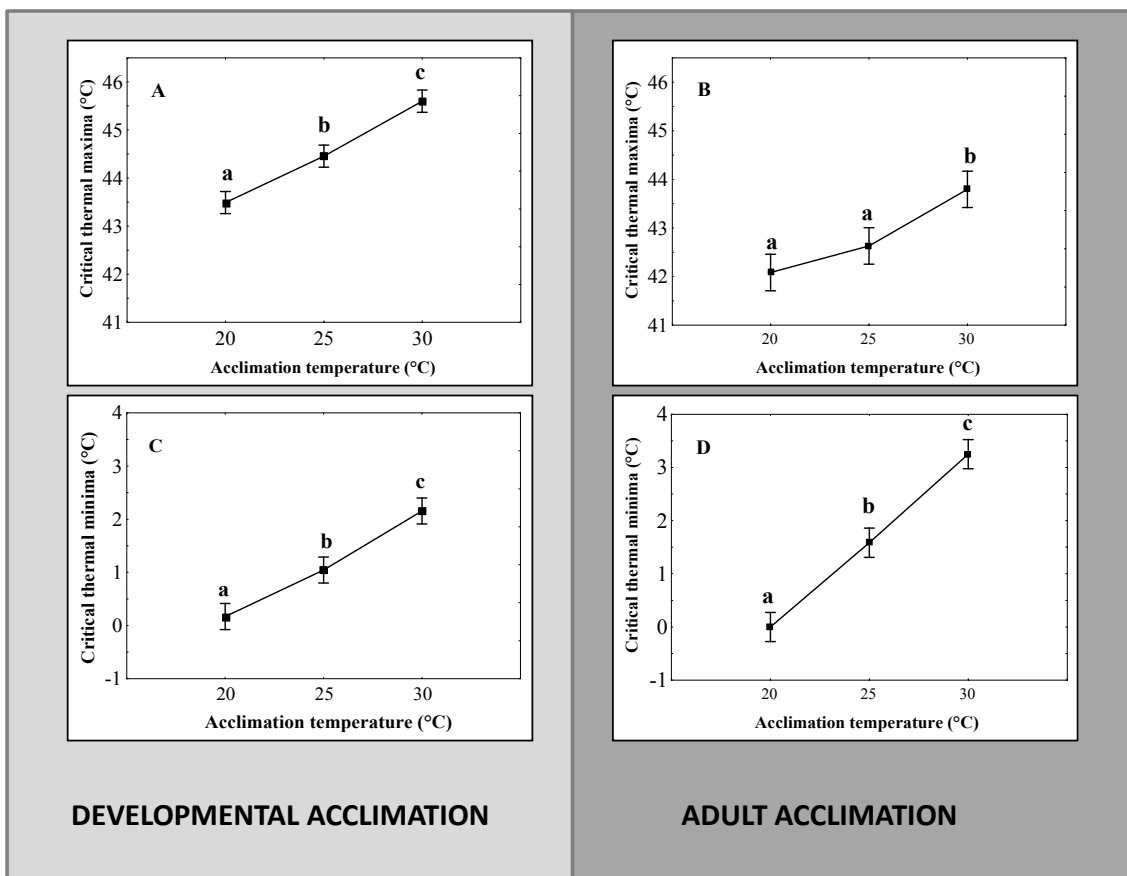


Figure 3.1 Effects of developmental acclimation temperature (20, 25 and 30 °C) on critical thermal limits; CT_{max} (A), CT_{min} (C), and adult acclimation temperature (20, 25 and 30 °C) effects on CT_{max} (B) and CT_{min} (D) of codling moth. Similar letters on each panel indicate statistically homogenous groups as determined by Tukeys' HSD post-hoc test. ($n=20$ in each group) (See Table 3.1 for statistics).

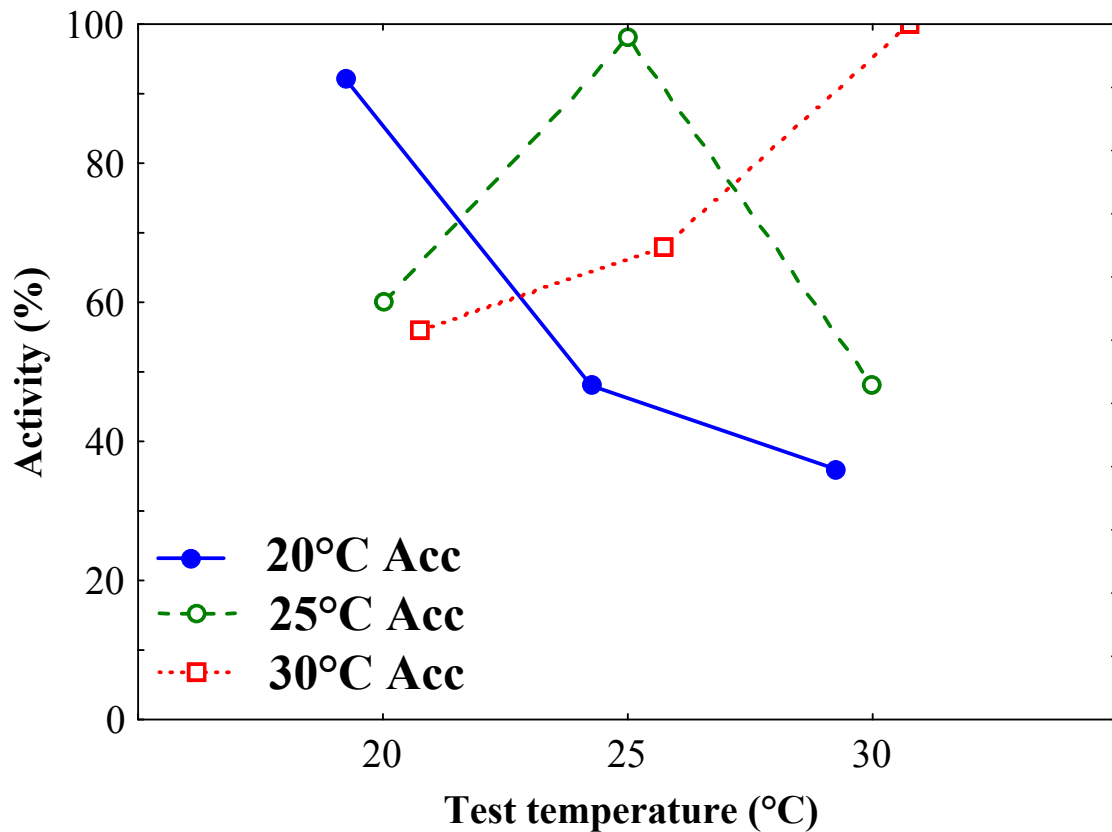


Figure 3.2 Effects of developmental acclimation temperature (20, 25 and 30 °C) on proportion of moths active in 1-2 day old adult laboratory-reared codling moth at three test temperatures (20, 25 and 30 °C) under controlled thermal conditions.

3.3.2 Field performance trials

The majority of moths which were recaptured were trapped within the first 24 h after release (>95%). On the second day, a maximum of 8 moths were recaptured in one event with a median recapture of 1 moth across all trials and treatment groups. In consequence, no significant effects were detected between acclimation treatments in either the 2nd or 3rd day or both days pooled ($p > 0.23$ in all cases). By the third day after release, no moths were ever recaptured. Therefore, we only focused on data from the first day's recaptures for further analyses of temperature effects on moth field performance. Field release trials showed a significant interaction effect between acclimation temperature and field temperature (Table 3.2, Fig. 3.3A). Cold-acclimated moths were recaptured significantly more under cold conditions at sex pheromone traps, but not under warm conditions, and that warm-acclimated

moths were recaptured in higher numbers under warm, but not cooler, conditions (Fig. 3.3A). Field release trials also showed significant effects of acclimation temperature on absolute recapture rates at different ambient temperatures in *C. pomonella* (Fig. 3.3A, Table 3.2). There is considerable variation in recapture rates among releases undertaken on different days. Hence, we also examined the possibility that this is a consequence of other varying abiotic factors, such as wind. Using type I model in least-squares regression we examined the residual variance in the recapture rates using a range of other climate variables, most significantly, maximum and minimum wind speed, average wind speed, wind direction, and maximum and minimum daily temperature (note: all wind data was taken from a nearby orchard weather station). However, after mean temperature during the release has been accounted for, no other climate variables we examined were significant explanatory variables in our release-recapture data ($p > 0.25$ in all cases). It is therefore unclear what determines the variation in recapture rates among trial days, but may be related to cohort effects.

However, to account for differences among releases we repeated analyses adjusting for control 25 °C moth recapture in each release trial. Here, using the proportion recaptured moths relative to intermediate (25 °C) temperature group numbers, the effects of acclimation temperature were still prominent (Table 3.2, Fig. 3.3B). As expected, using these control adjusted results, there are no significant effects of field average temperature as this has been standardized across releases ($\chi^2 = 0.03$, d.f.=1, $p = 0.8663$). Regardless, the interaction between field average temperature and acclimation temperature is still highly significant for moth recapture rates (Table 3.2, Fig. 3.3B). As in the uncorrected results of recapture proportions (above), this shows that warm-acclimated moths performed relatively well, and were recaptured in higher numbers, under warm conditions but poorly under cooler conditions. It also showed the opposite: cold-acclimated moths performed well in the field under cooler conditions but poorly under warmer conditions. Standardized for intermediate control group numbers, this translates to roughly a two-fold difference between acclimated and non-acclimated moths in either warm or cold environmental conditions (Fig. 3.3B).

Table 3.2 Results of generalized linear model (GLZ) analyses investigating the effects of developmental acclimation temperature (20, 25, 30 °C; ‘Acclimation’) on the proportion of recaptured moths relative to the intermediate control group (25 °C acclimation temperature) and absolute numbers of recaptured moths. Data for laboratory-reared moth recapture rates were regarded as count data and analysed using GLZ assuming a Poisson distribution and an identity link function in SAS statistical software (Proc Genmod), with corrections for overdispersion. In all cases investigated, the effect of acclimation temperature was the categorical variable and recapture ratios or absolute number captured as the dependent variable at varying average field temperatures as a continuous variable. The Wald χ^2 was used to test for significance differences between acclimation groups. Significant effects are highlighted in bold font.

Effect	d.f.	Wald χ^2	P
<i>Absolute moth recapture</i>			
Acclimation	3	26.68	<0.0001
Field temperature	1	23.04	<0.0001
Acclimation x Field temperature	2	15.13	<0.0005
<i>Proportion moth recapture relative to control numbers</i>			
Acclimation	3	63.23	<0.0001
Field temperature	1	0.03	0.8663
Acclimation x Field temperature	2	53.13	<0.0001

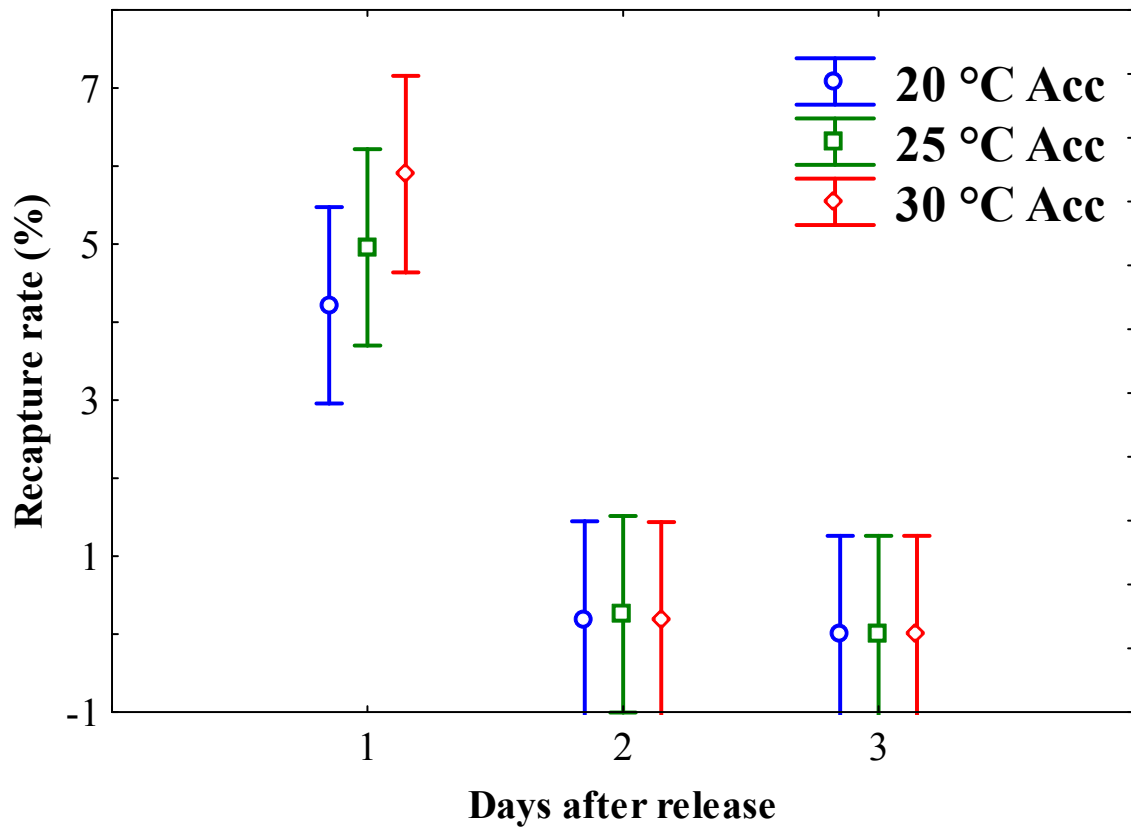


Figure 3.3 Codling moth recaptured (as % of total number of moths released) on each day after release under field conditions for each of the acclimation groups. Note releases are treated as replicates to obtain error bars (95% CLs).

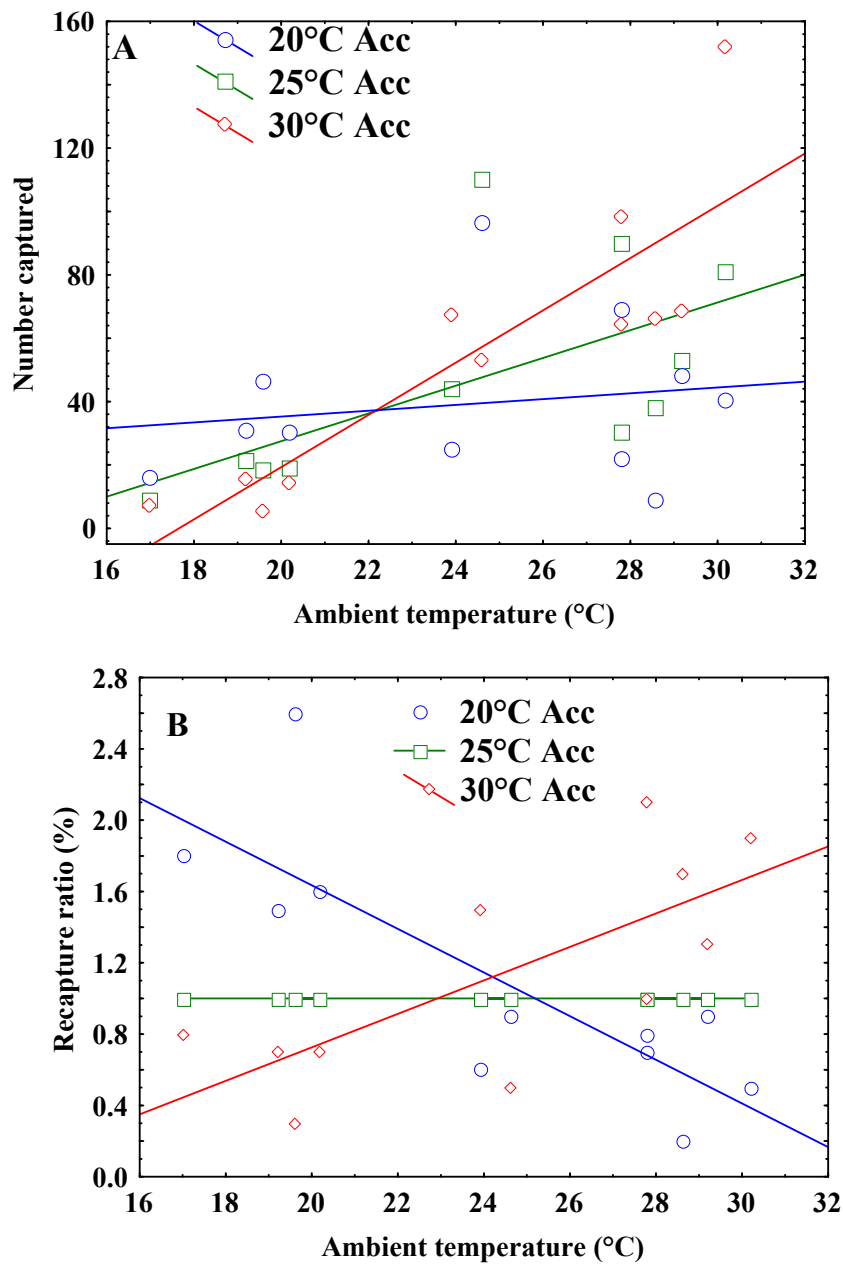


Figure 3.4 Developmental acclimation temperature (20, 25 and 30 °C) effects across a range of mean field average temperatures in laboratory-reared codling moth. Acclimation temperature effects are shown for the absolute number of moths captured per acclimation group (20, 25 and 30 °C) (A) and proportion recapture as a ratio of moths caught in the intermediate group (25 °C) to standardize effects across days (B). Regression equations for lines of fit in plot of (A) are: 20 °C: $y = 1.749 \pm 0.971x - 25.679 \pm 22.306$ ($R^2 = 0.094$, $p = 0.083$); 25 °C: $y = 3.505 \pm 1.096x - 62.950 \pm 25.177$ ($R^2 = 0.248$, $p = 0.003$); 30 °C: $y = 5.423 \pm 1.256x - 103.616 \pm 28.037$ ($R^2 = 0.376$, $p = 0.0002$) and those in plot of (B) are: 30 °C $y = 4.083 \pm 0.704x - 0.82 \pm 0.191$ ($R^2 = 0.673$, $p = 0.0002$); 20 °C: $y = -1.154 \pm 0.717x - 0.734 \pm 0.226$ ($R^2 = 0.539$, $p = 0.010$). All results presented are for moths captured in the first day after release.

3.4 Discussion

These results show that rearing protocols can have dramatic effects on field performance under variable ambient temperatures in *C. pomonella* and that inducible plastic physiological responses are of direct relevance to CM control programmes. Indeed, poor recapture rates of mass-reared CM under low T_a have been attributed to rearing under constant optimal growth temperature (Thistlewood and Judd, 2003; Judd et al., 2004; Judd et al., 2006b). These results demonstrate that plastic physiological responses gained during development may improve the performance of adult CM in the field such that thermal acclimation can be undertaken during development and pre-conditioning need not be performed only in adults. This may prove useful for rearing protocols of insects like CM which, because ageing and starvation may compromise the fitness and thermal tolerance of insects, could result in reduced reproductive success (Emlen and Oring, 1977; Bowler and Terblanche, 2008). Moreover, from our laboratory assays the magnitude of plastic responses induced during developmental acclimation appeared similar to adult acclimation effects (Fig. 3.1A vs B and 3.1C vs D) although older adults seem less tolerant of high temperatures. In our field assays, warm- or cold-acclimated CM were recaptured almost twice as much in T_a similar to their thermal history. This may be critical in reducing SIR programme costs as fewer moths will be required to achieve the current efficacy levels. Simple calculations suggest a roughly two-fold reduction in costs per hectare, or approximately double the efficacy of releasing thermally pre-conditioned CM. Alternatively, the same numbers could be released possibly with increased efficacy due to increased successful mating events. Hence, SIR programmes could incorporate rearing of CM at both increased and reduced temperatures, or perhaps increased variability of temperature, to improve moth performance during releases undertaken on days with adverse thermal conditions. Further work is required however to strengthen these results for SIR, since, for example, we did not include irradiation treatments owing to logistic constraints. Nevertheless, it might be argued that even the inclusion of an irradiation treatment on thermally-acclimated CM does not convincingly prove the method's efficacy. For example, assessing trap recapture rates alone does not demonstrate improved SIR efficacy (even if undertaken on irradiated moths) and, instead, future work will need to demonstrate improved mating success with wild females, a reduction in wild moth populations, and decreased fruit damage. Regardless, the present results are an important demonstration of the feasibility of such an approach. Overall, it appears that thermal acclimation probably gives mass-reared CM a significant performance advantage to cope better under adverse field

temperatures when released in temperatures similar to their thermal history, which will in turn allow the laboratory-reared CM to compete better with wild individuals immediately after release.

The present work can be interpreted as providing support for the beneficial acclimation hypothesis (BAH, reviewed in Angilletta, 2009; Chown and Terblanche, 2007). Formally, the BAH has been defined as ‘...*acclimation to a particular environment gives an organism a performance advantage in that environment...*’ (Leroi et al., 1994). In light of this definition, the results of both the laboratory trials for critical thermal limits and temperature-dependent activity assays can be argued to provide support for the BAH since in all cases CM performed best in environments they were acclimated to previously, and worse in environments not previously experienced (and see Kristensen et al. 2008). For the field recapture rates, a similar conclusion can be reached from the distinct increases in recapture rates when animals were released in temperatures they had been exposed to during development, probably indicating increased flight or dispersal performance. However, this interpretation presumes that increased recapture rates is an indicator of improved performance, and vice versa, and that this may be a reasonable proxy for field fitness (and see Loeschcke and Hoffmann, 2007 for discussion). This is probably a reasonable assumption given the other studies which have used similar methods and made similar assumptions for food bait traps (e.g. Kristensen et al., 2008) although verification of this assumption would be valuable. One potential weakness of this as a test of the BAH is that developmentally-acclimated moths were tested as adults, as these two forms of plasticity may be argued to be fundamentally different (Wilson and Franklin, 2002; Terblanche and Chown, 2006; Kristensen et al., 2008; discussed in Chown and Terblanche, 2007) especially if behavioural thermoregulation differs among stages and impacts acclimation responses (Marais and Chown, 2008).

Unlike Kristensen et al., (2008), we assessed the effects of acclimation on CM thermal tolerance in the laboratory using a dynamic protocol (ramping temperatures as opposed to constant temperatures) and found that these results were in close agreement with the field release-recapture results. Specifically, cold-acclimated CM in our study had generally lower critical thermal limits, and vice versa for warm-acclimated moths. This suggests that dynamic protocols may be more ecologically relevant for assessing field thermal tolerance, especially if starting conditions and thermal ramping rates (heating or cooling) can be modified to match the thermal environments experienced by the insect (Terblanche et al., 2007; Chown et al.,

2009; Mitchell and Hoffmann, 2010; Nyamukondiwa and Terblanche, 2010). Although our heating and cooling rates were not identical to those experienced in our field sites (approximately 3 times faster than natural rates) in order to maximise throughput of acclimation groups in our laboratory assays, the dynamic thermal tolerance protocols we used may still be more relevant to field performance than static (acute) thermal tolerance survival assays. The incongruence in results between Kristensen et al.'s (2008) laboratory assays of acclimation responses and our results for these experiments may therefore be attributed to their scoring of survival, rather than critical thermal limits, as a measure of thermal tolerance. This suggests that critical thermal limits may describe more closely the locomotor ability of the organisms to cope with diurnal changes in temperature and functional performance, and that CTL's are probably a better method for linking laboratory acclimation results with field performance (and see discussion in Chidawanyika and Terblanche, in press).

The five minute chilling at 5 °C of moths for handling and sorting purposes, in conjunction with transportation to the field, had no effect on the benefits of acclimation, suggesting the key physiological changes acquired during development persist into the field. This may prove critical for SIR programmes which rely on transportation of moths at low temperatures (Bloem et al., 2006) to the release sites because acclimatory benefits gained take considerably longer to be lost. Codling moth in the field may survive as adults for 2 to 4 weeks depending on the season (Thistlewood and Judd, 2003; Tyson et al., 2008) and have re-mating capacity (Knight 2007). Therefore, longer duration of survival in adult moths probably has a direct impact on population dynamics through reproductive output, and increased survival and performance of laboratory-reared moths released into the wild can also have additional benefits to pest control programmes. Under field conditions, such ability to retain acclimatory benefits even in this laboratory-strain of CM, may help in improving CM competitiveness and longevity in the field after release, but will likely depend on the duration and intensity of novel temperatures encountered after thermal pre-treatment (see discussions in Fay and Meats 1987; Nyamukondiwa and Terblanche, 2010) and the impact of irradiation on performance decay over time (e.g. Judd and Gardiner, 2006; and see discussions in Vreysen and Robinson, 2010).

Codling moths were attracted using CM1X Biolure[®] with E8-E10 dodecadienol (5.25g/kg) (Chempac, South Africa) sex pheromone located within yellow delta traps (Chempac, South Africa) containing Sticky Pads (Chempac, South Africa) and thus probably only recaptured

male moths. Despite the marked difference in lure and capture method (i.e. food vs. sex pheromone) between our study and Kristensen et al.'s (2008) study, the overall results for costs and benefits of thermal acclimation on field performance remained similar. This demonstrates that an important component of SIR success, namely mating attraction, is retained under variable thermal conditions (Castroville and Carde, 1979). In addition, time taken to locate calling females is probably an important aspect of competition between wild and mass-reared male CM (Thornhill and Alcock, 1983; Andersson and Iwassa, 1996; Judd et al., 2006a) and thus probably also represents an important aspect of field fitness. The results of the influence of thermal history on CM locomotor activity assays confirmed that T_a influences mobility in the laboratory. Indeed, Bloem et al. (2006) showed higher activity in moths at 25 °C and 20 °C than 15 °C in diapaused and non-diapaused CM which were reared in constant temperatures but kept under different durations of cold storage. Nevertheless, irrespective of specific differences in acclimation protocols and traits examined, our results clearly show that CM activity can be enhanced in both laboratory and field trials (Fig. 3.2 and 3.3), if moths experience T_a identical to their thermal history relative to CM which have not had the opportunity to acclimate.

One important issue this work raises is the general lack of information on behavioural thermoregulation, and the use of microsites to optimise body temperatures (T_b), in adult CM in the wild. It is clear that under controlled laboratory conditions larvae and adults of CM have the ability to behaviourally thermoregulate and thereby maintain T_b within a fairly narrow range of preferred temperatures (Kürht et al., 2006a; b). Moreover, many insect species can sense and respond to small increments in temperatures (Chown and Terblanche, 2007). Codling moth also prefer to oviposit within a relatively narrow range of temperatures (Kurht et al., 2006b) and experience a wide range of thermal conditions in orchard microsites (Kührt et al., 2006c). However, little work to date has been undertaken for adults in the wild and it is generally poorly understood if such regulation allow moths to avoid temperature stress under natural conditions (Kürht et al., 2006b; and see discussions in Chidawanyika and Terblanche, in press). Field observations showed that moths in our study clearly dispersed rapidly from the release point, using both short flights and walking and that traps were located within the expected daily dispersal distance of laboratory-reared moths. After 24 hours it was extremely difficult to observe released moths in the orchard, although traps captured moths relatively effectively, making the difficulties of microsite and thermoregulation work considerable. Nevertheless, this is an important area for future research, as behavioural

thermoregulation may partially or fully offset any potential benefits gained through laboratory acclimation. However, it will need to be borne in mind that any adjustments in Tb are largely dependent on microsite opportunities and operative environmental temperatures and that behavioural thermoregulation may impact on the form of acclimation responses (Stevenson, 1985; Marais and Chown, 2008).

In conclusion, this novel study shows plastic physiological responses of CM in response to thermal acclimation which clearly transfers to field performance. In addition, our results provide an important first demonstration that thermal acclimation during development could be a potential way to manipulate and enhance field performance of *Cydia pomonella* for pest control programmes, although further work is necessary to validate such an approach. Moreover, this study demonstrates clear costs and benefits of thermal performance depending on thermal conditions previously experienced. Finally, this study has shown the potential value and practical feasibility of thermal pre-treatments for offsetting negative efficacy in the SIR programme for *C. pomonella* control under cooler, springtime conditions, though also under potentially adverse warmer conditions typical of some growing regions in peak summer. These results are of direct importance to CM and other pest control programmes and the evolution of thermal performance in terrestrial ectotherms.

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Chapter 4
Conclusions

The results of the present study contribute to understanding how temperature affects the physiology of adult *Cydia pomonella* in the wild. First, lethality has been shown to be subject to both the magnitude of the temperature and the duration of exposure at both high and low temperatures (Chapter 2). This is similar to what has been observed in other Lepidoptera species such as false codling moth (FCM) (Stotter and Terblanche, 2009), and other insects more generally (e.g. Mironidis and Savopoulou-Soultani, 2010; reviewed in Chown and Terblanche, 2007). Cold temperature required to inflict 50% population mortality (LT_{50}) on the laboratory-reared adults of *C. pomonella* population at short intervals was -13.8 °C after 1 hr exposure and was much lower than microclimate temperatures in a local agroecosystem habitat. It is therefore highly unlikely for low temperature to directly cause mortality in adult *C. pomonella* in South African orchards during the months of peak moth activity and the months which were sampled. Moreover, *C. pomonella* seasonal occurrence is synchronised with their life cycle in such a way that the adverse effects of winter are escaped by going into diapause (Riedl, 1983). However, low temperatures occurring during spring, or sudden cold snaps which are characteristic of the Western Cape, South Africa (see Tyson and Preston-White, 2000 for review of local climate and e.g. Sinclair and Chown, 2006) could mean that adult moths are exposed to a wide range of diurnal temperatures even during their adult active season. Low temperature may also contribute to *C. pomonella* population dynamics in the wild by its effect on other adult activities such as mating behaviour, oviposition or fecundity, as reported by e.g. Saethre and Hofsvang (2002), and possibly also through “ecological death”- cessation of key activities contributing to population growth - due to chill coma (Lee and Denlinger, 2010). On the other hand, results of the laboratory trials of acute high-temperature mortality were 41 °C at 1 hour exposures, in comparison with high temperatures recorded suggest high temperature may be a potential factor determining mortality in the wild at diurnal scales. Mechanisms contributing to mortality of insects at acute high temperature exposure are reviewed in Chown and Nicolson (2004) and Denlinger and Yocum (1998) (see also Chapter 1 and Chapter 2, Introduction).

Apart from ecological and population dynamics implications discussed in Chapter 2, temperature/time mortality assay results are also important for post harvest treatment protocols of harvest bins and fruits. Whilst I did not conduct a full probit study design, the lethality curves I estimated may still help in prediction of temperature or time required to inflict a certain percentage of mortality on adult codling moth which is essential in post-harvest disinfestation of harvest bins (Hansen et al., 2007) and modified atmosphere

treatments of fruits (Neven, 2005). Moreover, these time-temperature mortality curves are likely a critical component of accurate forecasting of population abundances under future and present climate scenarios (Lima et al., 2009) and are discussed further in Chapter 2.

Second, the present study demonstrates phenotypic plasticity of *C. pomonella* with respect to temperature tolerance. Thermal pre-treatments to sub-lethal temperatures improved survival at otherwise lethal temperatures. For example, 55 % of moths survived a 2 hr exposure at -9 °C, while 85 % survived those same thermal conditions after pre-treatment at 5 °C for 2 hours. This is unlike the case in false codling moth which is also found in the Western Cape of South Africa and is a major pest of citrus (Stotter and Terblanche, 2009). While it appeared that low temperature is highly unlikely to determine mortality of adult *Cydia pomonella* in the wild through direct effects (see Chapter 2), some limited rapid cold-hardening (RCH) responses were detectable. The ecological significance of RCH may not be that of improving survival but rather of benefiting the organism to maintain fitness in less severe cold temperatures encountered (Kelty and Lee, 2001; discussed in Chown and Nicolson, 2004; Lee and Denlinger, 2010). This was also supported by results of critical thermal minima (CT_{min}) showing lower CT_{min} in individuals that were cooled at ecologically relevant rates which were slower and more gradual as opposed to plunge protocols which are less ecologically relevant. Moreover, RCH occurs in actively feeding, growing, reproducing as well as overwintering stages (Li et al., 1999) and thus, may help in preservation of reproductive behaviours (Shreve et al., 2004), enhancing fecundity and longevity (Rinehart et al., 2000; Powell and Bale, 2005) as opposed to the overwintering dormant diapause phase which occurs within a single life-stage (Lee and Denlinger, 2010). Apart from programmed, longer-term seasonal responses, these insects often encounter rapid temperature changes on a daily basis and the potential to respond to these transitions may thus be one of the important mechanisms which help CM to cope in its natural habitat.

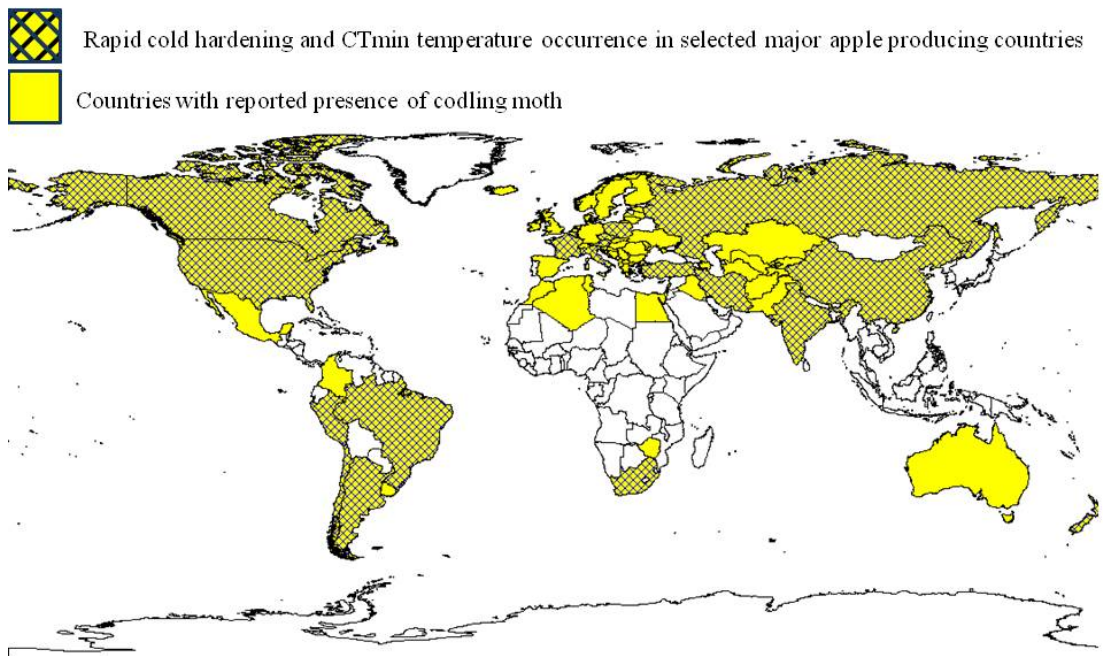
Cydia pomonella has continued to prevail in the Western Cape, South Africa. This may seem to be in contrast to the relatively high microclimate data recorded at Welgevallen farm suggesting high temperature may be a real potential threat to survival of CM, especially considering temperatures causing mortality in the laboratory assays. However, this potential conflict could at least partly be explained by the rapid heat-hardening (RHH) responses I detected, which may offset mortality risks to some degree. Indeed, *C. pomonella* showed marked improvements in survival upon pre-treatments to sub-lethal high temperatures in the

laboratory, as reported in *Drosophila* species (Loeschcke et al., 1994; Hoffmann et al., 2003; Dahlgard et al., 1998), in the Lepidopteran *Epiphyas postvittana* (Lester and Greenwood, 1997), and cross-tolerance (i.e. heat pre-treatment improving low temperature survival) in *Pringleophaga marioni* (Sinclair and Chown, 2003). A summary of past RHH and RCH work undertaken in Lepidoptera is given in Table 1.1. This review also suggests that further work investigating RCH, RHH and cross-tolerance in Lepidoptera would be useful for understanding ecological and evolutionary variation in these responses. Another explanation for why CM still persist despite relatively high microclimate temperatures could be behavioural thermoregulation, and thus, avoidance of damaging or lethal temperatures. These behavioural responses have been well documented in the larvae of CM, although to a much lesser extent in adults (Kuhrt et al., 2005; 2006, Notter-Hausmann and Dorn, 2010). Further work examining adult microsite use in orchards during high temperatures would be useful in this regard.

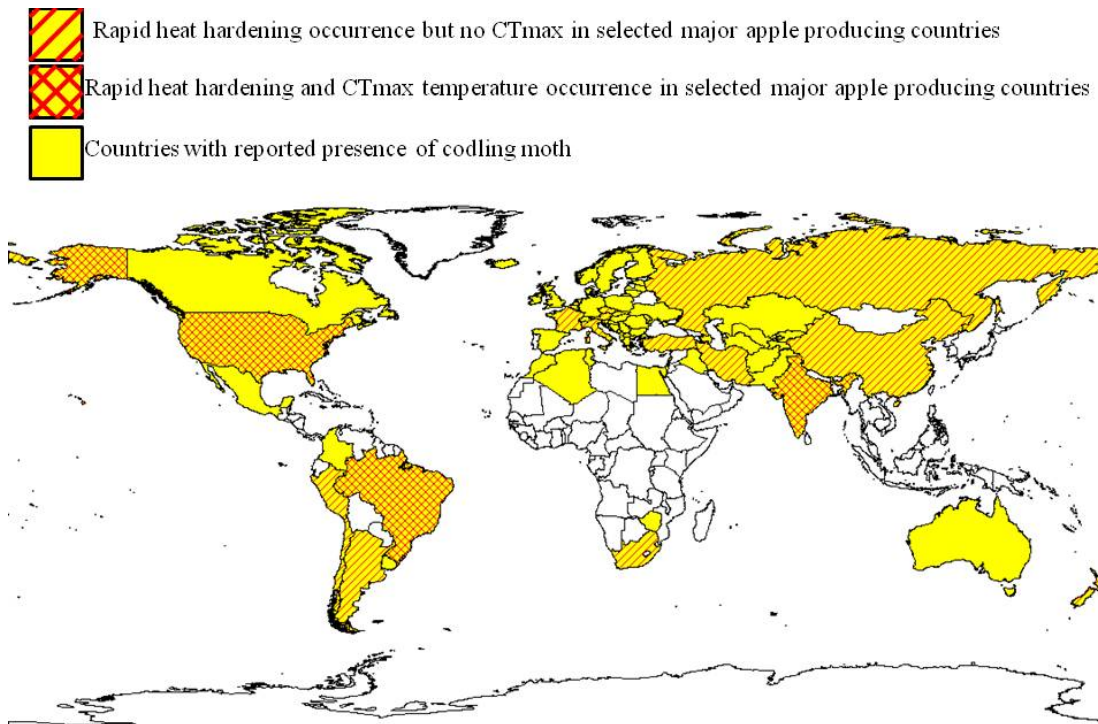
Table 1 Summary of Lepidoptera species examined to date for their rapid cold-hardening (RCH) and rapid heat hardening (RHH) responses

Species	Family	Life-stage	Pretreatment temperatures (°C) time (hrs)	Discriminating temperature (°C) time (hrs)	Trait examined	Survival Control: treatment (%)	Reference
<i>Danaus plexippus</i>	Nymphalidae	Adult	4(1)	-4(24)	Survival	40:80	Larsen and Lee, 1994
<i>Epiphyas postvittana</i>	Tortricidae	Larvae	28(8)	43	Survival	Lethal time recorded	Lester and Greenwood, 1997
<i>Thaumatotibia leucotreta</i>	Tortricidae	Adult	8(2)	-3(4)	Survival	65:70	Stotter and Terblanche, 2009
<i>Cydia pomonella</i>	Tortricidae	Adult	5(2)	-9(2)	Survival	55:85	Chapter 2
<i>Cydia pomonella</i>	Tortricidae	Adult	37(1)	43(2)	Survival	20:85	Chapter 2
<i>Pringleophaga marioni</i>	Tineidae	Larvae	-5(2)	-7.9(2)	Survival	5:25	Sinclair and Chown, 2003
<i>Chilo suppressalis</i>	Crambidae	Larvae	0(4)	-21(2)	Survival	25:50	Qiang et al. 2008
<i>Spodoptera exigua</i>	Noctuidae	Larvae	5(2)	-11(2)	Survival	30:65	Kim and Kim, 1997
<i>Spodoptera litura</i>	Noctuidae	3 rd instar larvae	0(2)	-15(1)	Survival	0:98	Kim et al. 1997
<i>Spodoptera litura</i>	Noctuidae	5 th instar larvae	0(2)	-7(6)	Survival	0:70	Kim et al. 1997
<i>Spodoptera litura</i>	Noctuidae	Eggs	0(2)	-10(1)	Survival	0:25	Kim et al. 1997

To place the present study into a more global perspective, I used DIVA-GIS (www.diva-gis.org) software and historical extreme temperature data (www.meteorologyclimate.com) for some of the major apple growing regions in the world (Fig. 4.1), to display the likelihood of temperature-related mortality and RCH or RHH events influencing *C. pomonella* survival. The lowest temperatures documented in apple-growing regions of Argentina, Austria, Belgium, Brazil, Chile, China, Czech Republic, France, India, Iran, Italy, Netherlands, New Zealand, Peru, Russia, South Africa, Turkey and the United States of America were low enough to induce both RCH and CTmin (Fig. 4.1A). Of the same nations, only Austria, Belgium, Brazil, Czech Republic, France, India, Iran, Italy, Netherlands, New Zealand, Peru, and United States experience temperatures high enough to elicit RHH or CTmax, with growing regions in Argentina, Chile, China, Russia, South Africa and Turkey experiencing only RHH temperatures but not CTmax (Fig. 4.1B). Therefore, on a global scale, both low and high temperatures could play a role in CM adult survival through direct mortality and thus, may influence, or have influenced in the past, population dynamics. However, the temperatures used for drawing these maps were the most extreme temperatures to have ever occurred in those areas over long periods (~ 40 years) and not necessarily during a single fruit growing season. Regardless, even within peak fruiting seasons, the temperatures which elicit these thermal responses do occur (e.g. Howell and Schmidt, 2002). In addition, adult survival might be affected, especially in more extreme conditions predicted under climate change scenarios (see e.g. Easterling et al., 2000; New et al., 2006).



A



B

Figure 4.1 Possible effects of (A) low or (B) high temperatures on the survival of adult *Cydia pomonella* in some of the major apple growing regions of the world.

Third, my release-recapture study of developmentally-acclimated *C. pomonella* show almost two-fold increases in numbers of individuals recaptured using sex pheromone traps in individuals released in ambient temperatures (T_a) identical to their thermal history when compared with non-acclimated moths. However, such benefits indicative of increased performance in the wild came at a cost when laboratory-reared *C. pomonella* were released in T_a not matching their thermal history. This therefore shows clear costs and benefits of acclimation on performance of insects, as reported by Kristensen et al. (2008) for *Drosophila*. My study therefore supports the beneficial acclimation hypothesis (BAH), as defined by Leroi et al. (1994), and can find application in current SIT programmes for a number of reasons: 1) My work shows that acclimatory benefits gained during development can be manifested in adult CM. This might be ideal for (SIT) as acclimation may be incorporated into current rearing protocols; 2) Warm- or cold-acclimated *C. pomonella* were recaptured almost twice as much in T_a matching their thermal history, but not the opposite conditions. In the latter, performance decreased dramatically.

This may be critical in minimising costs of SIT programmes as fewer moths will be required to achieve the current efficacy levels. On the other hand, efficacy can be increased when the same numbers are released as a chance of increased successful mating events is made possible, although the increase in mating events has yet to be demonstrated in the field through this approach; 3) The study demonstrates the potential practical feasibility of thermal acclimation for offsetting negative efficacy in the SIR programme for *C. pomonella* control especially under cooler, springtime conditions where low performance has been reported.

In conclusion, the outcomes of this work stimulate a number of issues which seem like valuable areas to explore in future research:

- Sterile insect technique is used as an area-wide technique (Judd and Gardiner 2005; Vreysen et al., 2006). In this study the benefits of acclimation were only demonstrated on a localised plot with additional support from laboratory activity and thermotolerance assays of *C. pomonella*. Future research should consider assessing the effects of acclimation on other desired traits of quality SIT insects such as longevity and dispersal on an area-wide basis. Whilst I used data on CM performance from sex pheromone trap catches, perhaps indicative of preserved mating behaviours, further research should consider evaluating if acclimation contributes to mating success with wild individuals under varying temperatures.

- Frazier et al. (2008) attributed improved low-temperature-flight performance in low-temperature-reared *Drosophila* to changes in wing morphology. Future research may consider testing the mechanistic basis of acclimation responses of *C. pomonella* at cellular, tissue or organ levels, and which was not tested in this study. For example, understanding heat shock protein responses (e.g. HSP70) may be useful for determining field conditions eliciting acclimation or hardening responses (e.g. Karl et al., 2009; Feder et al., 2000). Such knowledge may help to further understand the functional significance of acclimation (see discussions in Kingsolver and Huey, 1998).
- Climate change effects on pests of agriculture are a major concern for society (Thomson et al., 2010). To avoid erroneous predictions of the effects of climate change, future research may consider studying effects of climate change on adult *C. pomonella* under simulation models of expected climate change scenarios (Cannon, 1998) to better comprehend population dynamics and potential fruit damage. Observations which can be made may include mortality (longevity), fecundity, reproduction, viability of eggs, foraging activities of produced larvae and adults. With such data, active approaches such as mechanistic models for prediction of the pest's distribution can then be made possible and may be incorporated into management plans and eradication programs.

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Appendix 1

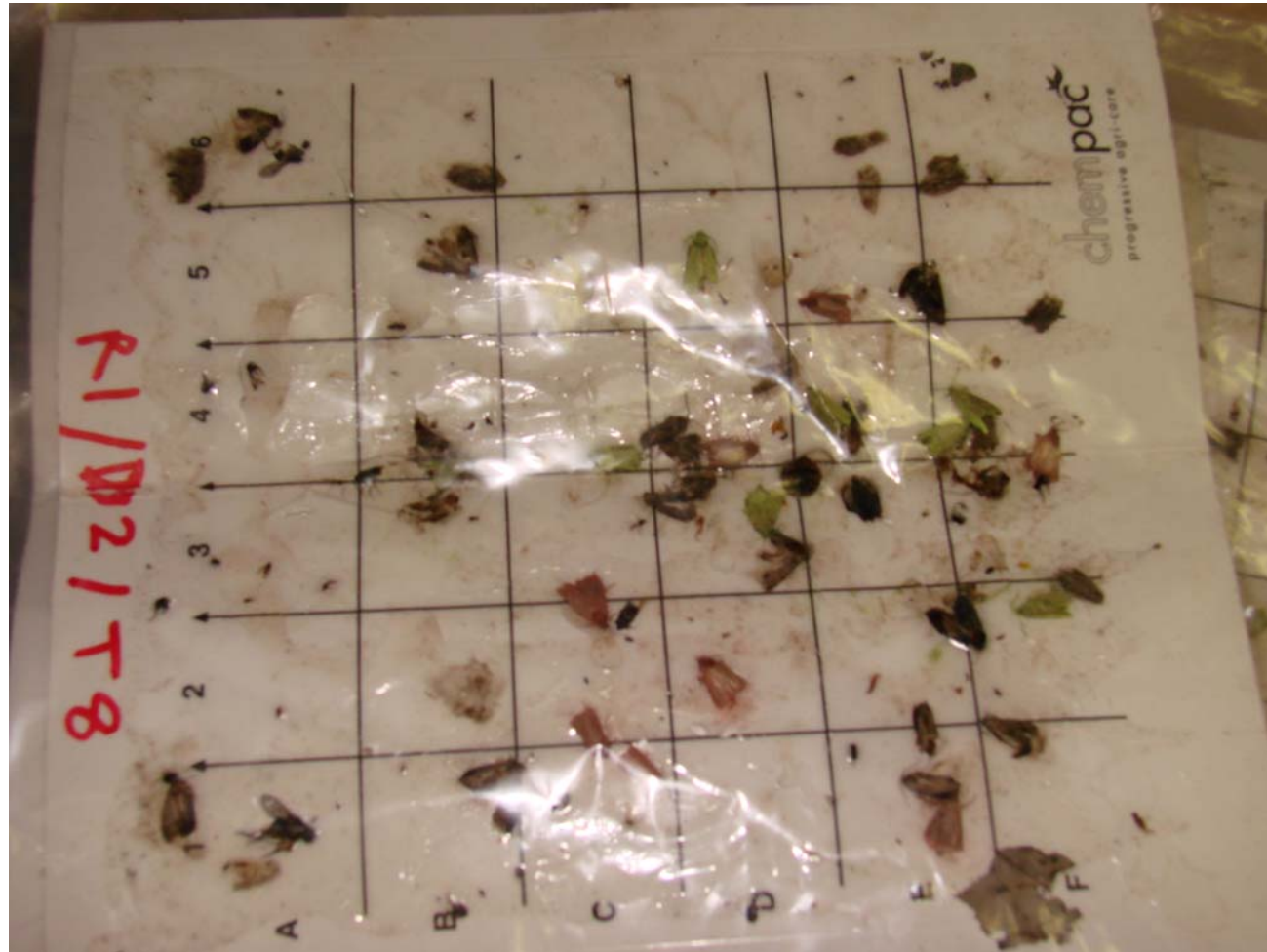
Photos from field release and recapture trials



Appendix 1A Ground release at the central release point with 3 different colours for each acclimation group (20, 25, 30 °C). These colours were randomised across release days.



Appendix 1B Trap visit for retrieval of sticky pads with catches. Trap height and orientation in the apple orchard.



Appendix 1C Retrieved sticky pad from one of the 8 traps showing insects from the red and green colour coded groups recaptured more than the blue one.