

**Thermal biology and behaviour of two
predatory Phytoseiid mites: *Amblyseius swirskii*
(Athias-Henriot) (Acari:Phytoseiidae) and
Phytoseiulus longipes (Evans)
(Acari:Phytoseiidae)**

by

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ABSTRACT

Amblyseius swirskii and *Phytoseiulus longipes* are targeted as biological control agents for the horticultural pest *Tetranychus urticae*. This study applies a standardised protocol to evaluate the risk of establishment of introduced species and investigates temperature related behavioural thresholds for all three species.

Laboratory results demonstrate a low level of cold tolerance in *A. swirskii* and no diapause. Field studies recorded 100% mortality within two weeks of outdoor winter exposure. *Amblyseius swirskii* has a higher activity threshold temperature than its target prey *T. urticae*. *Amblyseius swirskii* lacks cold tolerance and is unlikely to establish outdoors and thus can be considered a 'safe candidate' for release.

Laboratory results demonstrate that *P. longipes* can not diapause yet is more cold tolerant than *A. swirskii*. Field studies report 100% mortality after 73 days of winter exposure. *Phytoseiulus longipes* demonstrates mid-range cold tolerance yet is unlikely to survive an entire winter outdoors. *Phytoseiulus longipes* has lower activity threshold temperatures than *T. urticae*. Further studies are required on other factors attributable to establishment potential before it can be classified a 'safe candidate'.

As a consequence of the findings of the present study *A. swirskii* was granted a license for release into the UK in 2006.

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CHAPTER 1

Introduction

1.1 Biological control

Biological control is the manipulation, by mankind, of naturally occurring predation or parasitism of one organism by another. It is the regulation of plant and animal numbers by natural enemies. The phenomenon has long been applied by man, and involves the management of natural enemies, and sometimes the introduction of non-native species, to control pests. Pests are defined as a species whose activities cause damage to man. The principle definition of biological control is the action of parasites, predators and pathogens to maintain another organism's population density at a lower average than would occur in their absence (DeBach 1964). Biological control is a manifestation of the natural associations between different kinds of organisms. It is dynamic and subject to disturbances by other factors; environmental change and to the specific adaptations, properties and limitations of the organisms involved in each interaction (Van den Bosch 1973). In this way, biological control is a form of population management, whereby a pest organism may be eliminated from the local area or more often, its numbers are suppressed to a level that no longer poses a nuisance or economic damage.

The first orchestrated biological control programmes were set up by Chinese and Yemenite farmers who encouraged native predatory ants to protect citrus and date trees from insect pests (Van den Bosch 1973). In 1888 the predaceous *Vedalia* beetle,

Rodolia cardinalis (Mulsant) (Coleoptera: Coccinellidae), was imported to California from Australia to control the Cottony-Cushion Scale insect, *Icerya purchasi* (Maskell) (Homoptera: Margarodidae), a destructive pest in citrus groves and fruit plantations (Waage & Greathead 1988). This is considered the first example of a scientific and institutionally-backed biological control programme.

There are three types of biological control, described as: (i) Classical control, this is the control of alien species using small scale releases leading to permanent establishment of co-evolved natural enemies from the origin of the pest species; (ii) Augmentative (Inundative) control, involving a mass release of laboratory-reared populations (indigenous or non-native) to control open field or glasshouse pests and are designed not to persist; and (iii) Conservation control, is the sustainable use of indigenous biological control agents against indigenous or non-native pests (Van den Bosch 1982).

Biological control can be used as a pest control strategy on its own, or it may be integrated with other farming methods such as cultivation of resistant plant cultivars (including GM crops), pheromone traps and the application of chemical pesticides. This multi-strategy approach to pest control is termed Integrated Pest Management (IPM) and is thought to be the most cost effective measure of pest control. The central concept of IPM is often expressed in terms of the economic threshold, the point at which the cost of management intervention is less than the damage that can be expected if the problem is left untreated. Therefore the goal of pest management is to avoid losses associated with a particular intensity of a biological process which in this case is herbivory (Lockwood 2000; Hui & Zhu 2006; Estay *et al.*, 2009; Singh &

Vyas 2009). Ideally, in biological control, the agent would act in a density-dependent manner to suppress the target process as it proceeds towards the economic injury level (Coll *et al.*, 2007).

Biological control often involves the search for natural enemies in the native home of the pest species, where a regulatory pressure is applied to that pest. These enemies are then collected, reared and released in large numbers into the country or area where the pest outbreak is occurring (Samways 1981). An important point to remember is that for control to be successful, conservation of all natural enemies is essential; this includes indigenous and exotic species. Particular cultural practises should be adopted to promote natural enemies and discourage the improper use of insecticides (Wyss & Pfiffner 2008).

In recent years, as public awareness has increased on topics such as organic foods and environmental safety, interest in the biological control of crop pests has increased. Crop protection in the 1950s relied primarily on the use of chemical pesticides, particularly within glasshouse environments, leading to the development of pesticide resistance. A pest species may develop resistance by employing one or more of the following mechanisms: delaying the entry of pesticide into the body, increasing detoxification of the poison or becoming de-sensitised to the chemical at its site of action (Samways 1981). Chemical resistance often occurs when a pesticide has been re-applied time and again to the same area and is a result of underlying natural genetic variation in the pest population. Pesticide application also tends to kill a broad range of insect species and can lead to resurgence of the pest population, if a relatively greater number of natural enemies are killed. A secondary pest outbreak is also common in farming practises where a lot of chemical control is employed. If a large

proportion of natural enemies and primary pests are destroyed, this may leave room for normally innocuous organisms to become major pests due to a decrease in pressures of predation and competition (Vassiliou 2004).

Although chemical pesticides are cheap and often very effective, they do cause many problems. An alternative was needed and the first natural enemies for pest control on glasshouse vegetables occurred in Europe in the 1960s (van Lenteren 2000).

The use of Invertebrate Biological Control Agents (IBCA) for crop protection has many advantages, particularly in a controlled environment such as a glasshouse. These include: lack of residues on produce, the absence of phytotoxic effects, no environmental pollution, reduced exposure of people to toxic pesticides, and no build up of resistance. These features, along with added pressure and understanding of the need to balance intensive farming and environmental protection, have led to a substantial increase in the IBCA production industry.

Invertebrate pest populations may be biologically controlled by predators or parasitoids. In protected crops, such as those cultivated in glasshouses, two of the most well-known natural enemies are the predatory mite *Phytoseiulus persimilis*, used for the control of spider mites (mainly *Tetranychus urticae*), and the parasitic wasp *Encarsia formosa*, to control glasshouse whitefly *Trialeurodes vaporariorum* (van Lenteren & Manzaroli 1999).

1.2 Glasshouse agriculture

Between 1988 and 2000, the total area of the world covered in glasshouses has doubled from 150,000 ha to 300,000 ha. At present, biological control is employed on only 15,000 of that 300,000 ha (van Lenteren & Woets 1988; van Lenteren 2000). Growing vegetables and ornamental crops in such a protected environment is expensive, therefore pest damage can not be tolerated. If chemical control is cheaper and more effective it will be used. Irrespective of this fact, the development and application of biological control has been remarkably fast (Murdoch & Briggs 1996). Small scale application of biological control started in 1968 with the use of a predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae) (van Lenteren & Woets 1988). The glasshouse environment is characterised by conditions optimised for plant growth. However, stable glasshouse environments provide a favourable environment for herbivorous insects and mites. It is impossible to prevent accidental introductions of these organisms into the glasshouse or migrations through open vents and doors. Once inside, these pests will most likely find all the abiotic and biotic conditions favourable for rapid population increase. Major pest species include whiteflies, aphids, spider mites, rust mites, tarsonemid mites, thrips, leaf miners, lepidopterans and scale insects (Brodsgaard & Albajes 1999). In glasshouse agriculture, establishment of biological control agents is designed to be short term, at most the length of a growing season. After this, the glasshouse can be cleaned to remove all pests and predators before new plants are introduced. This is as opposed to an 'outdoor' environment, where it is necessary that the control agent can develop and reproduce in synchrony with the target pest species, and also be able to survive any adverse weather conditions for long term establishment to occur

(Caltagirone 1981). It seems then that glasshouse agriculture is most conducive to somewhat 'safer' short-term biological control programmes. This, combined with the rising costs of pesticides, increasing resistance and the overall desire to reduce pesticide use because of concerns for human health, has prompted a surge in growers' interest in the use of natural enemies in glasshouse agriculture and horticulture (van Lenteren & Manzaroli 1999).

1.3 Environmental concerns

Only about a third of the species used in biological control have been predators rather than parasitoids. This is because predators are not usually as specific as parasitoids and may switch to another form of prey when food becomes scarce. This is known as a 'non-target effect' of biological control (Simberloff & Stiling 1996). As interest in biological control has increased and the biological control industry has grown, these non-target effects of natural enemies have become more of a concern to conservation biologists. Is it ethical to import and release non-native organisms that could be potentially damaging to harmless, indigenous populations? Doult (1967) described the ethical dimension of biological control and in his opinion, "civilised societies need to protect and preserve natural and native values, including natural environments".

A major uncertainty in biological control is how effective the natural enemy will be when it is released. Extrinsic factors affecting an IBCA's efficacy include the climate, weather conditions, crop variety and age, farming practices and competition from other natural enemies. Alongside these, there is the intrinsic factor of genetic variation within the IBCA population. Biological control companies will generally collect their source populations from different areas so that commercial releases will include

sufficient genetic variation in the control agent population to survive exposure to a range of extrinsic variables (Samways 1981). Again, this mass release can have catastrophic consequences for non-target organisms. A study of the parasites and predators employed as biocontrol agents against forest insects in Canada between 1910 and 1958, found that 37.5% attacked more than one host species (McLeod *et al.*, 1962). One species from this study, *Compsilura concinnata* (Diptera: Tachinidae) is now known to attack over 200 species of Lepidoptera and Hymenoptera (Stiling & Simberloff 2000).

In the past 100 years many exotic natural enemies have been imported, mass-reared and released as biological control agents for pest control (Albajes *et al.*, 1999; van Lenteren 2000, 2003). Although the majority of these releases have not resulted in unwanted side effects, some serious cases of non-target hazards by exotic biological control agents against insects and weeds have been recently reported (e.g. Boettner *et al.*, 2000; Follett & Duan 2000; Wajnberg *et al.*, 2000; Louda *et al.*, 2003)

A famous non-target effect of a biological control programme involved the release of the predatory New World snail *Euglandina rosea* (Mollusca: Prosobranchia). This species was released in many parts of Asia, numerous islands of the Pacific and Indian oceans and the West Indies, to control an accidentally introduced giant African land snail *Achatina fulica* (Mollusca: Prosobranchia), which was causing widespread agricultural damage in these areas. The biological programme began in the 1950s and *E. rosea* has subsequently been blamed for the extinction of several endemic species of land snail (Civeyrel & Simberloff 1996). However, even with this startling knowledge, a further release of *E. rosea* was made in Samoa as recently as 1992. A

review by Stiling and Simberloff (2000) suggests that when studies on non-target effects are made, they are found to be frequent and substantial, which contrasts with the view of van Lenteren *et al.*, (2006). It is almost impossible to know all the non-target effects of past species introductions. Some of these effects would be direct (e.g. species extinctions due to predation), but the most difficult to study are the indirect effects, such as competition and niche extraction.

The scientific community tend to agree that introducing a non-native species as part of a biological control scheme requires extensive knowledge of the biology of that species (Jutsam *et al.*, 1988). However, even extensive research into an organism's biology and ecology will probably not be enough to predict the effects an introduced species will have on that new environment. A general consensus on the comprehensiveness of protocols for such introductions suggests that in the past they have fallen well below an acceptable standard (Simberloff & Stiling 1996a). For example, most glasshouse IBCAs introduced to the UK are of tropical, subtropical or Mediterranean origin (van Lenteren 1997), and it is often assumed that these organisms will be unable to survive through a UK winter and establish outdoors because they lack sufficient cold hardiness, thus ensuring no non-target effects. There have, however been two specific examples where this assumption has been proved wrong. *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) was released in the UK under licence in 1991 as a control agent for glasshouse spider mite, *Tetranychus urticae* (Acari: Phytoseiidae), with the expectation that any escaping individuals would die out in winter. However, wild populations have since been found established close to the release sites. This winter survival and cold hardiness has been attributed to a diapausing ability in some populations (Jolly 2000) and later research also found

that non-diapausing adults are able to survive and reproduce in winter for over 3 months (Hart, *et al.*, 2002a). In addition, the predatory mirid bug *Macrolophus caliginosus* (Heteroptera: Miridae), released as a control agent against glasshouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), in 1995, is regularly found outside of glasshouses in winter (Hart, *et al.*, 2002b). In both of these cases, the effect on native species and surrounding ecosystems is unknown. This shows that there needs to be a shift in the perspective that most introductions are not likely to cause trouble, to a view that thorough research is necessary to justify a judgement that an introduced species is likely to be innocuous (Simberloff & Stiling 1996a) – encapsulated in the concepts of ‘Qualified Presumption of Safety’ *versus* the ‘Precautionary Principle’. In particular, the above examples suggest that there is a need for greater consideration, prior to release, of the low temperature survival and establishment potential of any non-native species regarded as prospective candidates for the biological control of pests in UK glasshouses.

In addition, knowing the effects of climate and habitat on the distributions of insect pests and their natural enemies would help target the search for biological control agents, increase establishment of intentional introductions, improve risk assessment for accidental introductions and the effects of climate change (Ulrichs & Hopper 2008). Climate change is expected to alter trophic interactions within food chains, but predicting the fate of a particular species is difficult because predictions hinge on knowing exactly how climate influences direct and indirect interactions (Barton *et al.*, 2009). Kriticos *et al.*, (2009) explored the interactions between a biological control agent and its target host under changing climatic conditions using a process-based population dynamics model. They report that climate change could have a significant

impact upon the interactions between control agents and target pests. At the coolest sites the control effect was slowed, but it was still eventually capable of controlling pest populations. At the warmer sites, both populations grew faster, and control levels were only slightly higher than normal. Climate change within the UK is predicted to affect winter temperatures. Milder winter temperatures reduce energy budgets and increase the quality of the foraging environment (Creswell *et al.*, 2009) and this is likely to impact directly on the establishment potential of non-native species. This situation may lead to a necessity, that even a species that has been previously released may need to be periodically assessed in the light of changing climatic conditions. In these cases a simple yet robust methodology for predicting establishment potential relative to temperature constraints could be integrated into a simple and efficient monitoring system.

1.4 Regulation and legislation

The risks and benefits associated with efforts to control invasive alien species using biological control programmes are being subjected to increasing scrutiny (Kriticos *et al.*, 2009). Potential risks arising from the introduction of exotic biological control agents include those to the environment, human health and economy (van Lenteren *et al.*, 2006). At the present time, the UK operates a stringent regulatory framework for the release of biological control agents. The Wildlife and Countryside Act 1981 prohibits the release of non-native species into the UK and applications for release licences are reviewed by the Department for Environment, Food and Rural Affairs (DEFRA). The application involves a dossier of information on the organism's origin, biology, ecology, preliminary tests for suitability of pest control, and an

environmental risk assessment of likelihood of establishment in the UK (Bigler, *et al.* 2005).

In 2002, the proportion of commercially used biological control species in the EPPO (European and Mediterranean Plant Protection Organisation) region that were of non-native origin was 39% (van Lenteren & Loomans 2006) and research continues into novel IBCAs for use in pest control programmes in non-native countries.

However, in Europe, biological control is not used as widely or as effectively as it is in the USA, Canada, New Zealand and Australia (Barratt *et al.*, 1999). For this, there are two main reasons: firstly, the threshold for environmental safety is too high to invest in research and development for the introduction of new organisms; and secondly, there is only a patchy regulatory framework in European countries for the release of IBCAs. In some European countries there is regulation and in others there is none at all. This would appear an illogical situation considering that insects have no awareness of political boundaries and can move freely from one country to the next in mainland Europe. In theory, introducing non-native biological control agents to UK glasshouses is a safe option, as there would not be any interaction with the outside environment. However, insects can always overcome physical boundaries and escapees from the glasshouse are inevitable, thus allowing these introduced species to interact with their new surroundings (Howarth 1991). A famous and recent example where this loose regulatory framework in Europe has caused problems is the spread of the Harlequin ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). This species originating from Asia, is highly polyphagous and was first released in trials in France and Belgium in 1991 and commercially released in 1995 for the biological control of aphids and coccids. It was intentionally released into nine different

countries. Since then it has spread rapidly and in 2004 invaded the UK and is now considered established in at least 13 countries (Brown *et al.*, 2008).

There is an emerging consensus on the need to apply Europe-wide regulations that would set out the minimum level of biological information necessary to assess the likelihood of an outdoor establishment of an introduced biological control agent without forfeiting environmental safety; and also, similar information on host range and dispersal (van Lenteren *et al.*, 2006). In effect, a set of standardised protocols would be used to investigate the establishment potential of a non-native organism and this information would be an important component of the submitted dossier. The research should include the minimum temperature requirement for development and reproduction, cold tolerance and winter survival, and wild prey availability and target host.

A recently designed, environmental risk assessment (ERA) for invertebrate biological control agents has been put forward by van Lenteren *et al.*, (2006) to provide a method of comprehensively evaluating the risk potentiated by an introduced non-native species. The method consists of a stepwise procedure to evaluate the environmental risks based on the intended use, establishment potential, host range, non-target effects, dispersal and direct and indirect effects. The ERA scheme enables researchers and biocontrol companies to screen potential candidates one level at a time to make decisions on their appropriateness for purpose. Both native and exotic species can be included in the risk assessment. Figure 1.1 details a schematic of the ERA and it can be seen that establishment potential is the first screening test to be undertaken. When this ERA was applied to evaluate the environmental risks of

releasing *H. axyridis* the species was found to fail at the first assessment of establishment potential relative to thermal data (van Lenteren *et al.*, 2008). It concluded that this species is of particular high risk of establishment in Northwest Europe as a result of: it being very cold tolerant; having a very wide host range (including plant materials), it can cover large distances, it moves into non-target areas and attacks many non-target species (including beneficial insects and insects of conservation concern). Beyond this it's activities have resulted in the reduction of populations of native predators in North America, it is known as a nuisance in North America and recently also in Northwest Europe, and it may develop as a pest of fruit in North America. Retrospectively the environmental disaster of the *H. axyridis* release has only proved to strengthen the case for the usefulness of straightforward environmental risk assessments.

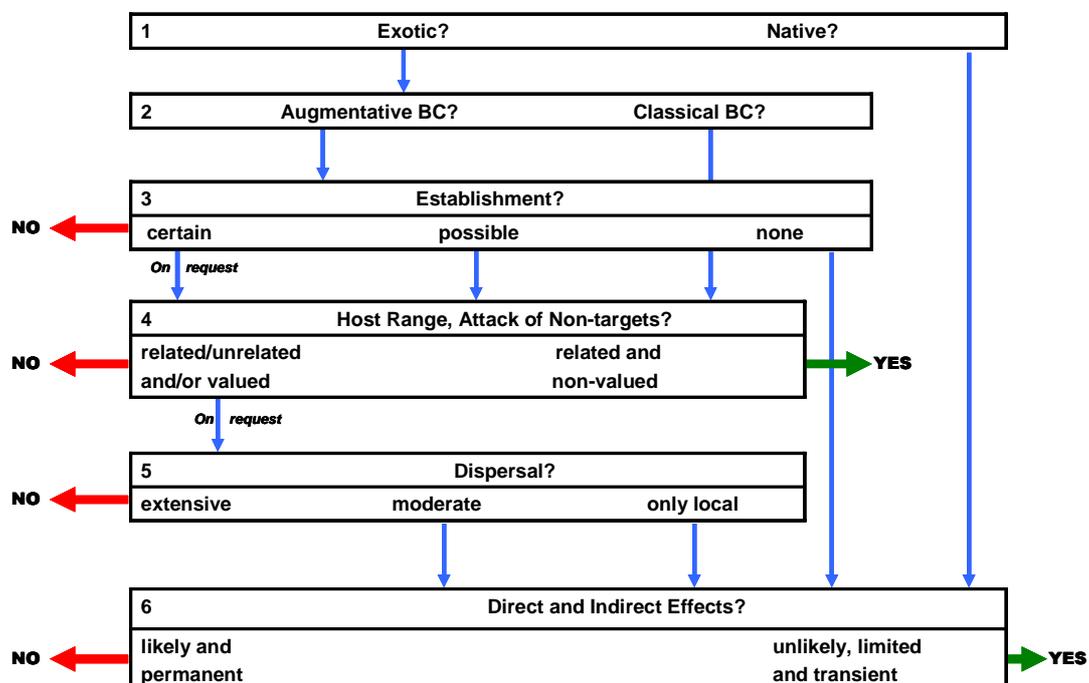


Figure 1.1: Environmental Risk Assessment (ERA) scheme for arthropod biological control agents NO: release is not recommended; YES: release is recommended as

safe; On request: when applicants desire, information about the following issue(s) in the ERA can be provided to allow reconsideration of the decision not to release the species (Adapted from van Lenteren *et al.*, 2006).

The assessment of risk-cost-benefit of a biological control agent should take into account the expected risks, costs and benefits to human and animal health, the environment and economy. Impact to the environment is not usually assessed in monetary values so they must be assessed qualitatively. The highest ranked adverse affect must be compared to the highest ranked benefit of the biological control agent and decisions should then be made on the overall value (Bigler & Kölliker-Ott 2006).

The IOBC (International Organisation for Biological Control of Noxious Animals and Plants) has organised an initiative with the objective of merging all relevant international recommendation documents of risk assessment, and provide more specific guidance to harmonise the regulation of IBCAs in European countries. This initiative is called CHIBCA (Commission on Harmonisation of Invertebrate Biological Control Agents) (Bigler *et al.*, 2005). Importantly, these guidelines have been produced by representatives from industry, regulatory bodies and science to try and achieve a balanced perspective of the risk-cost-benefit relationship.

Recently, an EU-funded project (REBECA) was established, to make recommendations on the future regulation of biological control agents across Europe. It has assessed protocols for macrobial invertebrate biological control agents, including insects, mites and nematodes. Its main objectives were to consider the mechanisms by which to produce a single regulatory system for macrobial agents encompassing different taxonomic groups and licensing situations, develop appropriate research methodologies to support the regulatory system and to make recommendations for the implementation of a pan-European regulatory system for

macrobial BCAs. REBECA is “an EU policy support action to review possible risks of biocontrol agents, compare regulation in the EU and the USA and to propose alternative, less bureaucratic and more efficient regulation procedures maintaining the same level of safety for human health and the environment but accelerating market access and lowering registration costs” (REBECA 2007). REBECA seems realistic in its aim of developing a balanced regulatory system that minimizes the costs imposed on industry without compromising risks to human health or the environment.

For an introduced biological control agent to successfully establish outside of the glasshouse environment, the organism will need an adequate thermal budget. That is sufficient day-degrees above the developmental threshold temperature of that organism. One day-degree is defined as the amount of development that occurs in 24h when the ambient temperature is 1°C above the developmental threshold. The organism must also be able to produce an overwintering stage and have a source of alternative wild prey (Leather *et al.*, 1993). Therefore, to be able to predict the establishment potential of a biological control agent these requirements would need to be rigorously tested. As ecological factors determining the environmental impact of an exotic agent are increasingly included in current evaluations, determination of the establishment potential of these species based on their thermal biology could provide a simple methodology of ability to establish in the target area (Boivin *et al.*, 2006; van Lenteren *et al.*, 2006). A realistic set of protocols to investigate the establishment potential in the UK of any introduced IBCA would have to consider the following thermal indices: developmental threshold temperature, day-degree requirement per generation, annual voltinism, lethal times, freezing temperature, acclimation response,

lethal temperatures and ability to diapause. These laboratory tests should be analysed alongside results from winter field survival experiments.

In the main commercial agricultural industry is still using large scale pesticide programmes (Gomiero *et al.*, 2008; Lichtfouse *et al.*, 2009) and convincing growers worldwide to adopt a systems approach to pest management and make greater use of biological control has been slow (Bale *et al.*, 2008). Barriers in technology and economy are somewhat to blame but mainly a problem in attitudes towards an IPM approach exacerbates the issue. Even so, biological control has made a way in, particularly for the management of pests that are difficult to control with insecticides and in the organic market. It is hoped that in future applications, data from the temperature-establishment assessment of both novel and currently used biological control agents, could be collated and applied to white list of supposedly safe candidates for specified regions (van Lenteren *et al.*, 2006). Diligence with the use of the quick scan method for cold tolerance and evolution of a regulatory process which is non-prohibitive and harmonious, might stimulate the wider utilisation of biological control.

1.5 Insects at low temperatures

Arthropods are ectothermic animals in which their body temperature is generally determined by the temperature of the local environment. The likelihood of insects and mites escaping from glasshouses into the surrounding area is high. Therefore, their impact on native flora and fauna should be thoroughly assessed before they are granted a release licence to be used as biological control agents. In the UK, the temperature regime is the primary factor that governs an insect's developmental period, reproduction and survival which ultimately contribute to its distribution and

abundance (Bale & Walters 2001). The severity of winter and the degree of cold tolerance an insect exhibits will ultimately determine the size of the population that is active in spring (Bale 1987). To survive outdoors in a new environment, a non-native species must have an adequate thermal budget above its developmental threshold temperature to complete its life-cycle and reproduce, and also, must be able to survive through winter (Leather *et al.*, 1993). If temperature regimes are favourable for development and survival, then its ability to find alternative sources of food, locate and utilise sheltered microhabitats and avoid predation must also be studied (Tauber *et al.*, 1986).

An insect's overwintering biology can be assessed using a number of thermal indices including, developmental threshold, thermal budget, voltinism and field survival (Bale & Walters 2001). These values can then be compared with measurements of cold tolerance (e.g. supercooling point and lower lethal times and temperatures) to estimate the organism's winter survival and consequently, its likelihood of establishment in the UK, and similar cool temperate climatic areas.

1.5.1 Developmental threshold

An ectotherm's rate of development is a function of its thermal environment, with warm temperatures accelerating, and cold temperatures decelerating, the developmental process. The lower developmental threshold is the temperature below which no development can occur. This threshold temperature varies between species and individuals and different life stages of the same species (Hart *et al.*, 1997). Developmental threshold temperatures are determined by rearing samples of an insect species at a series of different temperatures. This information can be plotted as a

linearised curve from which the temperature at which no development occurs can be estimated (see Figure 2). Day-degrees are used extensively to describe insect development over a growing season (Boivin *et al.*, 2006). When the daily average temperature is higher than the base temperature (developmental threshold), the difference is known as day-degrees. The accumulated day-degrees can be used to determine the developmental stage of the insect, estimating the growth of an insect population and the number of generations produced over a particular period or season.

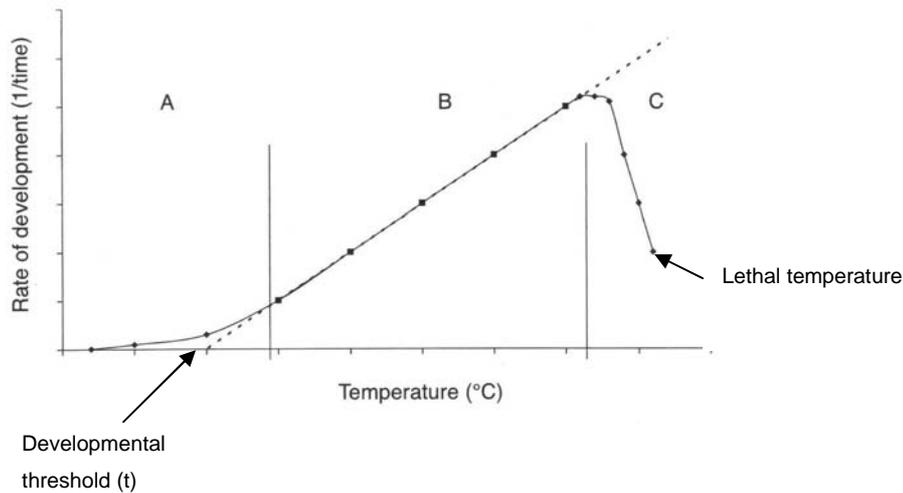


Figure 1.2: Relationship between the rate of insect development and temperature (after Campbell *et al.*, 1974).

Low temperatures within zone A can result in high mortality, especially of species of tropical and Mediterranean origin. The high temperatures in zone C will only be deleterious if they occur over an extended period of time (Campbell *et al.*, 1974). The linear relationship in zone B can be represented as a straight line:

$$R(t) = bT - a$$

where the rate of development (R) and rearing temperature (T) gives the developmental threshold (t).

1.5.2 Thermal budget

The thermal budget is the number of day-degrees required by a species to complete a generation. Therefore the base temperature must be established before this index can be calculated. The mean number of day-degrees available in a year, in a particular region, can be calculated from local temperature data records (Hatherly *et al.*, 2004). When the annual accumulation of day-degrees in an area is below that required to complete a generation, an insect will be unable to establish (Boivin *et al.*, 2006).

1.5.3 Voltinism

Voltinism is the number of generations per year and can be calculated from the thermal budget. The voltinism over a specified time period is calculated from the sum of the day-degrees available for each day in that time period and the thermal budget required by the species (Hatherly *et al.*, 2005). For example, if an insect has a developmental threshold temperature of 10°C, for each day of the year when the temperature is 11°C the insect gains 1 day-degree. If this insect requires 250 day-degrees to complete a generation and over the year the accumulated day-degrees is 1000, then the insect can theoretically complete four generations within that year.

1.5.4 Field survival

The level and duration of survival of populations of the subject species under winter field conditions should be assessed and compared alongside the other thermal indices. This index, above others, should give the clearest indication of winter survival and

establishment potential of an introduced species within that country/area. However, in terms of risk assessment, it can be a time-consuming and expensive process

1.6 Insect Cold hardiness

In 1961, Salt established the principles of cold hardiness. Cold hardiness is a term used to describe the ability of an organism to survive at low environmental temperatures (Salt 1961). It may vary between and within a species, particularly between developmental stages within a species (Leather *et al.*, 1993). For example, studies on the cold tolerance of Phytoseiid mites suggest that only diapausing females survive the winter months, while males and juvenile stages die at the onset of cold weather. The females are inseminated in the autumn and begin to reproduce the next spring when diapause terminates (van der Geest *et al.*, 1991). The distinction between species with the ability to tolerate freezing and those that die as a result of freezing is one of the most important principles of insect cold hardiness (Bale 1989). In evolutionary terms, freeze-tolerance is thought to be a more recent strategy for arthropod survival and freeze-avoidance is the primitive state (Vernon & Vannier 2002).

The cold hardiness of insects and mites is usually measured by indices such as the supercooling point (SCP), lethal temperature over a fixed time (LTemp), and lethal time at a fixed temperature (LTime).

1.6.1 Supercooling point

Supercooling is the phenomenon by which water and aqueous solutions remain unfrozen at temperatures below their melting point. The supercooling point (SCP) of

an insect is the temperature at which spontaneous freezing of their supercooled body fluids occurs (Leather *et al.*, 1993). In most species, the SCP is the theoretical lower lethal temperature limit, because death usually occurs from the cumulative effects of cold above the SCP (Bale 1989). The SCP is measured using a differential scanning calorimeter (or similar cooling methods), and is identified by the freezing exotherm which is produced by the latent heat of crystallisation (Hart *et al.*, 2002a). Using this strategy, a species can be determined as freeze tolerant or freeze intolerant. As the supercooling point is not considered a reliable measurement of the cold tolerance of many insects and mites, other indicators such as the lower lethal temperature and lethal time, should also be measured.

1.6.2 Lower lethal temperature

The lower lethal temperature (LTemp) is the temperature required to kill a certain proportion of the population in a specified time period (e.g. 1 min) (Bale & Walters 2001). For example, the LTemp₅₀ would be the lowest temperature that kills 50% of the sample population after 1 min of exposure at that temperature. Lethal temperatures are determined by exposing samples of insects to a series of sub-zero temperatures that are above the SCP for the set period of time and then re-warming to a favourable temperature. The insects may still die when exposed to temperatures above the SCP due to acute exposure to cold shock (Hatherly *et al.*, 2004). When compared to the SCP, lethal temperature data will indicate whether individuals die before they reach their freezing temperature (and in what proportions) (Hart *et al.*, 2002b) and therefore if the species is freeze-avoiding according to the categories proposed by Bale (1993).

1.6.3 Lower lethal time

The lower lethal time (LTime) is the time required to kill a given proportion of a population at a set temperature (e.g. -5°C) (Bale & Walters 2001). For example, the LTime₁₀ would be the time it takes for 10% of a sample population to die when exposed to a constant temperature. In this way the insect is being held at a temperature, again above the SCP, but dies from the effects of chronic cold exposure. For both of these measurements, the relationship between dose (temperature or time) and mortality is often represented as a sigmoid curve, but can be linearised using probit analyses (Hatherly *et al.*, 2004). Both methods can provide estimates of the lethal doses (LD) required to kill 10, 50, and 90% of the sample population.

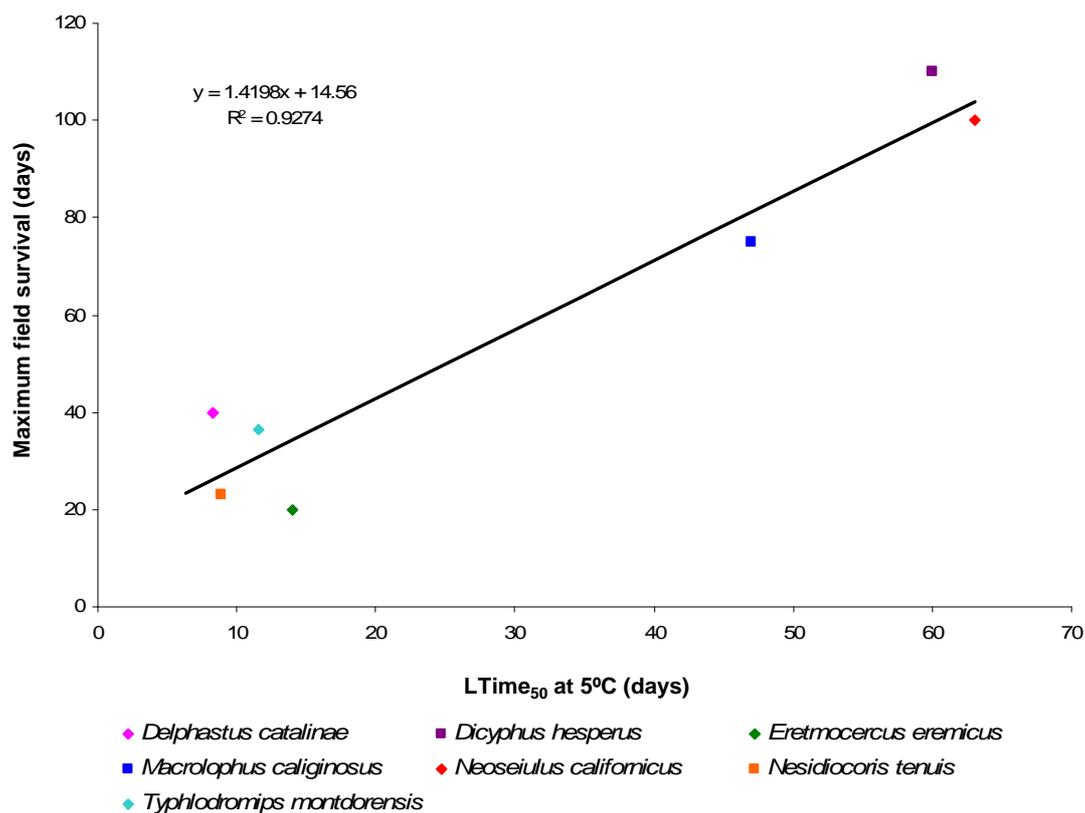


Figure 1.3: Relationship between maximum field survival (days) and LTime₅₀ at 5°C (days) for seven non-native biological control agents (adapted from Hughes *et al.*, 2009).

Analysis of low temperature biology data sets from five recently studied non-native (to the UK) biocontrol agents by Hatherly *et al.*, (2005) suggested that laboratory assessment of the LTime₅₀ at 5°C could be a reliable predictor of field survival, and this analysis has now been further extended to include seven non-native species. The seven species studied represent different taxonomic groups and trophic guilds. By using this graph, candidate biological control agents could be classified into different categories of risk with respect to their establishment potential. For example, using this system, the predatory beetle *Delphastus catalinae* (Coleoptera:Coccinellidae), the parasitic wasp *Eretmocerus eremicus* (Hymenoptera: Aphelinidae), the predatory mite *Typhlodromips montdorensis* (Acari: Phytoseiidae) and the predatory mirid *Nesidiocoris tenuis* (Heteroptera: Miridae) are classified as 'low risk' because 100% field mortality occurs within four weeks and any establishment is unlikely. In contrast, *N. californicus* (predatory mite) is in the 'high risk' group, where some strains overwinter in diapause and others survive long enough to develop and reproduce (Hart *et al.*, 2002a). *M. caliginosus* (predatory mirid bug) falls into the intermediate risk group because populations do persist outdoors in winter for extended periods but establishment is limited (Hart *et al.*, 2002b). Hatherly *et al.*, (2005) also suggest that this system has a much wider application; if the relationship proves to be consistent across other species, the LTime₅₀ could be used as a 'quick scan' initial screening protocol for any organisms deemed as having a potential role as biological control agents. This would then identify the need for further experimentation and the more costly field survival experiments.

1.6.4 Freeze tolerance

Freeze tolerance is the ability to withstand the formation of ice crystals in the body tissues and haemolymph. This freezing occurs extracellularly to prevent lethal intracellular freezing. Freeze tolerance is uncommon in arthropods and usually occurs in one life stage (Block 1990). Freezing in the extracellular spaces is promoted by the production of proteinaceous ice nucleating agents (INAs). In this way water is moved out of the cells via an osmotic gradient and hence freezing of the cell contents is avoided. In winter, most freeze tolerant insects accumulate polyols and sugars, such as glycerol, sorbitol, fructose and sucrose. These act to reduce the rate of ice growth, stabilise protein structure and maintain cell volumes, and in this way are thought to counteract the causes of freezing damage. These species also produce anti-freeze proteins (AFPs) which may act to protect freeze tolerant invertebrates from the effects of sub-zero temperatures in early winter before INAs have been synthesised (Bale 2002). Freeze tolerant insects usually freeze at temperatures between -5 and -10°C, and once frozen they can be cooled to much lower temperatures; for example, the carabid beetle *Pterostichus brevicornis* (Kirby) (Coleoptera: Carabidae) overwinters in Alaska and in the freeze-tolerant state can survive to -70°C (Miller 1969). On warming, freeze tolerant species thaw, recover and exhibit normal development, reproduction and behaviour (Bale 1993).

1.6.5 Freeze avoidance

Freeze intolerant species predominate in cool and cold climates rather than areas prone to extreme frost where most freeze tolerant species are found. The majority of cold-hardy insects are freeze intolerant and rely on supercooling to avoid freezing (Leather *et al.*, 1993) and for this reason are more commonly described as freeze

avoiding species. The freeze avoiding strategy has two phases: the first involves behavioural and physiological changes, including the location of an overwintering site that is buffered from the most severe low temperatures, a reduction in body water, an increase in body fat content, the cessation of feeding and evacuation of all gut contents, which may serve as ice nucleators. The second phase includes the production of polyols that depress the SCP by colligative properties, and AFPs to induce thermal hysteresis (when the freezing point of water is lowered relative to its melting point) and increase the supercooling capacity of the nucleator-free liquid compartments within the insect (Bale 2002).

On a world wide basis very few insect species are either freeze tolerant or truly freeze avoiding, if we use the definition that freeze-avoiding species should experience little or no low temperature mortality in the absence of freezing. As the majority of insects and other arthropods die as a result of the cumulative effects of cold exposure and not freezing, Bale (1996) has proposed three new groups of cold-hardy insects. 'Chill tolerant' species are defined as having a low SCP (-20° to -30°C) and a high level of cold tolerance but exhibit some mortality above the SCP, so are different from the freeze avoiding category which show little or no 'pre-freeze' mortality. 'Chill susceptible' species also supercool to very low temperatures but die after only brief exposures at temperatures significantly higher than the SCP. Finally, 'opportunistic survival' species are described as being unable to survive below the threshold temperature for development and therefore must find sheltered overwintering sites to survive (Bale 2002).

Many insects and terrestrial arthropods from temperate or tropical regions become immobilised in a chill coma when exposed to temperatures between 0° and 10°C . This

chill coma is reversible if the period of exposure is not too prolonged. Some winter active insects are lethally injured by cold shock even though their body fluids do not freeze (Lee 1991).

1.7 Diapause

Like all ectotherms, insects and acarines need a way to cope with fluctuating temperatures in their environment. Diapause is a physiological state of low metabolic activity with very specific triggering and releasing conditions. It is used as a means to survive predictable, unfavorable environmental conditions, such as temperature extremes, drought or reduced food availability. Its onset, maintenance and termination are characterized by a number of morphological, behavioural, physiological and biochemical features (Tauber *et al.*, 1986). Diapause synchronizes the insect's life cycle with seasonal changes in its environment, including winter conditions.

1.7.1 Induction of Diapause

Diapause occurs in response to a number of environmental stimuli that precede unfavorable conditions and is characterized as reduced morphogenesis, increased resistance to environmental extremes, and altered or reduced behavioural activity. The induction phase occurs at a genetically predetermined stage of life and occurs well in advance of the environmental stress (Kostal 2006). Of primary importance is that diapause is not only induced in an organism by specific stimuli, but once it is initiated, only certain other stimuli are capable of releasing the organism from this

state and this is essential in distinguishing diapause as a different phenomenon from other forms of dormancy such as quiescence and hibernation.

Studies of a number of mite species have shown that diapause occurs only in mated females and the most pronounced response is that no eggs are laid during the diapausing state (Overmeer 1985a). In similarity to hibernation, diapausing mites may seek shelter and their longevity is often increased following bouts of diapause compared to those that do not diapause (Veerman 1992). During diapause, females reduce their activity and feeding may cease entirely and this is accompanied with a change in appearance to a flattened and paler abdomen in comparison to non-diapausing females (Hoy & Flaherty 1975; Morewood & Gilkeson 1991; van Houten *et al.*, 1988). When inspected closely, the contents of the body cavity appears granular and this is thought to be due to an accumulation of lipids as energy reserves (Morewood & Gilkeson, 1991).

The primary cue for diapause induction in Phytoseiid mites is photoperiod and has been described for 15 species (Veerman 1992). Short day lengths will induce diapause but generally only in individuals that are exposed to the conditions during the immature life stages (Morewood 1993; Veerman 1992). However, van Houten and Veenendaal (1990) has described adults of *Amblyseius potentillae* (Garman) (Acari: Phytoseiidae) as entering diapause when subjected to a reduced photoperiod even when they have been reared in non-diapausing conditions. Veerman (1992) explains that the actual induction of diapause generally occurs in the life stage before the expression of diapause trait occurs. Length of photoperiod that induces differs between Phytoseiid species as summarised in Table 1.1.

Other influences on diapause induction include temperature and relative humidity. Temperature can act independently as a diapause stimulus or may modify the length of the critical photoperiod. It has been found that diapause may be prevented altogether if temperatures are kept above 20°C (Hoy & Flaherty, 1975; Morewood & Gilkeson 1991). Diapause induction in *Amblyseius andersoni* (Chant) (Acari: Phytoseiidae) and *A. cucumeris* (Oudermans) (Acari: Phytoseiidae) can occur even when they are kept in constant darkness, provided that the temperatures are optimal for induction.

Availability of prey may also play a part in diapause induction. Females of *Typhlodromus occidentalis* for example, reared under a photoperiod close to the critical value, entered diapause in higher percentages when prey individuals were lacking than when they were abundant (Field & Hoy 1985). The phytoseiid predatory mite *A. andersoni* and its prey *Tetranychus urticae* both exhibit a facultative reproductive diapause, which is expressed in females only (Veerman 1994). *T. urticae* and the predatory mite differ in their response to temperature. Whereas daily fluctuating temperatures induce diapause in *A. andersoni* in the complete absence of light, no such thermoperiodic response could be detected in *T. urticae*. Veerman (1994) also found similarities between the photoperiodic and thermoperiodic response mechanisms in *A. andersoni*, which strongly suggest that photoperiodic and thermoperiodic induction of diapause of this mite are based on the same physiological mechanism.

Diapause also occurs in the tropics and is often in response to biotic rather than abiotic factors. For example, food in the form of vertebrate carcasses may be more abundant following dry seasons, or oviposition sites in the form of fallen trees may be more available following rainy seasons. Also, diapause may serve to synchronize mating seasons or reduce competition, rather than to avoid unfavorable climatic conditions. Tropical diapause poses several challenges to insects that are not faced in temperate zones. Insects must reduce their metabolism without the aid of low temperatures and may be faced with increased water loss due to high temperatures. While low temperatures inhibit the growth of fungi and bacteria, diapausing tropical insects still have to deal with these pathogens. Also, predators and parasites may still be abundant during the diapause period (Denlinger 1986).

Table 1.1: Recorded critical photoperiodic ranges of Phytoseiid mites (adapted from Hatherly, 2005 p31)

Species	Critical Photoperiod (h)	Reference
<i>Metaseiulus (Typhlodromus) occidentalis</i>	10-12	(Hoy & Flaherty, 1975; James, 1988)
<i>Typhlodromus pyri</i>	12.5-13.5	(Fitzgerald & Soloman, 1991)
<i>Amblyseius potentillae</i>	14.5	(van Houten & Veenendaal, 1990)
<i>Amblyseius cucumeris</i>	12.45	(Morewood and Gilkeson, 1991)
<i>Neoseiulus fallacis</i>	11.75-12	(Rock <i>et al.</i> , 1971)

1.7.2 Maintenance of the diapausing state

The key hormones involved in the regulation of diapause include juvenile hormone (JH), diapause hormone (DH), and prothoracicotropic hormone (Gullan & Cranston, 2005). Prothoracicotropic hormone stimulates the prothoracic glands to produce ecdysteroids that are required to promote development. Larval and pupal diapauses

are often regulated by an interruption of this connection, either by preventing release of prothoracicotropic hormone from the brain or by failure of the prothoracic glands to respond to prothoracicotropic hormone (Denlinger 2002). The duration and intensity of the diapause state are specific to each species and the response to external stimuli during the diapause state varies in type and intensity (Tauber *et al.*, 1986) The rate of entry into diapause is influenced by the rate of change of photoperiod and temperature and the relationship between conditions and the intensity of the diapause state is proportional. In general, as conditions become more favourable for example, during late winter/early spring, the diapause state starts to decrease (Veerman 1992).

1.7.3 Diapause termination

Termination of diapause requires certain specific developmental stages to occur and usually in a very specific order (Veerman 1992). Diapause does not necessarily terminate as soon as favourable conditions return, often a number of day degrees must be accumulated before diapause can be exited (Mansingh 1971). For example, the flesh fly *Sarcophaga bullata* (Parker) (Diptera: Sarcophagidae) transfer from the diapause state to an intermediary 'post-diapause' developmental stage when environmental conditions become more favourable before exiting diapause entirely. Physiologically these two stages appear identical but at the molecular level they are distinguishable (see Denlinger 2002 for review). Some species do not have a specific termination stimulus for example, diapause ends in mid winter, but post-diapause development cannot occur because it is still too cold. It has also been found that after diapause termination certain physiological characteristics such as cold hardiness and drought tolerance are reduced (Broufas & Koveos, 2001).

1.8 Physiological behaviour at low temperatures

To survive at both high and low temperatures, insects must maintain their body temperature (T_b) within a range bounded by their tolerable maximum and minimum limits. Insect movement becomes progressively slower and less coordinated as T_b is reduced and the animals ultimately enter cold torpor, characterised by complete immobility. At the opposite extreme, as T_b is raised above normal levels, movements initially become increasingly rapid; this phase is followed by reduced coordination at higher T_b and finally by paralysis or heat torpor (Whitman 1987).

1.8.1 Cold Torpor

Direct transfer of organisms from their rearing temperature to low temperatures, and then to potentially injurious temperatures, while useful in extending our understanding of the physiological basis of low temperature mortality, can be misleading as most studies subject organisms to cooling rates and thermal extremes that would rarely, if ever, occur in nature. In insects and other poikilotherms, less severe chilling, and therefore more ecologically relevant exposure, often induces more subtle, yet significant deleterious effects (Hazell *et al.*, 2008). For instance, when chilled to moderate temperatures, well above those which directly cause death, many organisms enter a state of cold torpor. At or below the temperature at which this occurs (the critical thermal minimum or CT_{min}), they are unable to seek refugia or food, or to avoid predation (Kelty & Lee 1999). This torpor or chill coma can be assessed for a species by gradually lowering the temperature of the insect's environment and closely observing their behaviour. The chill coma temperature is the temperature at the insect becomes immobilised by the cold (Mellanby 1939). At a

critical temperature, walking will cease and at the CT_{min} , all body and appendage movements will cease and if upturned, the insect will be unable to right itself. Prolonged exposure to this temperature may cause an accumulation of deleterious effects of cold (Zachariassen 1985). Traditional methods for measuring the CT_{min} and chill coma of small insects has involved a vertical glass tube and lowering the temperature results in individuals losing muscle control and therefore falling from the edge and out of the bottom of the tube (Huey *et al.*, 1992).

1.8.2 Chill coma recovery

Following exposure to the CT_{min} temperature an insect may take a period of time and gradual warming before it will fully recover from the immobility constraints of the CT_{min} . The temperature at which the insect can move its appendages and then begin to walk freely is known as the 'Chill Coma Recovery' temperature (Hazel *et al.*, 2008).

1.8.3 Motility and ability to predate

It is suggested that the most important aspect of insect predator-prey dynamics is the difference between the lower temperature thresholds of predator and prey (Dixon 2000). If an insect's lower thermal threshold is higher than that of its prey it is unlikely to have much influence in controlling prey numbers at temperatures approaching these thresholds. As thermal thresholds are likely to be subject to natural selection (Campbell *et al.*, 1974) it is relevant to ask why should the lower thermal thresholds be lower than that of their prey species. In the case of non-native biological control agents this may simply be due to different strains of the prey species

originating in different geographic ranges and hence being selectively adapted for life in those conditions (Gaston 2009).

In matching biological control agents to target prey species and locations in which to act, it would seem sensible to acquire data on the thermal thresholds to predict the efficacy of the control species. Knowledge of thermal tolerance is crucial to our understanding of ecology, evolution, and physiology because these traits play an important role in determining species range distributions (Hazell *et al.*, 2008; Bryant *et al.*, 1997) and how they may be altered by climate change (Parmesan 1996; Walther *et al.*, 2002). Thermal tolerance traits are also important determinants of the likelihood that a species will become invasive (Slabber *et al.*, 2007; Ward & Masters 2007).

Investigation into the motility and predatory activity of species with potential as biocontrol agents at different temperatures can provide an insight into their efficacy against target prey. It is clearly beneficial for a control agent to be more active than its prey across a range of temperatures, and to remain active at a temperature at which the prey have become immobile. A number of techniques have been developed to investigate activity thresholds in arthropods such as heat and chill coma and chill coma recovery. These methods involve the observation of the 'righting response' of individual organisms (e.g. Lutterschmidt & Hutchison 1997; Gilbert *et al.*, 2001). This approach usually involves removing the subjects from a temperature controlled environment (such as a water bath) at regular intervals, turning the species onto their backs and monitoring their ability to right themselves (see Castaneda *et al.*, 2005). As well as being laborious and subject to error, there has been concern that these methods

result in disturbances which might affect both the body temperature and physiological status of the subjects and subsequently the trait being observed. A new technique for studying activity thresholds was recently described by Hazel *et al.*, (2008) in which multiple specimens can be cooled in a temperature controlled arena with the behaviour of the organisms recovered by video capture technology.

The ability of a predator to control prey numbers is influenced by different factors, including behavioural patterns in response to its own densities, developmental response, preference, utilization of alternative prey and intraguild interaction (Lester & Harmsen 2002). The functional response describes how the consumption rate of individual consumers changes with respect to resource density (Solomon 1949). However, the term behavioural response may be more appropriate because the response describes the hunting and attack behaviour of the consumer. There are three main predator responses to prey: Type I (linear) response in which the attack rate of the individual consumer increases linearly with prey density but then suddenly reaches a constant value when the consumer is satiated; Type II (curved) response in which the attack rate increases at a decreasing rate with prey density until it becomes constant at satiation; Type III (sigmoid) functional response in which the attack rate accelerates at first and then decelerates towards satiation. A type II response is typical of predators that specialise on one or a few prey whereas a type III response is typical of generalist natural enemies which readily switch from one food species to another (Real 1977). Holling's 'Disk' equation (1959) defines the number of prey attacked by a predator which instantaneously assimilates its prey by:

$$H_a = aH$$

where H_a is the number of prey attacked per predator per unit of time, H is prey density (assumed constant over the hunting period) and a is the rate of attack. The time taken by predators that have to handle, kill and devour its prey before it can search for another is called the "handling time". If the predator requires t_a time units to handle its prey then, of the total time T exposed to prey, it will spend:

$$T - t_a H_a$$

time actually searching for prey. Thus, the proportion of the total time spent in searching for prey is:

$$(T - t_a H_a)/T$$

To date, very little research has been done to study the predatory behaviour of biological control agents in response to their prey, in relation to temperature (Kim *et al.*, 2009; Simonsen *et al.*, 2009). However, the benefits in doing so would be to produce a suite of control species with specified activity levels that could be matched directly to suit the particular growing conditions of the crop and the activity thresholds of the target prey.

1.9 Phytoseiid mites

Phytoseiid mites (Acarina: Phytoseiidae) are small, free-living, terrestrial arachnids belonging to the order Mesostigmata. Anatomically, they have eight legs and the body is divided into two regions: the gnathosoma (jaw area where the chelicerae are located) and the idiosoma (distinct body on which the legs are attached). Phytoseiid mites are distinct in that the males exhibit a spermatodactyl on the chelicerae. Their life cycle has five developmental stages: egg, larva, protonymph, deutonymph and adult, where the larval stage has only six legs (Woolley 1988).

Phytoseiid mites have received global attention since the 1950s when it became clear that they have economic importance as natural predators of phytophagous mites and small insects, and therefore are useful in the biological and integrated control of crop pests (Swirskii & Amitai 1997). Most early studies focused on the ability of these predaceous mites to rapidly increase and overcome outbreak populations of spider mites (Tetranychidae) (McMurtry & Croft 1997). Predatory mites of the family Phytoseiidae are now valued with growers worldwide, as natural enemies that provide effective pest control in greenhouses and on agricultural crops (Bjornson 2008).

This study is concerned with two species of Phytoseiid mite: *Amblyseius swirskii* and *Phytoseiulus longipes*. The former is categorised as having a type III life style as classified by McMurtry & Croft (1997). These are generalist predators known to utilise several different prey species such as Tetranychid and Eriophyid mites and potential insect prey species including whitefly, mealybugs, and scale crawlers. Also, most type III Phytoseiid mites can reproduce on a diet of pollen alone. In comparison *P. longipes* has a type I life style. These mites are specialised predators of *Tetranychus* species and are unable to reproduce and develop on diets of other prey species and will not survive for long on a diet of pollen alone.

1.9.1 *Amblyseius swirskii*

Amblyseius swirskii (Athias-Henriot, 1962) is a phytoseiid mite originating from the eastern Mediterranean region, i.e. Israel, Italy, Cyprus and Egypt. It is found on a wide variety of host plants including crops such as apples, grapes, vegetables, citrus fruits and cotton. In some publications, it is referred to as *Typhlodromips swirskii* due to a taxonomic debate as to the genus of this mite.

A. swirskii is a generalist predator known to feed on other mites, whitefly, thrips, lepidopteran eggs and pollen (Teich 1966, Swirskii *et al.*, 1967, Ragusa & Swirskii 1975, Hoda *et al.*, 1986). Although *A. swirskii* can take advantage of a relatively wide variety of food sources, Momen and El-Saway (1993) found that when fed either tetranychid mites, eriophyid mites or pollen, development was faster and reproduction higher on a diet of mites than on pollen alone. *A. swirskii* will also feed readily on honeydew, although development on this diet is retarded and oviposition stops (Ragusa & Swirskii 1977). Honeydew may therefore serve as a beneficial supplement when other prey is available or as a survival diet when adequate food is limited. It has been hypothesised that the generalist feeding habit in the Phytoseiidae is the ancestral state. The family may have evolved with the Ascidae from a common ancestor that fed on small arthropods or micro-organisms in protected habitats such as bark crevices, mosses and leaf litter. Subsequently, they radiated to foliage habitats unoccupied by other predatory mites (McMurty & Croft 1997).

Experiments by El-Tawab *et al.*, (1982) on the effect of temperature and photoperiod on *A. swirskii*'s development found that an increase in temperature produced more rapid development, reduced adult longevity, and increased feeding capacity and fecundity; whereas, increasing the photoperiod results in lower prey consumption and an associated drop in fecundity. This phenomenon is explained by the tendency of the mite to occur on the lower leaf surface of the host plant. These results would be expected in a poikilothermic organism native to a Mediterranean climate.

El-Laithy and Fouly (1992) conducted a comparison between *A. swirskii* and *A. scutalis*, both of which in their native environments are found widely distributed and

in high abundances in association with the two spotted spider mite, *Tetranychus urticae*, suggesting some potential for use as biological control agents. They found the oviposition period of *A. swirskii* to be almost double that of *A. scutalis* (22.3 days compared to 12.9). The longevity for both mites was similar, but *A. swirskii* showed a much higher fecundity and prey consumption than *A. scutalis*. They concluded that *A. swirskii* had considerable potential as an agent against spider mites but is not as active as *Phytoseiulus persimilis*, another predatory mite, widely used and very effective against spider mite in glasshouses. The evaluation of a predator as a biological control agent depends on its reproductive rate and egg production which is positively correlated to prey consumption during the oviposition period. It is thought that prey consumption forms a ratio of about 70% of the biomass of deposited eggs (Sabelis 1981).

There have been several studies carried out since the initial discovery that *A. swirskii* has potential as a biological control agent. Most of this work has concentrated on development and predation against the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). This species occurs worldwide and causes considerable yield loss and economic injury in many crop varieties. Due to its polphagy and resistance to many chemical insecticides, *B. tabaci* has rapidly become a serious crop pest (Cahill *et al.*, 1995). Nomikou *et al.*, (2001) found *A. swirskii* to be an effective predator of the immature stages of whitefly. *A. swirskii* will also predate on the Japanese bayberry whitefly *Parabemisia myricae* which causes serious damage to citrus crops (Wysoki & Cohen 1983).

The majority of the work to study the efficacy of *A. swirskii* as a biological control agent has been carried out by biological control companies with an interest to rear and market predatory mites for commercial use. These studies have not been subject to rigorous peer review but carried out for the purpose of pursuing licence applications for release of this mite in non-native countries where it may be successful against many crop pests. Studies have shown that on sweet pepper and cucumber, *A. swirskii* can generate high population densities and effectively control whitefly and spider mite. However, on aubergine and tomato the population diminishes rapidly. This is thought to be as a result of the dense hairs on the leaves of these plants making it difficult for the mites to move about and locate prey. *Amblyseius swirskii* is also less effective at combating dense ‘hot-spots’ of spider mite. On strawberry plants, *A. swirskii* will predate strawberry mites *Tarsonemus pallidus* and *T. fragariae*. *A. swirskii* can establish on roses but the population build up is slow and gives only some control of whitefly and spider mites (personal correspondence from Dr. Mark Whittaker, Koppert UK Ltd). Recently *A. swirskii* has been shown to act effectively in an IPM biological control programme with *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae), to control whitefly on sweet pepper crops (Calvo *et al.*, 2009). Work is being carried out to measure the efficacy of *A. swirskii* on other horticultural plants such as gerbera, chrysanthemum and carnation.

1.9.2 *Phytoseiulus longipes*

Phytoseiulus longipes is a Phytoseiid mite with a geographical distribution limited to the Southern Hemisphere. It occurs primarily in South America and southern Africa between latitudes of 15° and 35°S (Takahashi & Chant 1993; Moraes *et al.*, 2004) In South America it has been reported in Argentina (Herrero *et al.*, 1990), Chile

(Gonzalez & Schuster 1962) and Brazil (Furtado *et al.*, 2006). It has been targeted as a potential biological control agent because it is often found in close proximity to the two spotted spider mite *Tetranychus urticae* and life functions of all *Phytoseiulus* species are carried out exclusively in spider mite colonies (McMurty & Croft, 1997). Mites of the genus *Phytoseiulus* are considered specialised predators and their reproduction seems dependent on Tetranychid mites as prey and reproductive rates are usually higher on prey of the genus *Tetranychus* than on species of other genera (Ashihara *et al.*, 1978, Badii and McMurty, 1984). *P. longipes* is observed to walk well on infested leaves and does not seem hampered by the heavy webbing produced by *T. urticae* or by leaf appendages, such as tomato trichomes (Furtado *et al.* 2006). A morphological characteristic of the genus *Phytoseiulus* is the presence of long setae in the medial position of the dorsal shield (Sabelis & Bakker 1992). It may be these setae that enable the predators to move about more easily in the prey webbing. The eggs are also laid on the webbing and all immature stages are able to move unhindered through the webbing. *Phytoseiulus* species have probably the highest capacity for population increase in the whole Phytoseiidae family of mites (Zhang 1995; Kazak *et al.*, 2008). The larvae of *P. longipes* do not feed and do not require food in order to moult to the protonymph stage (Takahashi & Chant 1992). The percentage development per day and survival of protonymphs and deutonymphs at different densities of spider mite eggs exhibit a curvilinear rise to a plateau (Badii *et al.*, 1999). This indicates that as prey density increases, the searching efficiency of immature stages is not affected by predator hunger level or rise in handling time of the prey. Badii and McMurty (1988) stated that *P. longipes* is an optimal forager. This means it spends more time and hence energy, in prey patches where availability is greatest.

All of these traits in the biology of *P. longipes* suggest that it could be very useful as a biological control agent, particularly of spider mites, especially as it can tolerate temperatures of up to 38°C (100°F) when humidity is high and also low relative humidities (down to 40%) at 21°C (70°F) (Henn *et al.*, 1995).

1.10 Aims of study

The main aims of this project are to:

1. Investigate the thermal biology of *Amblyseius swirskii* by studying its development at different temperatures, cold tolerance and field survival. A thermal screening protocol developed by Hart *et al.*, (2002a) and further refined by other members of the Arthropod Ecophysiology Group at the University of Birmingham (Hatherly *et al.*, 2005) will be used and the data analysed to create a risk assessment of the likelihood of establishment for this species if it were used in UK glasshouses as a biological control agent.
2. Investigate the thermal biology of a second species of predatory mite *Phytoseiulus longipes*, using the same protocols as above to assess its establishment potential in the UK. This species has been proposed as a biocontrol agent of spider mites on tomato plants and other vegetables at higher temperatures and humidities.

3. Investigate the activity thresholds for both species of mite including CT_{\min} , chill coma, recovery from low temperature exposure, walking speeds and predatory abilities at a range of temperatures, to further inform the likely efficacy value of *A. swirskii* and *P. longipes* as biological control agents in glasshouse environments.

4. Carry out comparisons with other non-native biological control agents previously subjected to the same methods and analyses.

CHAPTER 2

General Methods

2.1 Introduction

This chapter gives details of the culturing techniques for rearing populations of *Amblyseius swirskii* and *Phytoseiulus longipes* in the laboratory and provides an overview of the methods used to quantify arthropod cold tolerance and physiological behaviour at low temperatures.

2.2 Culturing mite populations

The culturing techniques were developed to achieve two objectives: firstly, to ensure the long term viability of the stock culture, and secondly, to provide the required numbers of individuals in synchronised cohorts for the planned experiments. Temperatures throughout all experiments were recorded using Tinytalk® dataloggers (Gemini, UK) and light regimes were monitored using HOBO Onset(R) light loggers (Tempcon, UK).

2.2.1 *Tetranychus urticae* culture

Tetranychus urticae were taken from an established culture maintained by the Arthropod Ecophysiology Group at the University of Birmingham. The *T. urticae* population was reared on Dwarf French beans, *Phaseolus vulgaris* (Linnaeus)

(Fabaceae) in a controlled environmental room at 25°C, 18:6 LD and a relative humidity of approximately 50-70%. At any one time the population existed on 48 healthy plants and a supply of 8 young 20cm tall plants were added to the *T. urticae* culture twice weekly. Heavily webbed and infested leaves were removed from the older plants and placed onto the younger plants to encourage transfer of the mites on to the new plants. Unused and wilting plants were removed from the culture to reduce intermittent infestation of the plants by species of thrips. Occasional infestation with thrip pests was found to be largely unavoidable but removal of the older plants kept the possibility to a minimum. Under the culturing conditions, the developmental time of thrips was slightly longer than that of *T. urticae*, hence removal of older plants usually contained the problem (Larentzaki *et al.*, 2007). The bean plants were grown in an isolated temperature controlled growth room (20°C, 18:6 LD) to ensure a clean, healthy stock of plants, avoid premature infestation by mites and to ensure that no other pest species established on them.

2.2.2 *Amblyseius swirskii* culture

Amblyseius swirskii were supplied by Koppert Biological Systems, Netherlands, and reared under quarantine conditions at the University of Birmingham, U.K.. The mites were transported in a plastic bottle filled with a mixture of cereal bran, dust mites *Dermatophagoides pteronyssinus* (Trouessart) (Acari: Pyroglyphidae) as prey, and over 1000 *A. swirskii* individuals in each container. Mites had to be extracted from the mixture using a fine artist's paintbrush; *A. swirskii* could be encouraged to move away from the mixture by addition of water droplets.

The initial mite population was reared at 25°C, L18:D6 and a humidity of 70-75% (Baier 1991). These mites were used in experiments as the non-acclimated treatment. Mites classed as acclimated were held at a temperature of 10°C for 3 days prior to experiments, with the same LD regime and humidity as for the non-acclimated population. This acclimation regime was selected on the basis of previous studies with non-native (to the UK) biological control agents originating from tropical, semi-tropical and Mediterranean climates (Hart *et al.*, 2002a, b; Hatherly *et al.*, 2004). In essence, the acclimation treatment is not intended to produce fully acclimated mites, but rather, to detect an acclimation ability. For species originating from 'warm climates', an extended acclimation regime at 5° or 0°C can be deleterious or lethal.

The culturing method was adopted from Hatherly *et al.*, (2004) and involved a plastic box, the lid of which had ventilation holes cut out and then covered with gauze. The arrangement inside the box consisted of a sponge block, semi-submerged in water and upon which a black ceramic tile (13×17×2cm) was mounted. The mites were confined to the black tile using OecoTak® A5, a non-drip insect adhesive that does not set or dry. A thin layer was piped around the edge of the tile to create a physical barrier for the mites that they did not attempt to cross. A water source was applied to the rearing stage by soaking a 0.5cm wide, 10cm long piece of cotton wool and lying one end across the tile and the other down into the water. Strands of cotton thread, 2cm in length, were frayed at each end and placed on the tile to act as oviposition sites. Each tile provided enough space to rear 250 predatory mites. Depending on the number required, eggs were collected either daily or every 2 days and transferred to an egg stage, a smaller but otherwise identical stage to that used for rearing adult mites.

Isolating the eggs and larvae from the adults prevented cannibalism of the immature stages.

Food provisioning was dependent on the numbers of mites and age group needed for each experiment. *Amblyseius swirskii* were fed on both *T. urticae* and maize pollen (*Zea mays*); *A. swirskii* can be reared solely on a diet of pollen but oviposition rates are usually reduced (Momen & El-Saway 1993). *Tetranychus urticae* were cultured on Dwarf French beans (*Phaseolus vulgaris*) and were brushed from the leaves, onto the *A. swirskii* stages at least every 2 days. New culture stages were set up every 4 weeks when debris made it difficult to see the mites and eggs clearly.

2.2.3 *Phytoseiulus longipes* culture

Phytoseiulus longipes were obtained from Koppert Biological Systems, Netherlands, and reared under quarantine conditions at the University of Birmingham, U.K. The mites were transported in batches within Petri dishes. The Petri dish was half filled with agar jelly and a cucumber leaf (*Cucumis sativus*) was placed into the jelly with the underside facing up. The leaf was infested with *T. urticae* eggs as a food source, and this arrangement supported 10-15 adult *P. longipes* during transportation.

Phytoseiulus longipes is a specialist predator of *T. urticae* and carry out all of their life functions exclusively in spider mite colonies (McMurty & Croft 1997), Necessitating that the rearing of *P. longipes* had to be carried out in very close association with *T. urticae*. The rearing chamber consisted of a plastic box with ventilation holes cut out of the lid. The box contained a sponge block, semi-submerged in water and upon which a black ceramic tile (13×17×2cm) was mounted.

The tile was covered in a large square of filter paper the edges of which draped over the edge of the tile and into the water. A fresh cucumber leaf was soaked in water and placed underside up on the filter paper. The surface was then blotted dry and an excess of *T. urticae* were brushed onto the leaf. A thin layer of OecoTak® A5 was piped on to the filter paper following the edge of the tile to create a physical barrier for the mites which they did not attempt to cross. The chamber was then left for 24 h to allow time for *T. urticae* to establish, produce webbing and oviposit. Following this time, approximately 50 adult *P. longipes* were introduced to the leaf. The entire chamber was then placed into a larger plastic box (30x20x15cm), filled to a depth of 5 cm with salt water, and a solid plastic lid was placed on the top. This arrangement enabled a high relative humidity to be maintained, which is essential for successful predator egg hatch. Numerous attempts were made to discover the optimal rearing conditions for *P. longipes* and finally conditions of 28°C, 18:6 LD cycle and approximately 80-90% relative humidity were found to be most favourable for egg hatch (personal communication Yvonne van Houten, Koppert, Netherlands, 2007). After a further 24-48 h the adult *P. longipes* were removed and placed on to a fresh leaf chamber. This was found necessary as *P. longipes* are highly cannibalistic and will predate on the immature stages of their own species if the numbers of their target prey falls below a certain density. This rearing technique was used to generate high numbers of *P. longipes* for use in experiments.

A second rearing technique was used to create a stock culture of *P. longipes* that was easier to maintain but produced fewer individuals. Old leaves from the rearing tiles were placed in a small plastic tray (10x8x4cm) with squares of plastic webbing and fresh cucumber leaves placed on top in alternating layers. This system provided a

sheltered overlapping leaf arrangement and the plastic webbing allowed for air spaces between the leaf layers. The mites on the lower old leaves gradually moved up to the younger top leaves onto which to which a fresh supply of *T. urticae* were added every 2 days. The plastic tray was mounted on a sponge block in a plastic box half filled with water. The box was ventilated with holes cut into the lid and covered in gauze. This entire chamber was placed into another larger plastic box half filled with salt water to maintain the required high relative humidity. This culturing technique provided a close to self sufficient colony of *T. urticae* and *P. longipes*. The oldest, dried up leaves were removed every week and replaced by leaves from the rearing tiles and the trays were replenished with *T. urticae* once a week.

2.3 Studying individual mites

In order to study individuals of *A. swirskii*, a black ceramic tile was divided into a 6×7 grid using thin layers of OecoTak®. This created 42 (2cm²) ‘arenas’ where an individual mite could be contained and studied, and kept separate from neighbouring arenas. As before, the tiles were placed on sponge blocks, in water-filled boxes with ventilated lids. A water source was provided for each mite by the addition of a single water droplet to the arena twice a day. Food was provided in the form of maize pollen.

This method was modified for studying individual *P. longipes*. The tile was covered with a moist sheet of filter paper which draped either side into the water and the Oekotak® barrier was then placed on top of the paper to divide it into 42 arenas. A 1cm² of cucumber leaf was moistened and placed underside up into each arena and a

supply of *T. urticae* were brushed onto each leaf section daily; the filter paper was kept moist by adding a single water droplet every day.

CHAPTER 3

Thermal biology of *Amblyseius swirskii*

3.1 Introduction

Amblyseius swirskii (Athias-Henriot 1962) is a phytoseiid mite originating from the eastern Mediterranean region. It is a generalist predator known to feed on other mites, whitefly, thrips, lepidopteran eggs and pollen (Teich 1966, Swirskii *et al.*, 1967, Ragusa & Swirskii 1975, Hoda *et al.*, 1986). *Amblyseius swirskii* will also feed on Honeydew in the absence of other food (Ragusa & Swirskii 1977). It is thought that the generalist feeding habit in the Phytoseiidae is the ancestral state (McMurty & Croft 1997). In the wild it is found on a wide variety of host plants including crops such as apples, grapes, vegetables, citrus fruits and cotton. It has been targeted as a potential biological control agent because in its native environment, *A. swirskii* is found widely distributed and in high abundances in association with the two spotted spider mite, *Tetranychus urticae* a highly persistent, phytophagous crop pest.

In comparison to other closely related mites *A. swirskii* has an oviposition rate of almost double that of *A. scutalis* (22.3 days compared to 12.9). *Amblyseius swirskii* also demonstrates a much higher fecundity and prey consumption than *A. scutalis* (El-Laithy & Fouly 1992). *Amblyseius swirskii* is of considerable potential as a biological control agent against spider mites but is not as active as *Phytoseiulus persimilis*, another predatory mite that is widely used and very effective against spider mite in glasshouses. The evaluation of a predator as a biological control agent

depends on its reproductive rate and egg production which is positively correlated to prey consumption during the oviposition period. (Sabelis 1981).

The majority of the work to study the efficacy of *A. swirskii* as a biological control agent has been carried out by biological control companies with an interest to rear and market predatory mites for commercial use. Studies have shown that on sweet pepper and cucumber, *A. swirskii* can generate high population densities and effectively control whitefly and spider mite. *Amblyseius swirskii* is less effective at combating dense ‘hot-spots’ of spider mite where webbing is heavy and mobility of the predatory mite is restricted (personal correspondence from Dr. Mark Whittaker Koppert UK Ltd).

3.2 Aims

The aims of this chapter were to:

1. Determine the developmental threshold of *A. swirskii* and predict its potential voltinism in the UK.
2. Investigate any possible diapause response to low temperature.
3. Investigate laboratory low temperature biology of *A. swirskii*.
4. Undertake field survival experiments and make comparisons of relative survival with laboratory data.

3.3 Methods

Mites were reared in populations of 250 individuals on each rearing stage at 25°C, 18:6 LD and approximately 70-75% relative humidity. Non-acclimated mites used in the following experiments were kept under these conditions; acclimated mites were subjected to 3 days at 10°C. Initially an acclimation period of 7 days was selected because this has been shown to be an adequate time for the full development of an acclimation response in critical thermal limits in a variety of species (Klok & Chown 2003; Terblanche *et al.*, 2006). However it was rapidly recognised that durations longer than 3 days at 10°C was deleterious to a high proportion of the mites sampled and lethal to a few. All mites were fed with *T. urticae* and maize pollen (*Zea mays*) every 2 days and had continuous access to a water source.

Detecting insect survival after any kind of stress induction can be difficult to quantify. According to Baust and Rojas (1985), survival after a cold hardiness experiment is best assessed by the ability of the individual to reproduce. However, due to the large sample sizes used in these experiments and the limited rearing resources, this was not feasible. In all low temperature laboratory and field experiments, mites were considered alive if they responded to (walked away from) a tactile stimulus applied directly after the low temperature exposure, and after a further 24 and 48 h..

3.3.1 Developmental time

Developmental times were studied in temperature-controlled incubators at 10°, 15°, 18°, 20°, 23°, 25°, 27°, 30° and 35°C, L18:D6 and 70-75% r.h. At each temperature there were two tiles containing in total 84 mites. The developmental times of the first

50 mites to complete their development at each temperature were recorded. Each arena was checked every 12h to record the date of egg hatch and the time taken (days) for development to larval, protonymph, deutonymph and adult stages. Development to each successive stage was recognised by the appearance of the moulted cast skins. Water was provided every 12 h in the form of a small water droplet pipetted onto the arena. A light sprinkling of pollen was provided as the only food source, based on the assumption that pollen is sufficient for *A. swirskii* to complete its development (Swirskii *et al.*, 1967), and due to the fact that feeding with *T. urticae* causes the arenas to 'dirty' with debris, which makes it more difficult to see the nymphal cast skins shed at each moult. Developmental times were log transformed and the differences between temperatures and life stages analysed by Two-way ANOVA and then by pairwise comparisons using Tukey's honest significant difference (HSD) method.

The data were also analysed by a simple linear regression between temperature and rate of developmental (Draper & Smith 1981) from which the developmental threshold temperature was extrapolated. The day degree requirement of *A. swirskii* was determined by taking the reciprocal of the slope (Campbell *et al.*, 1974). These data were used in conjunction with temperature recordings taken by the School of Geography, Earth and Environmental Sciences at the University of Birmingham to calculate the available day degrees each year and from this, to estimate the number of generations of *A. swirskii* possible per year under outdoor conditions.

3.3.2 Diapause

Induction of diapause was investigated by lowering the rearing temperature to 18°C and changing the light regime to 6:18 LD. These conditions have previously been shown to induce diapause in *Neoseiulus californicus* (Jolly 2000) and are a commonly used diapause inducing regime (Hart *et al.*, 2002). 100 eggs were collected and left to hatch and develop to the adult stage. Two further days were allocated to allow time for mating. Females were then isolated onto individual arenas and provisioned with a water source, food and an oviposition site. The females were examined every 24 h and the proportion of females ovipositing and number of eggs laid by each female was recorded. After 2 weeks all mites were transferred back to their original rearing conditions (25°C, 18:6 LD) and oviposition was continually monitored. Mites which did not oviposit in the diapause regime but then resumed oviposition in the original rearing conditions, after a lag period of 4 days (Jolly 2000), were considered to have been in diapause. Any eggs laid by these first generation females under the diapause conditions were kept in the same regime, reared to adulthood (becoming the second generation) and monitored for oviposition. Data were analysed by One-way ANOVA.

3.3.3 Supercooling points

Supercooling points (SCP) of adult mites were measured using PicoLog data acquisition software. Individual mites were attached to single T-type thermocouple probes (Bale *et al.*, 1984) using a small amount of Vaseline grease. The probes were connected to a multichannel temperature terminal panel (TC-08) which relays information to the PC software. Temperature was continuously monitored and recorded at 1 s intervals. The mites were held individually within size 3 Beem capsules (1 cm) inside glass boiling tubes in an antifreeze solution in a low

temperature programmable alcohol bath (Haake F8-C50, Thermo Haake, Germany). The temperature was lowered from 25° to -25°C at a rate of 1°C min⁻¹. The data were illustrated as a continuous graph of decreasing temperature over time. The software recorded a peak (exotherm) in the temperature profile at the point at which each mite froze, usually referred to as its ‘supercooling point’ (SCP). The SCPs of acclimated and non-acclimated adult mites were recorded by this method. At the end of each experiment the capsules were opened and each mite was examined to determine if any individual had survived the freezing event.

Larval mites were too small to be securely attached to the thermocouple probes and the probes were not sufficiently sensitive to detect the smaller ‘freezing exotherms’ produced by the larval mites. The SCPs of larval *A. swirskii* were therefore measured by a differential scanning calorimeter (DSC) at the British Antarctic Survey, Cambridge. This method involved placing 5 mites of each treatment into a small aluminium pan which is then sealed and placed inside the DSC. The mites were cooled at a rate of 1°C min⁻¹ to their SCP (onset of the freezing exotherm). The DSC software recorded the SCP of each individual in the pan, and multiple cooling runs were carried out to achieve the required sample sizes. The pans were re-opened at the end of each experiment to check for any survivors. The SCPs of acclimated and non-acclimated larvae were measured using this method.

3.3.4 Lower lethal temperatures

Acclimated and non-acclimated adult and larval *A. swirskii* were placed individually into size 3 Beem capsules along with a moist strip of filter paper (1 cm). Ten capsules were placed into a boiling tube, each sealed with a cotton wool bung and then held in

a rack suspended in an alcohol bath. A thermocouple probe was placed into an empty capsule in one of the tubes to monitor the temperature experienced by the mites. Five replicates of 10 mites of each treatment group were exposed at each temperature. The mites were cooled at a rate of $0.5^{\circ}\text{C min}^{-1}$ from 20°C to a range of temperatures between 5° and -15°C . Acclimated mites were cooled from 10°C (the acclimation temperature). Mites were held at the required exposure temperature for 5 min and then re-warmed to 20°C at the same rate. A control sample of 50 mites was placed in Beem capsules and held at 20°C for 75 min, equivalent to the maximum time of exposure for the treated groups. After the required exposures, the mites were then removed from the capsules and placed onto individual recovery arenas with food and water provided. Recovery tiles were held at 20°C 18:6 LD and survival was assessed immediately after exposure and then after a further 24 and 48 h. All surviving individuals were transferred to individual arenas at 25°C 18:6 LD and provisioned with water and food. The arenas were monitored to determine if the larvae would develop through to adulthood and the adult females would oviposit as normal.

The results were log transformed and assessed using Probit analysis (Finney, 1971) to estimate the lethal temperatures at which 10, 50 and 90% of the sample population are killed ($\text{LTemp}_{10, 50, 90}$). Negative values were ignored as required by Probit and the analysis was run in MINITAB v 14.0.

3.3.5 Lower lethal times

Acclimated and non-acclimated adult and larval *A. swirskii* were placed individually into size 3 Beem capsules along with a moist strip of filter paper (1cm). Batches of ten capsules were placed into glass boiling tubes and sealed with a cotton wool bung.

The tubes were held in a rack and stood in a plastic box (20x15x10cm) half filled with antifreeze solution (20%) to act as a thermal buffer against any fluctuations in the surrounding environmental temperature.

This experiment was run at 5°, 0° and -5°C. To avoid the possible effects of cold shock, the mites were held at 10°C for 30 min before being placed in an incubator for the 5°C exposure. For the experiment at 0°C mites were held at 10°C for 30 min and then at 5°C for a further 15 min before being placed in an incubator for the 0°C exposure. For the -5°C exposure, mites were held at 10°C for 30 min and then at 5° and 0°C for 15 min each before being transferred to the -5°C alcohol bath. Five replicates of ten capsules of each treatment group were taken from the three exposure temperatures at a variety of time intervals so as to determine the progress in mortality with increasing periods of exposure.

At end of each exposure period, mites were held at 0° and 5° (for 15 min) and then at 10°C for 30 min to avoid possible mortality due to heat shock. Capsules were then opened, mites transferred to individual arenas at 20°C and survival was assessed immediately, 24 and 48 h after exposure.

In addition, a control population of 50 adult and 50 larval *A. swirskii* was placed at 5°C with water and food provided. The mites were monitored every day to ascertain the maximum survival time for individuals with food and water available.

3.3.6 Field exposures

Outdoor winter temperatures were tested on eight treatment groups: adults and larvae, acclimated and non-acclimated, fed and unfed. Adults had moulted from the deutonymph stage no more than 48 h previous to being put out into the field and the larvae were less than 12 h old.

Individual mites from each of the 8 treatment groups were placed separately into a 'field test vial'. This was constructed from a 1.5ml Eppendorf™ tube with a 0.5cm layer of agar (2%) in the base and a circular piece of filter paper resting on top of the agar. The agar provided a moisture source for the mite and the filter paper prevented the mite having direct contact with the agar. The lid of each Eppendorf™ tube was hole-punched for ventilation and then covered in 75µm muslin. The mites were exposed singly within each vial because of the difficulty of loading multiple mites into one vial with the risk of escape. Also, *A. swirskii* are known to cannibalise each other (Rasmy *et al.*, 2004) and this would make it difficult to identify the mortality attributable to low temperature. The 'unfed' treatment was provided with no food. The 'fed' treatment included the addition of maize pollen. Food was provided to investigate whether, in the absence of diapause, field survival might be extended by the availability of food.

Twenty five vials, each containing an individual mite, of each treatment group were placed upright in a transparent plastic box. In total a box could hold 200 vials. The boxes were sealed apart from four ventilation holes covered with muslin in the box side. Enough vials to sample each treatment group at ten separate time intervals and to

take four replicates of ten individuals each time, were placed in the boxes. The boxes were put in a sheltered field location at the University of Birmingham. Each box was covered with a plastic tray to provide protection from direct sunlight and Tiny Talk™ data loggers (placed inside the box) were set to record the temperatures at 30 min intervals. At each sampling interval, 4 replicates of 10 vials from each treatment group were brought into the laboratory and held at 15°C for 1 h. After this time, the vials were opened and the surviving mites were transferred to recovery arenas. These recovery arenas were constructed in the same way as the tiles used for studying individual mites. One mite was placed into each arena, provided with pollen and a water droplet, and held at 20°C, 18:6 LD. Three records of survival were made: immediate after placing on the tiles and after a further 24 h and 48 h. Two field experiments were carried out, one in early winter (November-December 2005) and the second in late winter (February-March 2006). By selecting these times of year, it was envisaged that a range of temperatures would be experienced, and the exposures would give some indication as to whether escapes from glasshouses at different times in winter were more or less likely to survive.

The mortality data of field populations were transformed, tested for normality and analysed using a General Linear Model and pairwise comparisons were made between treatments using Tukey's HSD. A control sample of 40 non-acclimated adult and 40 non-acclimated larval mites were placed in individual vials and kept in an incubator at 25°C, 18:6 LD for one week to test for any deleterious effects of the experimental set up.

Short term field exposures were also carried out to investigate outdoor survival over 1 and 2 week periods throughout the winter months of 2006 and the spring and summer

months of 2007. It was hoped that these short field experiments would highlight any temperature regimes where 100% mortality would occur in all groups of *A. swirskii* and also conversely, which temperature regimes supported 100% survival.

Individual mites from 4 treatment groups (acclimated and non-acclimated adults and larvae), were placed separately into field test vials. Food was provided in all vials because the mini-trials were extended into the spring and summer season to investigate survival at higher temperatures, and it is envisaged that a lack of food at these temperatures would have a deleterious effect and contribute to mortality. Two boxes were placed in a sheltered field location at the University of Birmingham, each containing 40 vials of each mite group and a data logger. After each week of exposure, 4 replicates of 10 samples for each group were brought into the laboratory and the percentage mortality was assessed. The data from the short term field exposures were collated and analysed by Probit to estimate the temperatures required to cause 10, 50 and 90% field mortality of *A. swirskii*.

3.4 Results

3.4.1 Developmental time

At 10°C, 11% of individuals hatched after 10-15 days, but none of these moulted to protonymph and all were dead by day 30. At 12°C, 27% of individuals hatched after 7-17 days, and again, none moulted to protonymph and all were dead by day 28. At 35°C, 28% of individuals hatched, 80% of which moulted to protonymph but all died by day 5. The data from all of these temperatures were omitted from analyses on

development. Also at 30°C, the developmental time from egg to adult was longer than at 25° and 27°C and therefore these data were also omitted from the analysis to estimate the developmental threshold. The proportion of individual eggs that hatched at each temperature are shown in Figure 3.1. These results give a preliminary indication of the survival and developmental potential of *A. swirskii* at a variety of temperatures.

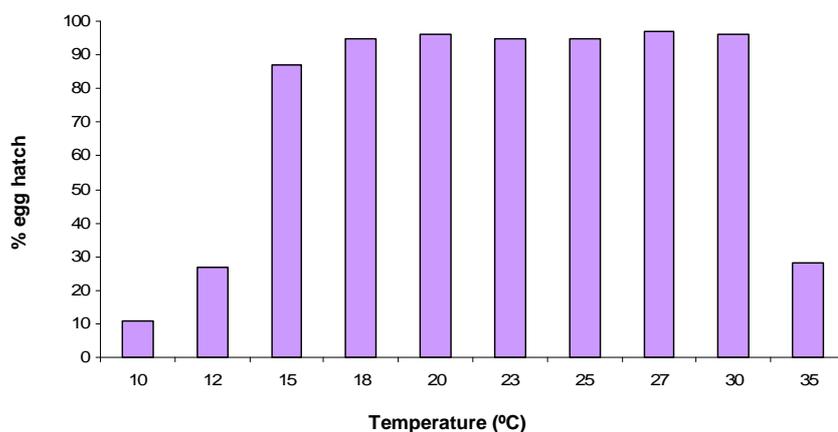


Figure 3.1: Percentage egg hatch of *Amblyseius swirskii* at temperatures ranging from 10 to 35°C (n = 84).

The developmental times for each life stage of *A. swirskii* at different temperatures are shown in Table 3.1. Between 15° and 27°C, the time taken to complete development to the adult stage decreased with temperature. Total developmental time from egg to adult at 15°C was significantly longer than at all other temperatures ($P < 0.01$). Similarly, the time to mature to the adult stage at 18° was longer than at 20°C ($P < 0.01$), longer at 20° than 23°C ($P < 0.01$) and longer at 23° than 25°C ($P < 0.01$). Development at 25° and 27°C were both faster than at 30°C ($P < 0.05$ and $P < 0.01$ respectively). Therefore, the optimum temperature for development in the present study was around 27°C.

When comparing developmental rate at each life stage, the time to egg hatch and time spent as larvae were significantly longer at 15°C ($P<0.01$) than at all other temperatures and shorter at 27°C ($P<0.05$) than at 30° and 25°C. There was no significant difference between time to egg hatch at 25°C compared to 30°C. In general, all developmental stages were significantly ($P<0.05$) shortened as the temperature increased from 15° to 27°C. In all cases, 27°C was the optimum temperature for development to the subsequent stage.

For the larval and deutonymph stages, these were shorter at 25° than at 30°C, but for the egg and protonymph stages there were no significant differences between development times at these temperatures. When comparing the time spent at each life stage, the larval stage at all temperatures was significantly the shorter than all other stages.

Table 3.1: Mean developmental time (days \pm SE) at each life stage of *Amblyseius swirskii* at temperatures between 15° and 30°C (n=84)

Temperature (°C)	Egg	Larvae	Protonymph	Deutonymph	Total time egg-adult
15	7.2 \pm 0.06	3.4 \pm 0.05	6.5 \pm 0.07	7.0 \pm 0.06	24.1 \pm 0.09
18	4.5 \pm 0.08	2.8 \pm 0.05	6.1 \pm 0.07	7.1 \pm 0.06	20.4 \pm 0.17
20	3.3 \pm 0.06	2.2 \pm 0.05	4.0 \pm 0.07	3.3 \pm 0.06	12.8 \pm 0.16
23	2.9 \pm 0.07	1.9 \pm 0.06	1.9 \pm 0.07	3.0 \pm 0.05	10.7 \pm 0.12
25	2.0 \pm 0.06	1.2 \pm 0.03	2.4 \pm 0.15	2.6 \pm 0.04	8.3 \pm 0.09
27	1.8 \pm 0.05	0.9 \pm 0.04	2.0 \pm 0.05	1.5 \pm 0.04	6.1 \pm 0.04
30	2.0 \pm 0.06	1.4 \pm 0.06	2.7 \pm 0.05	2.8 \pm 0.07	8.8 \pm 0.10

The developmental rate of *A. swirskii* is shown plotted against temperature in Figure 3.2. The temperature at which development can first occur is the lower developmental threshold. When estimated by simple linear regression, the threshold temperature for *A. swirskii* is 11.9°C. Developmental rate (1/days) and temperature forms an approximately linear relationship between 12° and 27°C.

Amblyseius swirskii requires a thermal budget (K) of 101 degree days above the threshold temperature to complete development from egg to adult. This is calculated from the linear regression and is the reciprocal of the slope of the line (1/0.0099).

The mean number of day degrees available each year to from 1987-2007 was calculated from the available temperature data as shown in Table 3.2. Alongside this information is the theoretical number of generations *A. swirskii* would be able to complete each year based on the number of available day degrees. Summer and winter have been arbitrarily separated to show the potential for population growth within each season. Between the beginning of April and the end of September the mean theoretical number of possible generations was 4.7 compared to 0.17 during the winter period i.e. the mite would not be able to complete one generation in winter.

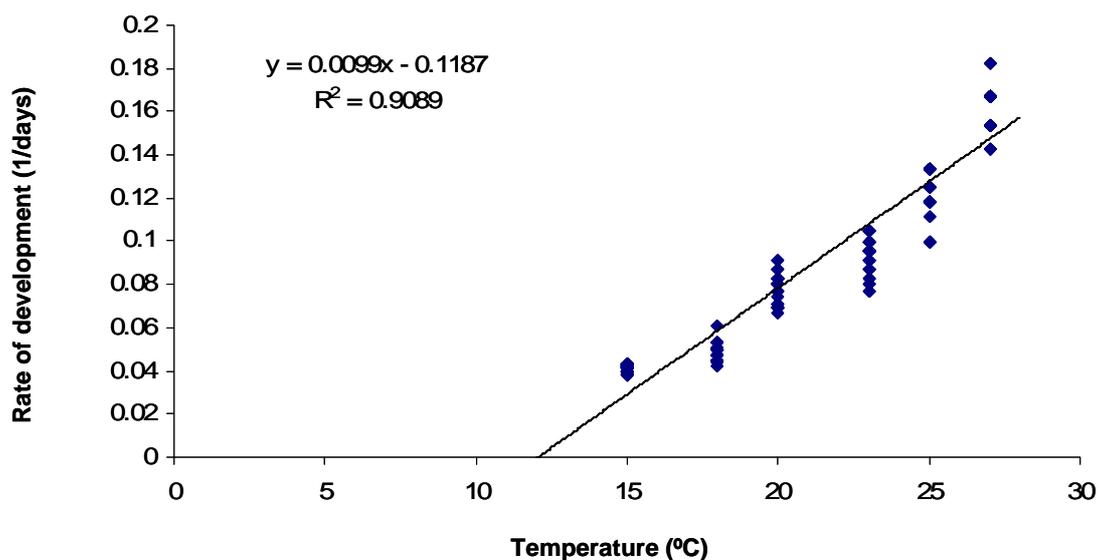


Figure 3.2: Egg to adult development of *Amblyseius swirskii* at temperatures between 15° and 30°C. Line fitted by simple linear regression $y = 0.0099x - 0.1187$ ($R^2 = 0.9089$).

Table 3.2: Theoretical number of generations of *Amblyseius swirskii* each year in summer (between April and September) and winter (between October and March) from 1987-2007 in Birmingham, UK using simple linear regression (bracketed values refer to the actual number of possible generations).

Year	Available °d per year	Max. no of generations per year	Available °d Apr-Sept	Max. no of generations Apr-Sept	Available °d Oct-Mar	Max. no of generations Oct-Mar
1987	358.3	3.5 (3)	350.5	3.5 (3)	7.8	0.08 (0)
1988	322.6	3.2 (3)	312.9	3.1 (3)	9.7	0.10 (0)
1989	584.1	5.8 (5)	570.2	5.6 (5)	13.9	0.14 (0)
1990	551.9	5.5 (5)	511.9	5.1 (5)	40.0	0.40 (0)
1991	497.3	4.9 (4)	483.1	4.8 (4)	14.2	0.14 (0)
1992	478.7	4.7 (4)	478.5	4.7 (4)	0.2	0.00 (0)
1993	347.1	3.4 (3)	346.3	3.4 (3)	0.8	0.01 (0)
1994	450.9	4.5 (4)	438.8	4.3 (4)	12.1	0.12 (0)
1995	644.4	6.4 (6)	606.7	6.0 (6)	37.7	0.37 (0)
1996	449.4	4.4 (4)	432.4	4.3 (4)	17.0	0.17 (0)
1997	545.6	5.4 (5)	516.7	5.1 (5)	28.9	0.29 (0)
1998	440.3	4.4 (4)	423.5	4.2 (4)	16.8	0.17 (0)
1999	504.4	5.0 (5)	491.0	4.9 (4)	13.4	0.13 (0)
2000	460.9	4.6 (4)	454.7	4.5 (4)	6.2	0.06 (0)
2001	516.6	5.1 (5)	473.5	4.7 (4)	43.1	0.43 (0)
2002	460.3	4.6 (4)	448.5	4.4 (4)	11.8	0.12 (0)
2003	603.1	6.0 (6)	591.7	5.9 (5)	11.4	0.11 (0)
2004	544.9	5.4 (5)	536.9	5.3 (5)	8.0	0.08 (0)
2005	540.9	5.4 (5)	506.6	5.0 (5)	34.3	0.34 (0)
2006	686.6	6.8 (6)	661.5	6.5 (6)	25.1	0.25 (0)
2007	421.5	4.2 (4)	407.8	4.0 (4)	13.7	0.14 (0)
Mean	495.7	4.9 (4)	478.3	4.7 (4)	17.4	0.17 (0)

The estimates of developmental threshold and voltinism indicate that, in reality, in the years from 1987-2007 *A. swirskii* could complete a total of 4 generations per year in the Midlands region of the UK. Development and consequently population increase would be confined to the summer months only (April-September) of each year. Temperature data from the years 1987-2007 show that temperatures above the developmental threshold of *A. swirskii* (11.9°C) are exceeded only between the beginning of May and middle of September (Figure 3.3). During this time, between 4 and 5 generations would be expected to occur. However, *A. swirskii* would then have to survive over 6 months of the year at temperatures below its developmental threshold and during exposures as low as -7°C.

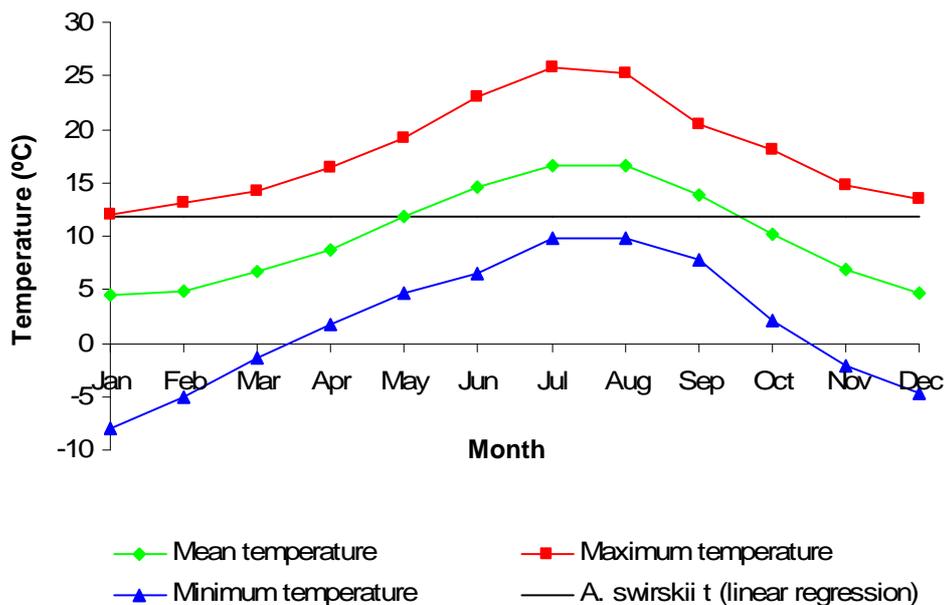


Figure 3.3: Average, maximum, mean and minimum temperatures recorded for each month from 1987-2007 in Birmingham, UK. *A. swirskii* developmental threshold (t) as calculated by simple linear regression, shown as a dotted line.

3.4.2 Diapause

The number of eggs laid per female mite under the control and the first and second generation females under diapause inducing conditions are shown in Figure 3.4. The total mean numbers (\pm SE) of eggs laid per female were 19.6 ± 0.22 , 17.6 ± 0.48 and 18.3 ± 0.46 . The number of eggs laid in the control population peaked on day 6 and then declined steadily to day 16. For the first generation diapause population, egg laying peaked at day 17, three days after being transferred back to the original rearing conditions (25°C, 18:6 LD). However, oviposition occurred from day 1 and 100% of the females were ovipositing by day 7 in the diapause inducing conditions. Egg laying declined rapidly from its peak at day 17 to zero at day 23.

For the second generation females, slightly higher egg production was seen in the first seven days than the first generation females. Oviposition peaked at day 17, three days after being transferred to the original rearing temperatures and again rapidly decreased to zero after 6 days.

Statistical analysis showed that the numbers of eggs laid in the control population was significantly different to that laid by the first and second generations under diapausing conditions ($F_{2,117} = 7.98$, $P < 0.05$).

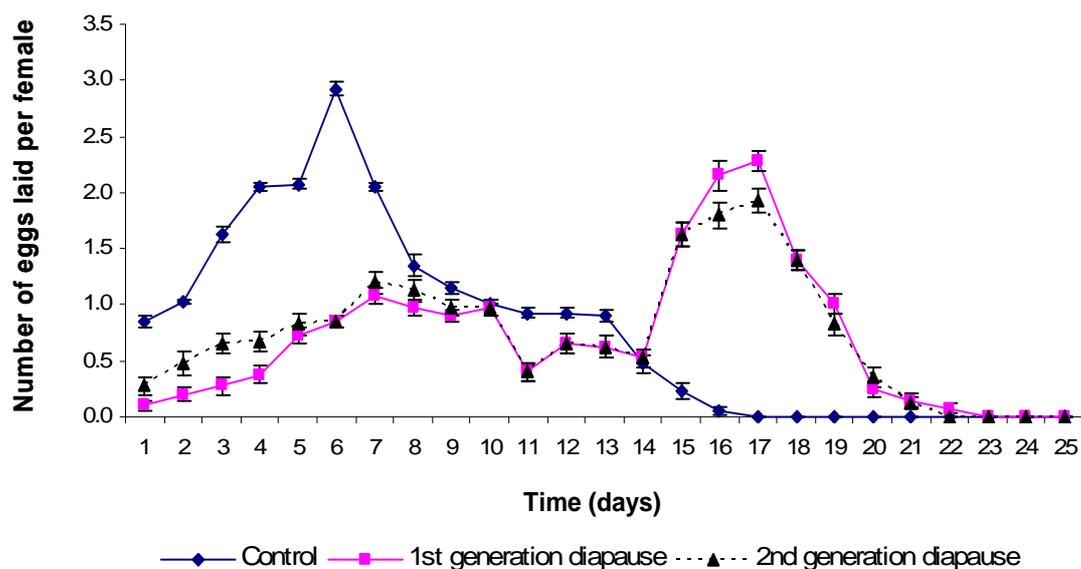


Figure 3.4: Mean number of eggs laid (\pm SE) per female *Amblyseius swirskii* per day under control conditions (25°C, 18:6 LD) and for first and second generation females reared under diapause inducing conditions (18°C, 6:18 LD) (n=40).

3.4.3 Supercooling points

The mean and range of SCPs of mites from each of the four treatment groups is shown in Table 3.3. All individuals tested were found to be dead after freezing. The mean larval SCPs were significantly lower than the adults ($F_{3,83}=51.07$, $P<0.05$). There were no significant differences between the SCPs of acclimated and non-acclimated *A. swirskii*.

Table 3.3: Mean (\pm SE) and range of supercooling points of non-acclimated and acclimated female and larval *Amblyseius swirskii*.

Treatment group	n	Mean \pm SE (°C)	Range (°C)
Acclimated adults	28	-18.3 \pm 0.28	-14.9 to -21.5
Non-acclimated adults	29	-18.1 \pm 0.39	-13.0 to -21.0
Acclimated larvae	15	-23.5 \pm 0.60	-17.7 to -27.1
Non-acclimated larvae	15	-24.9 \pm 0.77	-17.3 to -28.1

3.4.4 Lower lethal temperatures

No adult or larval individual from the control sample died after confinement to the Beem capsule and exposure to 20°C for 75 min suggesting that neither the Beem capsules nor the vibrations from the alcohol bath were producing a deleterious effect. The lethal temperatures for 10, 50 and 90% mortality of the sample of acclimated and non-acclimated adult and larval *A. swirskii* are shown in Figure 3.5. Non-overlapping fiducial limits indicate that there is a significant difference in the data at the level of 5% (StatsDirect 2008).

Overall, adult mites survived at lower temperatures than larval mites and this is a significant difference at 10 and 50% mortality levels. At 90% mortality, acclimated adults survived at significantly lower temperatures than both larval groups but the overlapping fiducial limits between non-acclimated adults and acclimated larvae indicate no difference in survival temperatures. A period of acclimation at 10°C did not increase survival at lower temperatures, as the data shows overlapping fiducial limits between all acclimated and non-acclimated treatments with the exception of acclimated larvae at 90% which survived significantly lower temperatures than the non-acclimated larvae.

100% of the larvae recovered alive from the Beem capsules and transferred to recovery arenas continued to develop through to the adult stage. The vast majority of the surviving adult females oviposited within a few days of exposure illustrating the ability to form viable populations following a low temperature event.

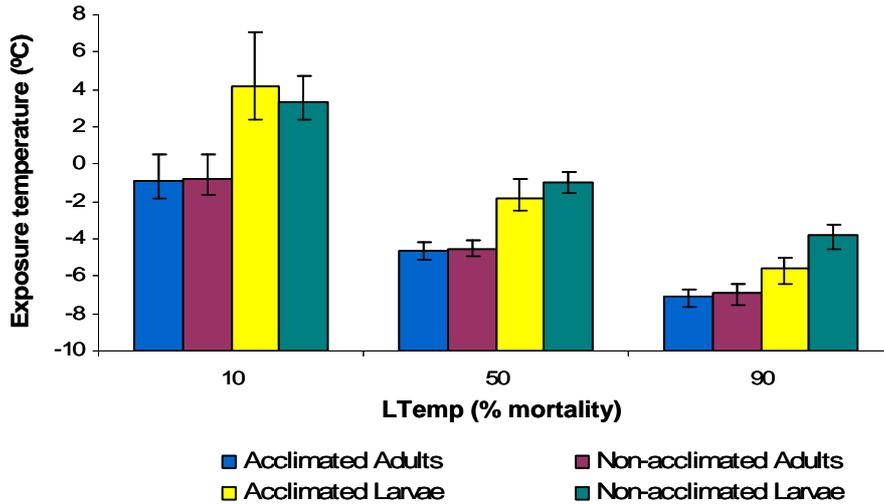


Figure 3.5: LTemp₁₀, ₅₀ and ₉₀ mortality (\pm fiducial limits) of acclimated and non-acclimated adult and larval *Amblyseius swirskii* after cooling at $0.5^{\circ}\text{C min}^{-1}$ to temperatures between 5° and -15°C .

3.4.5 Lower lethal times

The lower lethal times for 10, 50 and 90% mortality (LTime₁₀, ₅₀, ₉₀) of acclimated and non-acclimated adult and larval *A. swirskii* at -5° , 0° and 5°C are shown in Figure 3.6. The LTime₅₀ for non acclimated adults were 9.2 min, 1.6 and 2.7 days for -5° , 0° and 5°C respectively and 2.5 min ,0.6 and 1.1 days respectively, for non-acclimated larvae.

Overall, at -5°C , survival times were comparatively short, with exposures of minutes rather than days resulting in mortality. At the 10, 50 and 90% levels, there were significant differences in mortality between life stages as the adults survived consistently longer. There were no significant differences in mortality rates between acclimated and non-acclimated larvae at any of the intervals (shown by overlapping fiducial limits bars). However at 10 and 50% mortality, a period of acclimation did significantly increase the survival time in the adult groups.

At 0°C, acclimated adult mites survived significantly longer than non-acclimated at the 10 and 50% mortality levels. Adults survived longer than larvae at all levels, but acclimated and non-acclimated larvae showed no difference in survival.

Mites exposed to 5°C survived for the longest period of time. Again acclimation in the larval groups resulted in no significant differences in survival. Acclimated adults survived significantly longer at the 50 and 90% mortality levels than non-acclimated adults, and non-acclimated adults proved hardier than any of the larval groups at all three mortality indices. The maximum survival time for individuals at 5°C in the LTime experiments was 8 days by which time 100% mortality had occurred in the fed control population held at 5°C. This suggests that death was due to the cold stress and not to desiccation or starvation.

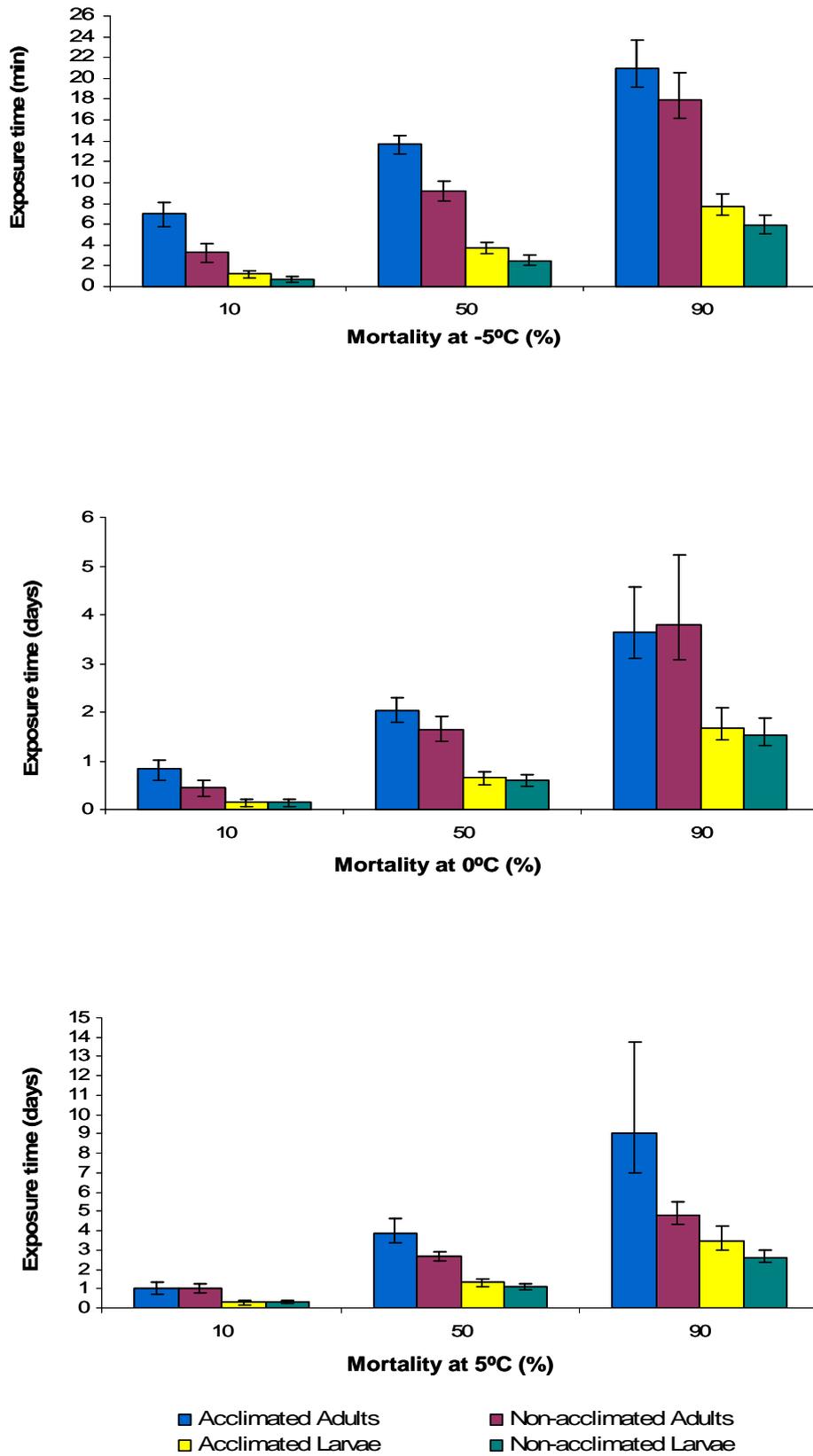


Figure 3.6: LTime₁₀, ₅₀ and ₉₀ mortality (\pm fiducial limits) of acclimated and non-acclimated adult and larval *Amblyseius swirskii* at -5°, 0° and 5°C (NB: different scales of x and y axes).

3.4.6 Field exposures

Field mortality rates of non-acclimated and acclimated, adult and larval *A. swirskii*, recorded in the first field exposure (November-December 2005) are shown in Figure 3.7. Each colour represents one of the mite treatment groups and solid and dashed lines represent unfed and fed treatments respectively for each group. The corresponding field temperatures are shown in Figure 3.8. Mortality of all treatment groups increased with duration of outdoor exposure. Mortality levels at day 1 were significantly lower than day 2 which were significantly lower than day 5 ($P < 0.01$). There was no significant difference in the percentage mortality across the groups between day 5 and 8. After 8 days of exposure, 100% mortality was recorded in all mite groups. The average daily temperature recorded during this time was 2.3°C , with a maximum of 8.2° and a minimum of -2.8°C . The results show a rapid population decline in all groups within 24 h of first exposure when the minimum overnight temperature was -2.8°C and the field study area was subjected to a heavy frost. Mortality in the larval groups was particularly rapid. At day 1 there was no significant difference between mortality rates in the larval groups. There was no significant difference between mortality rates in the adult groups but acclimated and non-acclimated fed adults survived significantly better than acclimated fed larvae ($P < 0.05$). After 2 days field exposure there was a significant difference between survival of acclimated and non-acclimated adults ($P < 0.01$) but no difference between fed and unfed adults within each acclimation treatment. Survival of acclimated adults and acclimated larvae showed no significant difference and the same is true of non-acclimated adults and larvae. The greatest difference in survival was the comparison between acclimated adults and non-acclimated larvae ($P < 0.001$). After five days of

exposure, only the acclimated, unfed larval group showed any survival (5%) but this was not statistically significant from any of the other treatment groups.

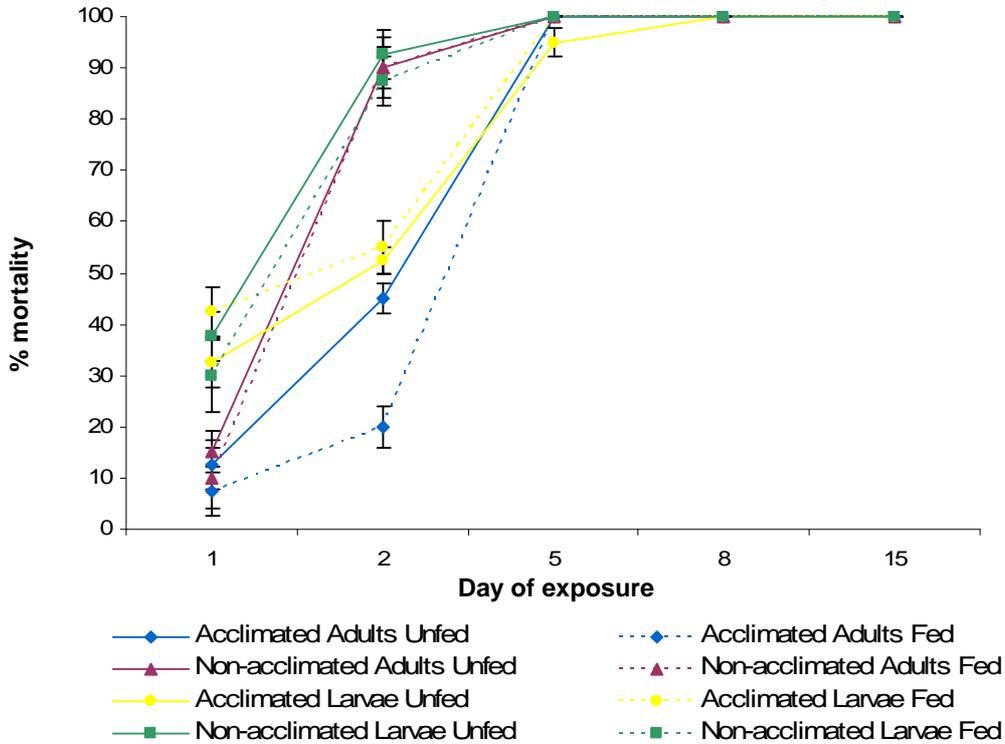


Figure 3.7: Field mortality of fed and unfed, non-acclimated and acclimated, adult and larval *Amblyseius swirskii* from 16 November to 1 December 2005.

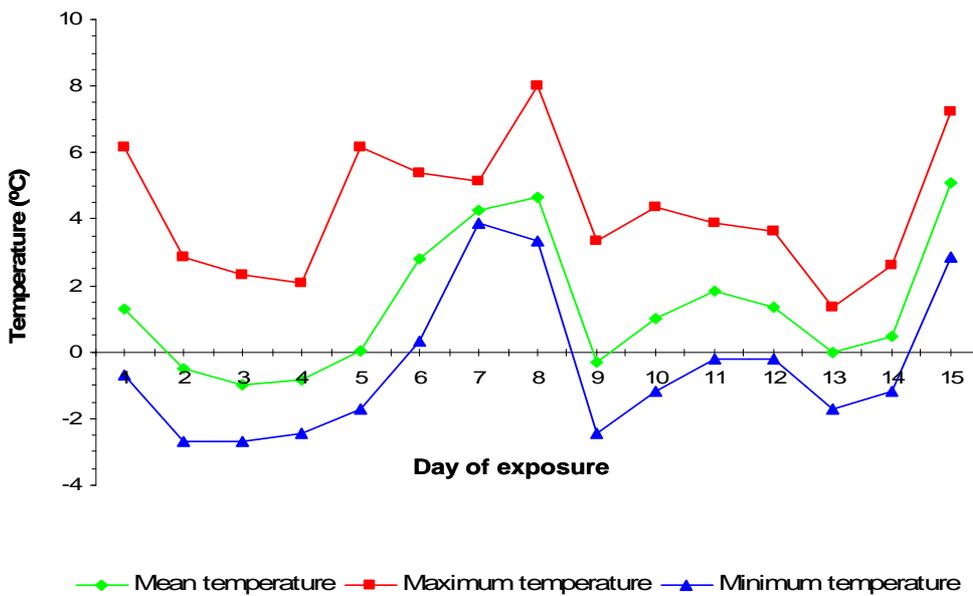


Figure 3.8: Mean, maximum and minimum outdoor temperatures experienced by *Amblyseius swirskii* in the field from 16 November to 1 December 2005.

Field mortality rates of non-acclimated and acclimated, fed and unfed adults and larvae recorded in the late winter field experiment (February to March 2006) are illustrated in Figure 3.9. The average daily temperatures recorded during the field exposures are shown in Figure 3.10. The average temperature throughout the trial was 3.2°C and temperatures fluctuated between a maximum of 11.2° and minimum of -3.2°C. Levels of mortality between day 2 and 4 did not differ significantly from each other. Mortality at day 1 was lower than day 2, day 4 was lower than day 6 and day 6 was lower than day 8 ($P < 0.01$ for all observations); 100% mortality in all groups was reached within twelve days of outdoor exposure. At day 1 acclimated adults showed significantly lower ($P < 0.001$) mortality than all other treatment groups but the fed and unfed treatments did not differ significantly from each other. After 2 days of outdoor exposure acclimated adults had significantly lower ($P < 0.01$) mortality rates than all other treatment groups with the exception of unfed acclimated adults and acclimated fed larvae ($P > 0.05$). Acclimated larvae showed lower mortality rates than the non-acclimated adult and larval groups ($P < 0.05$). On day 4 acclimated adults had significantly lower mortality than acclimated larvae which also had significantly lower mortality than the both adult and larval non-acclimated groups ($P < 0.001$ and 0.01 respectively). Data from day 6 show increasing mortality levels across all groups. Acclimated larvae showed significantly lower mortality than all non-acclimated groups ($P < 0.01$) and acclimated adults showed lower mortality than acclimated larvae ($P < 0.05$).

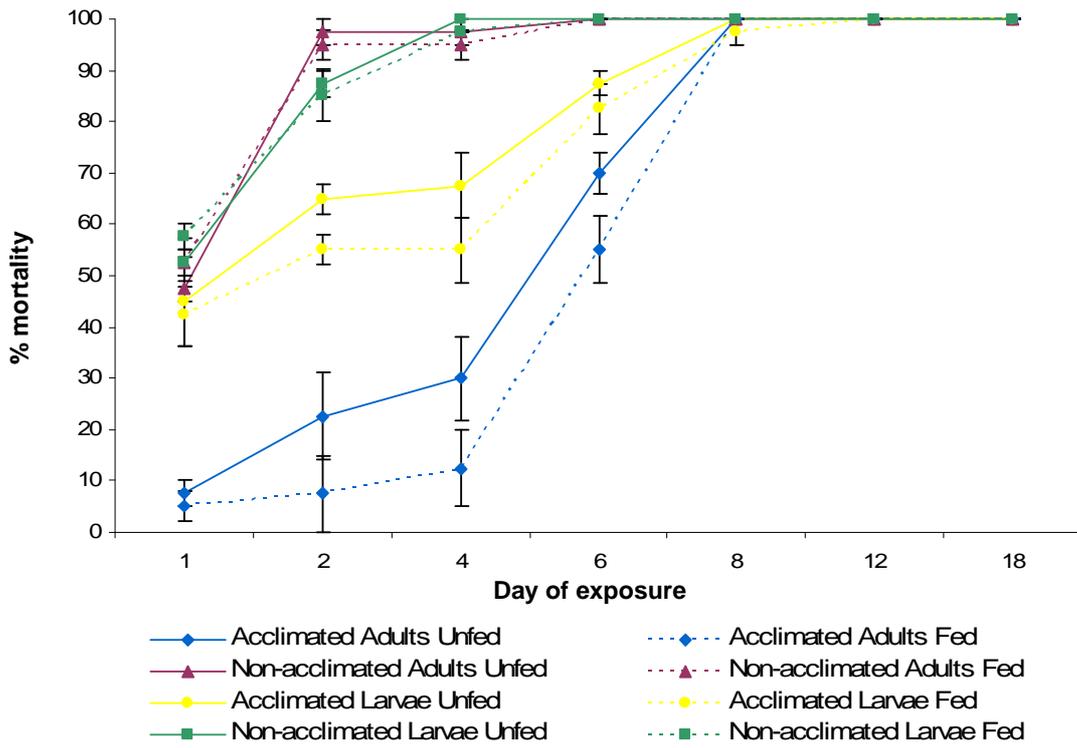


Figure 3.9: Field mortality of fed and unfed, non-acclimated and acclimated, adult and larval *Amblyseius swirskii* from 23 February to 7 March 2006.

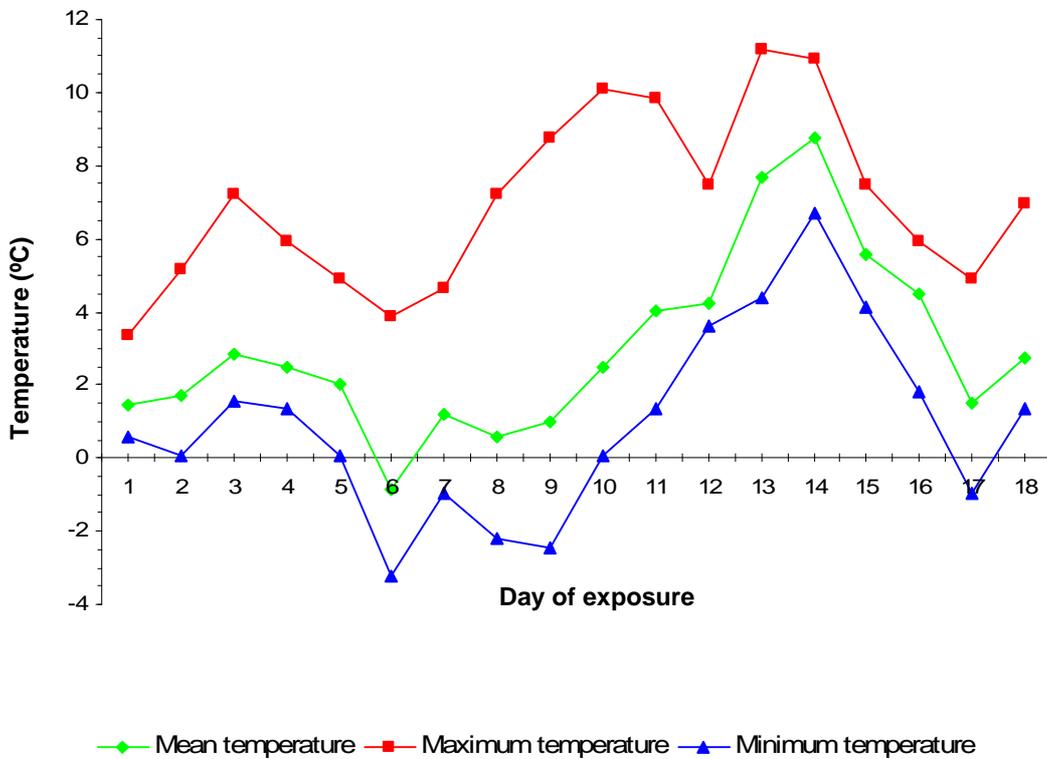


Figure 3.10: Mean, maximum and minimum outdoor temperatures experienced by *Amblyseius swirskii* in the field from 23 February to 7 March 2006.

Mortality data from the short term field trials are shown in Table 3.4. Mortality increased from week 1 to week 2 for all of the field trials with the exception of the January trial where 100% mortality was reached within the first week during which the minimum temperature was -0.4°C . Where survival occurred, adult survival was higher than larval survival in all samples. Minimum temperatures above 5.1°C resulted in 48.5% survival or more for *A. swirskii* larvae, with adult mites surviving at 77.5% and higher.

Table 3.4: Mean % mortality (\pm SE) of acclimated (AA) and non-acclimated (NA) adult and larval (AL and NL) *Amblyseius swirskii* after 7 and 14 days of outdoor temperature exposure. Also displayed are the mean, maximum and minimum temperatures for each 7 day period and the mean temperature of the total 14 day period.

Date into field	Mite treatment group	Mean % mortality (\pm SE)		Mean, max and min temperatures ($^{\circ}\text{C}$)		Mean temperature ($^{\circ}\text{C}$) days 1-14
		7 days	14 days	days 1-7	days 8-14	
01/12/2006	AA	37.5 \pm 4.8	100 \pm 0.0	7.8, 14.0, 4.9	6.4, 10.9, 1.1	6.9
	NA	52.5 \pm 6.3	100 \pm 0.0			
	AL	87.5 \pm 2.5	100 \pm 0.0			
	NL	92.5 \pm 2.5	100 \pm 0.0			
22/01/2007	AA	100 \pm 0.0	100 \pm 0.0	3.1, 8.8, -0.4	4.6, 11.2, -0.7	4.0
	NA	100 \pm 0.0	100 \pm 0.0			
	AL	100 \pm 0.0	100 \pm 0.0			
	NL	100 \pm 0.0	100 \pm 0.0			
26/02/2007	AA	5.0 \pm 2.9	27.5 \pm 4.8	7.0, 18.6, 1.3	8.3, 14.0, 3.9	7.5
	NA	27.5 \pm 4.8	55.0 \pm 6.5			
	AL	87.5 \pm 4.8	100 \pm 0.0			
	NL	85.0 \pm 2.9	100 \pm 0.0			
04/04/2007	AA	7.5 \pm 4.8	10.0 \pm 4.1	12.9, 23.1, 5.1	15.8, 27.2, 8.5	14.4
	NA	17.5 \pm 7.5	22.5 \pm 7.5			
	AL	22.5 \pm 6.3	30.0 \pm 7.1			
	NL	45.0 \pm 8.7	52.5 \pm 4.8			
17/05/2007	AA	0.0	2.5 \pm 2.5	15.2, 24.4, 8.5	11.9, 22.4, 6.2	13.6
	NA	5.0 \pm 2.9	12.5 \pm 2.5			
	AL	5.0 \pm 2.9	12.5 \pm 4.8			
	NL	7.5 \pm 4.8	10.0 \pm 4.1			
22/06/2007	AA	0.0	2.5 \pm 2.5	15.2, 23.4, 8.3	15.6, 21.8, 12.0	15.4
	NA	5.0 \pm 2.9	5.0 \pm 2.9			
	AL	10.0 \pm 4.1	15.0 \pm 2.9			
	NL	12.5 \pm 4.8	12.5 \pm 7.5			

The mortality data from all 6 field trials were collated and analysed against the mean and minimum temperatures experienced by the mites over the exposure period. The results of the Probit analysis are shown in Figure 3.11. Significant differences between the temperatures at which 10, 50 and 90% mortality occurs in each group are indicated by non-overlapping fiducial limits.

Mean and minimum temperatures that cause 10 and 50% field mortality in acclimated adult *A. swirskii* are significantly lower than the temperatures required to cause the same level of mortality in the other three groups; 50% field mortality of non-acclimated adults occurs at significantly lower temperatures than that which causes 50% mortality in either larval group. The 90% field mortality temperatures are significantly lower for adult mites than larvae.

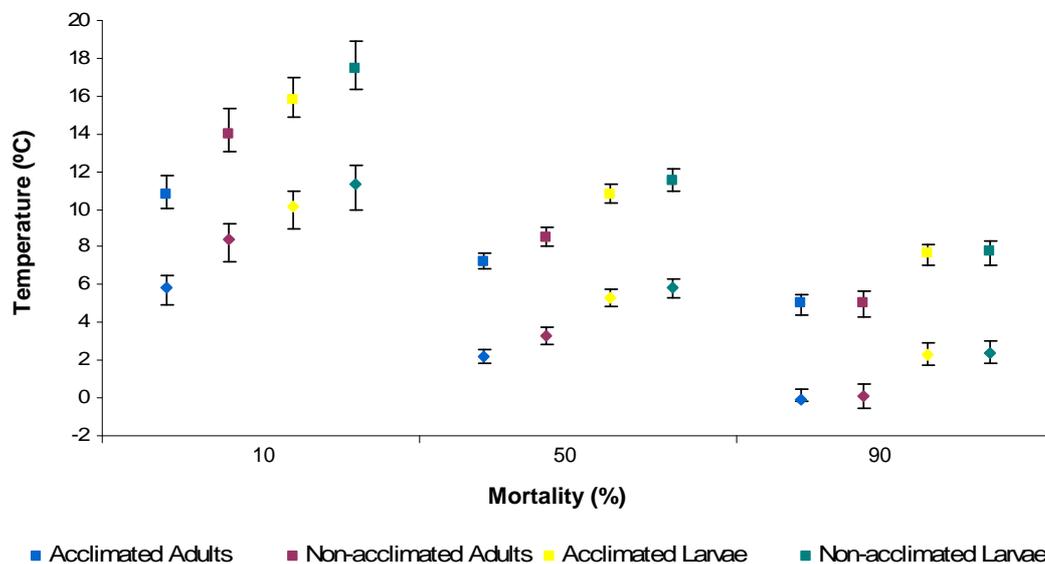


Figure 3.11: Mean (■) and minimum (◆) temperatures (\pm 95% fiducial limits) predicted to cause 10, 50 and 90% field mortality of acclimated and non-acclimated adult and larval *Amblyseius swirskii*. Data collated from the short term field trials and analysed by Probit (n=480).

3.5 Discussion

The main aim of the work described in this chapter was to acquire a comprehensive dataset on the thermal biology of *A. swirskii* that could be compared with comparable information on other predatory mites and non-native biocontrol agents more generally, as part of an environmental risk assessment.

In the present study, the optimal temperature for *A. swirskii* development was approximately 27°C and at this temperature mean egg to adult development occurred in 6.1 ± 0.04 days. *Typhlodromips montdorensis* and *N. californicus*, two other predatory mites, complete their development in 6.3 days (Hatherly *et al.*, 2004) and 8.1 days (Hart *et al.*, 2002a) respectively. At 30°C, *A. swirskii* development is slower than at 27°C and at temperatures below 12°C development does not occur beyond the larval stage.

A. swirskii responds to an increase in rearing temperature by increasing its rate of developmental at each immature stage. The larval stage was the shortest life stage at all temperatures which may be due to the larvae being a non-feeding stage and therefore it's growth restricted and also it is the most vulnerable stage to predation and cannibalism. This relationship is linear between 15° and 27°C. The relationship between temperature and developmental rate outside of this range is more likely to be curvilinear (Lamb 1992; Wagner *et al.*, 1984) but a linear approximation of the data is acceptable as long as the temperatures are considered to be in the linear region (Higley *et al.*, 1997). Simple linear regression results in a thermal budget estimation of 101 day degrees (°d) per generation and a lower developmental threshold

temperature of 11.9°C. This is the temperature, below which development does not occur. This temperature would appear to be accurate estimate because in the developmental time experiments it was found that at 12°C and below development does not progress beyond the larval stage. The developmental threshold for *A. swirskii* is relatively high compared to other biological control agents. For example, from simple linear regression analysis *T. montdorensis* has a developmental threshold of 10.7°C and a thermal budget of 105.3 °d (Hatherly *et al.*, 2004) and *N. californicus* has a threshold of 9.9°C and budget of 123.5 °d (Hart *et al.*, 2002a).

The thermal budget requirement and developmental threshold of *A. swirskii* were used to produce estimations of annual voltinism. Based on temperature data collated over a 20 year period, it can be predicted that *A. swirskii* would be able to have completed a maximum of 6.8, a minimum of 3.2 and an average of 4.9 generations per year. All population growth would have occurred solely during the summer months (April to September) as the mean number of day degrees during the winter period reaches only 17.4°d. In reality, during the years analysed, *A. swirskii* development would only have been possible from the beginning of May and middle of September as this is when mean temperatures rose above the developmental threshold of 11.9°C. Even considering *A. swirskii* could produce 6 generations in a particularly warm year, it would still find it necessary to survive over 6 months of the year at temperatures below its developmental threshold and some exposures as low as -7°C to establish a permanent population in the UK. The maximum developmental temperature for *A. swirskii* was between 30 and 35°C and no development occurred at 35°C. According to Higley *et al.*, (1986) the day degree requirement may be overestimated if no maximum developmental temperature is calculated. However, if the maximum developmental temperature is above the range of normal UK daily temperatures,

which is true of temperatures between 30° and 35°C is (see Figure 3.3), then an overestimation is much less likely.

In the present study *A. swirskii* demonstrated no ability to diapause under conditions which have been shown to induce diapause in other species of mite (Jolly 2000). Oviposition in individuals reared in the diapause inducing conditions was lower than the control individuals and oviposition rapidly increased both in the first and second generation individuals reared under these conditions immediately after transfer to the 'optimal' conditions and peaked three days after the transfer. As egg production was continuous during the diapause inducing conditions for both the first and second generations and none of the females were observed to have a pale and flattened appearance, the ability to diapause has been discounted (Morewood & Gilkeson 1991; Overmeer 1985a; Veerman 1992). Fewer eggs were laid in the first 7 days in either of the 'diapause' generations than in control population and far more successful predation events were observed after transfer to the original rearing conditions. This suggests that reduced egg numbers were due to the initial lower temperature of the diapause regime and the resulting lowered metabolic rate of *A. swirskii*.

The low temperature laboratory experiments confirms that *A. swirskii* does possess some cold tolerance. However, it is not a freeze tolerant species as none of the individuals survived below their freezing point (SCP) nor a true freeze avoiding species as there was high mortality above the SCP. The mean supercooling points for acclimated and non-acclimated adult and larval *A. swirskii* are -18.3° (\pm 0.28), -18.1° (\pm 0.39), -23.5° (\pm 0.60) and -24.9°C (\pm 0.77) respectively. Larval mites survived significantly lower temperatures than the adults but there was no difference in

survival between acclimated and non-acclimated treatments. The range of SCPs for adult *A. swirskii* was -13.0° to -21.5° and for larvae -17.3° to -28.1° . The adult SCPs seem relatively high when compared to other mite species that have been studied as potential biological control agents. *T. montdorensis* and *N. californicus* have mean SCPs of -24.1° and -21.6°C respectively (Hatherly *et al.*, 2004; Hart *et al.*, 2002a). Other Phytoseiid mites have also been tested for their SCPs, such as *Phytoseiulus persimilis* and *Amblyseius cucumeris* which have SCPs of -22.5° and -20.7°C respectively for non-acclimated mites (Morewood 1992).

SCP data alone is not generally accepted as a reliable indication of cold tolerance (Bale 1987; Bale 1996) because the vast majority of species are freeze avoiding and SCP temperatures are rarely experienced by the individuals in natural habitats. Although the larval supercooling points recorded in this study are of a similar nature to those obtained for other mites this should give no reliable indication as to a similarity in overall cold hardiness. *Amblyseius swirskii* exhibit pre-freeze mortality, as all individuals died during the lethal temperature experiments before reaching their SCP.

Lethal temperature data provides a more reliable estimation of cold tolerance than SCPs, as the temperatures experienced by the mites are more realistic to outdoor winter temperatures. Across the *A. swirskii* sample groups the range of temperatures at which 10, 50 and 90% of individuals died are 4.1 to -0.9 , -1.0 to -4.7 , -3.8 to -7.1°C respectively. The greatest significant difference was between acclimated adults and non-acclimated larvae. Adults survived significantly lower temperatures than larvae at all three mortality intervals. There was no significant difference between acclimation treatments of the same life stage with the exception of acclimated larvae at 90%

mortality surviving significantly lower temperatures than non-acclimated larvae. The LTemp₅₀ for non-acclimated adult *A. swirskii* was estimated as -4.5°C and the range in which 10 to 90% mortality occurred was -0.8° to -6.9°C. This is a wider temperature range when compared to non-acclimated adults of *T. montdorensis* which die at temperatures between -5.6° and -8.1°C (Hatherly *et al.*, 2004). However it is clear that *A. swirskii* can not tolerate low temperatures as well as *T. montdorensis*. Non-acclimated adult *N. californicus* survived temperatures of -15.1°C (Hart *et al* 2002a), this temperature being only 7°C above their SCP. In contrast, *A. swirskii* die 11°C above their SCP, indicating that extensive pre-freeze mortality is a characteristic of this species. *T. montdorensis* has a SCP up to 16°C lower than the LTemp₅₀ and first instar larvae of the aphid *Myzus persicae* (Hemiptera: Aphidoidea) have an LTemp₅₀ of -8°C but a SCP of -27°C (Bale *et al.*, 1988). This provides further evidence that the SCP is not a reliable indicator of cold tolerance, whereas lethal temperature data provides a valuable gauge of an organisms pre-freeze mortality. Normal development and oviposition was observed in the survivors from LTemp experiments, suggesting that there is a very fine line between cold tolerance and death for this species. Alternatively, any deleterious effects accumulated by the cold exposure led ultimately to death rather than to a sub-optimal surviving individual.

Lethal time data best represents naturally occurring cold stress because it tests not only low temperature but also exposure time. The lethal time experiments are the closest laboratory measure of field survival because it takes into account mortality over time at temperatures likely to be encountered during an outdoor UK winter season.

For non-acclimated *A. swirskii* adults 10% survival times at -5°, 0° and 5°C were 17.9 minutes, 3.8 days and 4.8 days respectively. By comparison, cold hardy Phytoseiid mite species such as *N. californicus* and *T. montdorensis* the equivalent values for 5°C survival times are 3 months and 22 days respectively (Hatherly *et al.*, 2004; Hart *et al.*, 2002a). The range of lethal times for *A. swirskii* at 50% mortality ranged from 2.5 to 13.7 min, 0.6 to 2.0 days and 1.1 to 3.9 days for -5, 0 and 5°C respectively. It can be noted from this data and the comparisons with other biological control agents that *A. swirskii* has a very low level of cold tolerance. At all temperatures, adults survived significantly longer than larvae and a period of acclimation at 10°C did appear to be in general advantageous to the adult life stage with a significantly longer survival for acclimated adults at 5 and -5°C. However, any increases in survival time were small; the LTime₅₀ was 3.9 compared to 2.7 days at 5°C and 13.7 compared to 9.2 min at -5°C for acclimated and non-acclimated adults. Acclimation of larval groups did not significantly alter survival times.

In the laboratory it does seem apparent that a short period of acclimation prior to cold exposure can convey a limited advantage to *A. swirskii*, particularly in the adult groups. However, there is no response in the larval stage for which the acclimation regime is deleterious to the mites. The acclimation treatment was at 10°C for three days and as seen in the developmental time experiments, individuals did not progress beyond the larval stage and survived between 10 and 15 days at this temperature. This provides evidence of cumulative deleterious effects of temperatures around 10°C for larval *A. swirskii*.

The objective of the field exposures was to determine the ability of *A. swirskii* to survive outside the glasshouse environment. Escapees from glasshouses are difficult to prevent and depending on the frequency and timing of these occurrences, would affect outdoor population growth and establishment. The laboratory data suggest that *A. swirskii* is weakly cold tolerant but may show some survival in the field. Quantification of this ability is necessary to perform a complete assessment of risk for such biocontrol agents. Field exposure experiments also provide a unique opportunity to investigate the effect of fluctuating temperatures on outdoor populations. As overwintering is likely to occur in sheltered locations such as leaf litter, bark or building crevices as shown in other Phytoseiid mites (Broufas *et al.*, 2002; Veerman 1992) the field trials were conducted in concealed boxes, sheltered from the effects of wind and direct sunlight, to provide a more realistic assessment of actual survival outside of a glasshouse.

Field survival was also extended slightly by a period of acclimation, but in general, mortality rates were so high and rapid that even after acclimation the maximum winter field survival time was 11 days. In both winter field exposure experiments, 100% mortality was reached in all treatment groups within two weeks. Mean temperatures experienced in the field during this time were 2.3°C for the early winter trial (November-December 2005) and 3.2°C for the late trial (February-March 2006). In all groups, initial population decline was rapid, occurring in the first two to four days of exposure (Figures 3.7 and 3.8). A period of acclimation seemed to slightly improve cold hardiness but provision of food had no effect on survival times. Acclimated adults seemed to cope better with the initial cold shock than the larvae and therefore survived slightly longer. The average temperature throughout the

second trial was 1°C higher than the first trial, yet the temperatures fluctuated more widely during this time and a maximum of 11.2°C and minimum of -3.2°C were recorded.

Non-acclimated adult and larval *A. swirskii* survived for a maximum of 5 days in the early winter trial and 4 days in the late winter. Acclimated adults and larvae survived for a maximum of 8 days and 12 days in the early and late trial respectively. These extremely short field survival time confirms the idea that *A. swirskii* lacks cold tolerance, more so than other species of Phytoseiid mite.

The short term field exposures that ran from December 2006 to June 2007 give a clearer indication of the effect of different temperature regimes on the survival of *A. swirskii*. The data show that fluctuating low temperatures and exposure to sub-zero temperatures is lethal across all groups. Even when the mean temperature is relatively high, it appears that exposure to low overnight temperatures exert considerably stress on the population. Acclimated adult mites showed the highest level of cold tolerance throughout all field exposures. The short term field exposures (Figure 3.11) indicate that a combination of mean temperatures over time and minimum temperatures both contribute to mortality. Due to the closeness of the data for mean and minimum temperatures for mortality in each treatment group, it seems that it is the low overnight minimum temperatures that have the greatest deleterious effect on *A. swirskii*, particularly sub-zero temperatures. This is corroborated by the LTemp₅₀ data which show that survival at -5°C is measured in minutes not hours or days. It would appear that a cold night with several hours of sub-zero temperatures would cause very high mortality of all life cycle stages of *A. swirskii*.

When considering the introduction and release of a non-native biological control agent, climate models can be used to study the effect of temperature on the development of both the target pest and the proposed control agent (Barrett *et al.*, 1997; Barrett *et al.*, 1999; Goldson *et al.*, 1992). This type of analysis together with a detailed assessment of an organism's laboratory cold tolerance and field survival can be collated to predict establishment potential of that subject species. However, this research and analysis is costly both in time and resources. For biocontrol companies looking to market new agents this research can be 'wasteful' if it transpires that the organism in question is not suitable for release because its cold hardiness might lead to permanent outdoor establishment.

Work has been carried out to find a reliable and effective way to classify candidate biological control agents into risk categories with respect to establishment potential within the UK (Hatherly *et al.*, 2005). Figure 3.12 shows the relationship between maximum field survival time and $LTime_{50}$ at 5°C for eight non-native biological control agents.

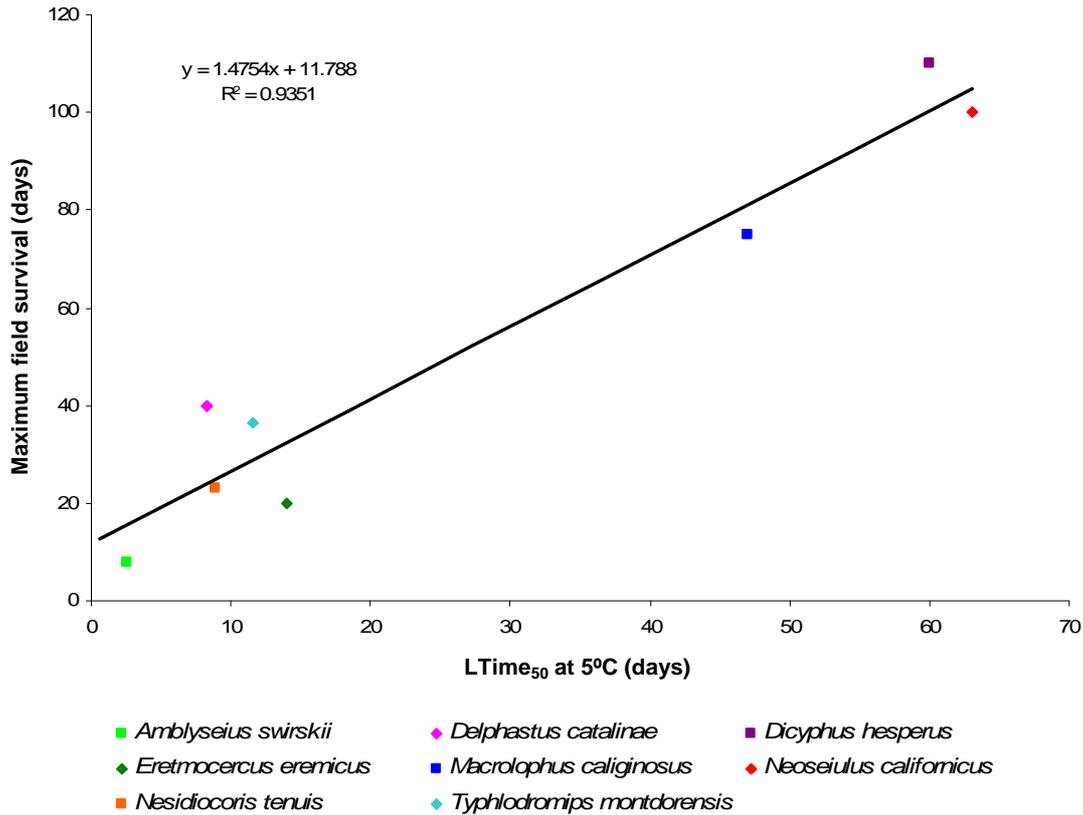


Figure 3.12: Relationship between maximum field survival (days) and LTime₅₀ at 5°C (days) for eight non-native biological control agents (expanded on from Hatherly *et al.*, 2005). Sources of data: *Delphastus catalinae* (Tullett 2002), *Dicyphus hesperus* (Hatherly *et al.*, 2008), *Eretmocerus eremicus* (Tullett *et al.*, 2004), *Macrolophus caliginosus* (Hart *et al.*, 2002b), *Neoseiulus californicus* (Hart *et al.*, 2002a), *Typhlodromips montdorensis* (Hatherly *et al.*, 2004)

The relationship described in Figure 3.12 appears to be robust and in the future it would seem possible to use the LTime₅₀ at 5°C derived from a laboratory experiment as an indicator of likely survival in the field. The main benefit of this approach is that it would avoid the need for field exposure trials (which can be expensive in time and resources), especially for species that are obviously too cold tolerant for release in the UK. However, if an organism falls at the ‘safe’ end of the graph (as is the case for *A. swirskii* and several other species, then any further research needed to comply with the information requirements for the licensing of non-native species could be justified.

The present study has expanded on this theme and Figure 3.12 illustrates the position of *A. swirskii* on a sliding scale of risk. The laboratory data shows that *A. swirskii* does not diapause, suffers high pre-freeze mortality and has very limited survival under moderately cold conditions. Winter field exposure cause high mortality and rapid population decline. In combination, these results give a strong indication that under current climatic conditions, the establishment of *A. swirskii* in the UK outside of a glasshouse environment (and elsewhere in northern Europe) is extremely unlikely and hence this mite can be regarded as a 'safe' biological control agent.

CHAPTER 4

Low temperature activity thresholds of *Amblyseius swirskii*

4.1 Introduction

The main focus of studies on insects and mites at low temperatures has been on survival and on temperature as a constraint to establishment (Hart & Bale 1997; Hart *et al.*, 2002; Hatherly *et al.*, 2004). Although cold tolerance is recognised as a stringent assessment of an organism's potential to establish, consideration into the effect of other 'thermal thresholds' which may also affect the distribution and therefore potential impact of a control agent. Such thresholds include temperatures above or below which a species becomes incapable of activity or development. With the movement of opinion away from the importance of the supercooling point as a measure of cold tolerance, the same may be suggested for a preference to factor in activity thresholds alongside data on the lethal affects of temperature in an assessment of risk for releases of non-native biological control agents. Knowledge of these activity thresholds may hold a particularly pertinent advantage in temperate regions where conditions are rarely severe enough to directly result in mortality, yet due to these sub-lethal constraints many species are unable to survive or breed successfully (Mellanby 1939). Temperature also affects other thermal activity thresholds that can indirectly influence survival such as prey finding ability, predator-prey interaction, mate finding and dispersal. Activity thresholds play an important role in determining

species range distributions (Bryant *et al.*, 1997) and how climate change may affect them (Parmesan 1996; Walther *et al.*, 2002). In the case of introduced species for use as biological control agents, these activity threshold temperatures can inform not only to the establishment potential of the species but also its efficacy as a control agent. When comparisons are drawn between predatory control species and their pest species prey, activity thresholds can indicate the ability of a predator to be active in response to prey, to a similar level as the prey and at the same environmental conditions. These species-specific sub-lethal thermal tolerances therefore not only determine the balance in predator-prey relationships that are fundamental to modern biological control and pest management, but can contribute to the optimal selection of the most successful control agent for particular prey and glasshouse conditions.

Thermal activity traits of insects and other terrestrial arthropods can be measured by making careful observations of their behaviour and movements at a series of temperatures. Traditionally several terms have been used to describe the behaviour of insects at low temperatures including chill coma, cold torpor, critical thermal value and knock down (Mellanby 1939; Colhoun 1960; Gaston & Chown, 1999). To add confusion, different researchers have used these terms interchangeably to describe the same and different behavioural indices. Hazell *et al.*, (2008) has recently described a method of measuring thermal activity thresholds of small insects which attempts to not only standardise the terminology used to describe the behaviour observed at these threshold temperatures but also to provide a more robust and accurate method of recorded the absolute values of the thresholds. Briefly, the experimental set up consists of an arena where the insects are placed and the temperature of which can be remotely controlled. Continuous video recording of the occurrences within the arena

are made and consequently the data can be reviewed at a later time and played back in reverse if necessary. In this way information concerning the thermal activity thresholds of small insects can be observed without the need for any intervention from the observer thus reducing any disturbance to the organisms and risk of human error and which could affect the results. This set-up has several advantages over the traditional vertical glass column method described by Huey *et al.*, (1992). In the present method tiny movements made by very small arthropods such as mites (body length <0.5cm) can be recorded, played back, reversed and played in slow-motion to ensure the observations are accurate. Video recording allows multiple, simultaneous observations to be made without direct handling or disturbance of the organisms, a permanent record of the experiment is captured allowing retrospective analysis and the activity arena itself is less conducive to escaping individuals and there is less risk of individuals knocking into each other and effecting the results of the behavioural thresholds.

When an organism is systematically cooled and then warmed, there are four clearly distinguishable behaviours; cessation of walking; cessation of all appendage movement; resumption of movement of body parts and resumption of walking.

Therefore during the cooling process there are two thresholds of behaviour. The lowest temperature at which the organism can show coordinated walking movement is it's 'lower activity threshold' or 'critical minimum temperature' (CT_{min}) and the lowest temperature at which the organism can make controlled movements of antennae or legs is the 'chill coma' temperature. As the organism is re-warmed the two following behavioural thresholds can be observed; the ability to move appendages is the 'chill coma recovery' temperature and the ability to resume

coordinated walking is the ‘activity recovery’ temperature. These temperatures can be coded T_1 , T_2 , T_3 and T_4 .

These temperatures at which activity ceases and recovery occurs can provide valuable data as part of an assessment of the physiological and behavioural response of *Amblyseius swirskii* across a range of ecologically relevant temperatures. Subsequently these data can be taken alongside lethal temperature constraints to provide a more complete assessment of risk of establishment and estimation of efficacy as a biological control agent. In the present study, these four measurable levels of thermal tolerance are referred to as the CT_{min} (T_1), chill coma (T_2), chill coma recovery (T_3) and the activity recovery (T_4).

The following experiments investigated the effect on *A. swirskii* of gradual changes in temperature down to the CT_{min} and then to chill coma. Studies were conducted to determine the effect of temperature on the walking speed of *A. swirskii* and one of its target prey, *Tetranychus urticae*, and also on the predator-prey interactions in such a mite-mite system. This type of study can provide information on the relative efficacy of different control agents in relation to their prey and the environmental conditions under which they would be required to act.

4.2 Aims

The aims of this chapter were to:

1. Determine the CT_{min} and the recovery temperature of *A. swirskii*.
2. Investigate the effect of temperature on walking speeds of *A. swirskii* and *T. urticae*.

3. Assess the effect of temperature on the predator-prey interactions between *A. swirskii* and *T. urticae*.

4.3 Methods

Investigations into low temperature activity thresholds were carried out on four *A. swirskii* treatment groups: acclimated and non-acclimated adults and larvae, and on acclimated and non-acclimated *T. urticae* adults. In the predation experiments, only adult *A. swirskii* were used as the larvae are a non-feeding life stage. The acclimation treatment was the same as that previously described for the cold tolerance experiments i.e. a period of three days at 10°C 18:6 LD cycle. Both *A. swirskii* and *T. urticae* colonies were reared using the same methods as described in Chapter 2.

In the experiments described below it was necessary to monitor the mites continuously to detect the behavioural and physiological thresholds at low temperatures. The experimental technique was similar to that described by Hazell *et al.* (2008) for monitoring aphid behaviour, but was adapted for the smaller size and different activity patterns of the mites. All experiments were performed within an aluminium block, constructed to provide a viewing arena for the mite behaviour (see Figure 4.1). A circular depression (10mm deep, 25mm diameter) was milled in the aluminium block to create the arena in which the mites were housed. A thin layer of OecoTak® was brushed onto the arena wall to prevent escapes and to ensure the mites remained within the field of view. A network of channels was drilled through the block to allow circulation of the cooling or heating fluid and the block was attached to a programmable alcohol bath (Haake Phoenix 11 P2, Thermo Electron Corp.,

Karlsruhe, Germany) by input and output plastic tubes. This enabled the alcohol fluid to be pumped directly underneath and around the mite arena and so the temperature experienced by the mites could be controlled by the selected programme. A small hole was drilled through the block and through the side wall of the arena. This was just large enough for a thermocouple probe to enter into the arena about 1 mm above the arena floor. The probe, attached to an electronic thermometer (Tecpel, Taipei, Taiwan) measured the air temperature within the arena and was linked to the video recording software (Studio Capture DT, Studio86Designs, Lutterworth, U.K.) so that the temperature and activity within the arena could be recorded simultaneously. Activity during the experiment was monitored using a digital video camera (Infinity1-1, Lumenera Scientific, Ottawa, Canada) with a macro lens (Computar MLH-10X, CBC Corp., U.S.A.). During the video recording of each experiment, the arena was covered with a thin sheet of Perspex to minimise the effect of fluctuating laboratory ambient temperatures. The experimental set up allowed for rapid quantification of data for large samples of mites (n=30). For all experiments, the temperature within the arena was initially set at 25°C, the rearing temperature for non-acclimated mites, or at 10°C, for acclimated treatment groups.

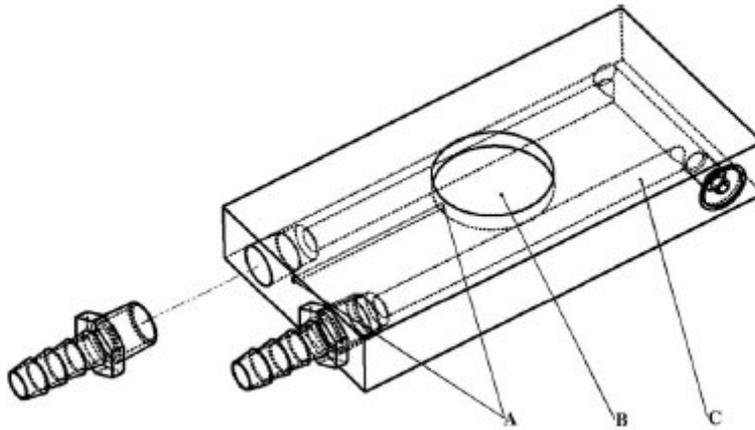


Figure 4.1: Design of the aluminium block system to allow control of the temperature within the mite arena. Point A shows the passage for the thermocouple from the exterior of the block into the side wall of the arena; point B is the mite arena; point C, the channels bored into the block to allow heated or cooled fluid to be pumped around the block (diagram taken from Hazell *et al.*, 2008).

4.3.1 CT_{min} and chill coma

The temperature was reduced from the rearing temperature to 10°C at a rate of 0.5°C min⁻¹ and was then further lowered from 10°C to -5°C at 0.1°C min⁻¹. Mite behaviour was recorded continuously during the experiment and the video footage was reversed before playback (using StudioPlayer, Studio86Designs, Lutterworth, U.K.). The temperatures at which each mite stopped walking (CT_{min}) and then moved an appendage (leg or antennae) for the last time (chill coma) were recorded. Acclimated and non-acclimated adult and larval mites of *A. swirskii* and acclimated and non-acclimated adult *T. urticae* were tested and the CT_{min} and chill coma temperatures were recorded for each mite. Data were tested for distribution and analysed by ANOVA and Tukey's HSD using MINITAB v 14.0.

4.3.2 *Chill coma recovery and activity recovery*

Using a new sample of mites, the arena temperature was lowered from the rearing temperature to 10°C at a rate of 0.5°C min⁻¹ and then further lowered from 10°C to 1°C at a rate of 0.1°C min⁻¹ to 1°C below the previously recorded chill coma temperature. After a hold period of 15 min, the mites were heated back up to 25°C at a rate of 0.1°C min⁻¹. The experiment was recorded and the recovery phase was played back as previously described. The observed temperatures at which the mites began to move an appendage for the first time (chill coma recovery) and then to walk spontaneously (activity recovery) were recorded. Data were tested for distribution and analysed by ANOVA and Tukey's HSD using MINITAB v 14.0.

4.3.3 *Walking speed*

Walking speeds of adult and larval acclimated and non-acclimated *A. swirskii* and *T. urticae* mites were measured at a range of temperatures. The mites were held for 10 min at each test temperature: 30°, 25°, 20°, 15°, 10° and 5°C. Non-acclimated mites were exposed to the highest temperature first and then the arena was progressively cooled to the lowest temperature. Acclimated mites were first exposed at 10°C and then cooled to 5°C, after which they were warmed back up to each higher temperature. This method was chosen so as not to remove any acclimation response that had been acquired with the 10°C treatment. Mite behaviour and movement was recorded during the entire experiment and video footage was examined at each temperature interval to measure the distance covered per unit time from which the walking speeds of the mites could be calculated. The walking speed of 100 mites from each treatment at each temperature was recorded. The mean walking speeds of the

different treatment groups of *A. swirskii* were compared against each other and to that of *T. urticae* by one-way ANOVA and Tukey's HSD.

4.3.4 Predation

Interactions between predatory mites (*A. swirskii*) and their prey (*T. urticae*) were observed at a range of temperatures. Initially, a partition (a 2.5cm x 2cm glass cover slip) was placed into the arena to divide it into two halves. Ten adult *A. swirskii* were placed into one half and 30 *T. urticae* into the other half and allowed to settle for 5 min. The partition was then removed and 10 min of 'interaction behaviour' was recorded by video capture. Experiments were run at 30°, 25°, 20°, 15°, 10° and 5°C. Both acclimated and non-acclimated *A. swirskii* were tested, and for the experiments with acclimated predators, *T. urticae* were also subjected to the same pre-exposure acclimation. After each experiment, the arena was cleaned with 70% ethanol to remove traces of dead or injured mites and then wiped clean with a cotton bud soaked in water. New mites, both predator and prey, were used for each experiment. *A. swirskii* were starved for 12 h prior to the experiments. Observations were made on (i) the frequency with which a predatory mite made contact with a prey mite, (ii) the number of attempts made to handle and attack a prey item, and if successful, (iii) how long the predatory mite remained engaged with its prey. Each experiment was repeated three times at each temperature and the data analysed by one way ANOVA.

4.4 Results

4.4.1 CT_{min} and chill coma

The temperatures at which the last mite in each sample of acclimated and non-acclimated adults and larval *A. swirskii* ceased walking (CT_{min}) were 3.4°, 5.0°, 4.0° and 5.6°C respectively. The equivalent mean values for these groups were 4.7°, 6.2°, 7.4° and 9.9°C. The lowest chill coma temperatures for individual mites in the four treatment groups were 2.7°, 4.4°, 3.0° and 3.6°C respectively, and the mean values were 3.4°, 5.0°, 5.2° and 5.2° (shown in Table 4.1). Figure 4.2 illustrates the mean CT_{min} and chill coma temperatures for each of the mites sampled for acclimated and non-acclimated adult and larval *A. swirskii* and adult *T. urticae* respectively. CT_{min} is represented by a closed symbols and a solid line and chill coma is represented by open symbols and a dashed line. Different colours distinguish the different treatments. All three graphs show the CT_{min} occurring at a higher temperature than chill coma and gives some indication as to the difference in temperature at which these two thresholds occur. Figure 4.2 shows that for *T. urticae*, CT_{min} and chill coma occur at temperatures relatively close to each other.

For all *A. swirskii* treatment groups, the mean chill coma threshold was recorded at a significantly lower temperature than the mean CT_{min} ($F_{7,348} = 44.54$ $P < 0.01$), with the exception of non-acclimated adults where the temperature at which walking ceased (CT_{min}) and the last appendage movement (chill coma) were similar. Acclimated adults had the lowest mean CT_{min} and chill coma threshold of all treatments ($P < 0.05$), but these values did not differ significantly from those of acclimated adult *T. urticae*. Non-acclimated adult *A. swirskii* had a lower mean chill coma than non-acclimated

adult *T. urticae* ($P < 0.05$) and non-acclimated adults had lower mean CT_{\min} and chill coma than larvae of the same treatment. The only effect of acclimation was to significantly lower the temperature difference at the chill coma threshold. Both treatments of *T. urticae* had lower mean CT_{\min} and chill coma threshold temperatures than the larval groups of *A. swirskii* ($P < 0.05$). At temperatures below the chill coma threshold, rapid and violent shivering was observed for all treatment groups of *A. swirskii* at temperatures ranging from 0.3° to -2.9°C .

T. urticae showed no difference between acclimation treatments or between the thresholds CT_{\min} and chill coma, suggesting the temperature at which they stop walking is very close to the temperature at which they enter chill coma.

Table 4.1: Mean (\pm SE) temperatures ($^{\circ}\text{C}$) at which walking stops (CT_{\min}), movement stops (chill coma), movement starts (chill coma recovery) and walking starts (activity recovery) for four treatment groups of *Amblyseius swirskii* ($n=30$).

Mite treatment group	Mean (\pm SE) activity threshold temperatures ($^{\circ}\text{C}$)			
	CT_{\min}	Chill coma	Coma recovery	Activity recovery
Acclimated Adults	4.7 ± 0.24	3.4 ± 0.08	4.3 ± 0.40	7.6 ± 0.63
Non-acclimated Adults	6.2 ± 0.12	5.0 ± 0.09	4.8 ± 0.13	7.6 ± 0.58
Acclimated Larvae	7.4 ± 0.36	5.2 ± 0.30	9.6 ± 0.58	16.4 ± 0.56
Non-acclimated Larvae	9.9 ± 0.54	5.2 ± 0.24	7.8 ± 0.37	14.3 ± 0.47
Acclimated Adult <i>T. urticae</i>	4.3 ± 0.43	2.8 ± 0.21	11.8 ± 0.93	9.3 ± 0.52
Non-acclimated Adult <i>T. urticae</i>	5.5 ± 0.39	4.4 ± 0.26	13.2 ± 1.12	10.8 ± 0.59

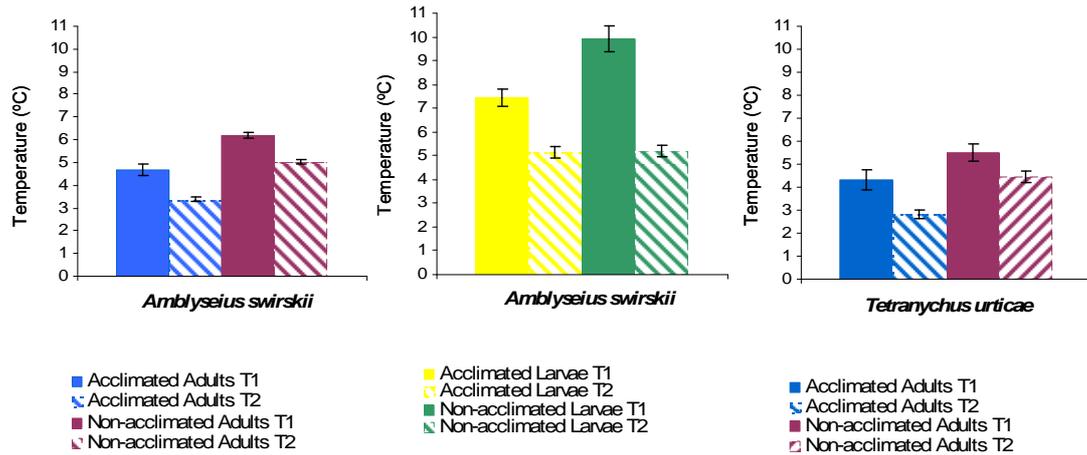


Figure 4.2: Mean threshold temperatures (\pm SE) for acclimated and non-acclimated adult and larval *Amblyseius swirskii* and adult *Tetranychus urticae*: CT_{\min} (T_1) represented by a closed bars and chill coma (T_2) represented by hatched bars. Different colours distinguish the different treatments.

4.4.2 Chill coma recovery and activity recovery

The temperatures at which the first mite from those sampled, began to recover from chill coma were 2.2°, 3.8°, 5.0° and 5.3°C for acclimated and non-acclimated adult and larval *Amblyseius swirskii* respectively. Subsequently the temperature thresholds for resumption of walking activity for the first mite of each treatment group were 4.0°, 4.2°, 12.1° and 9.5°C. It must be noted that the walking activity threshold was not measured for every larval *A. swirskii* observed because even after arena conditions had returned to 25°C, many larvae had failed to begin to walk within the experimental time period. However movement (chill coma recovery) was observed in each mite sampled.

The mean temperatures at which mites began to recover from chill coma were 4.3°, 4.8°, 9.6° and 7.8°C for the first movement and 7.6°, 7.6°, 16.4° and 14.3°C for the first walking step (activity threshold) for acclimated and non-acclimated adults and larval mites respectively (see Table 4.1).

Figure 4.3 illustrates the mean chill coma recovery and activity recovery threshold temperatures of acclimated and non-acclimated adult and larval *A. swirskii* and adult *T. urticae*. Different colours distinguish the different treatments. All three figures show walking activity occurring at a higher temperature than the coma recovery and gives some indication of the difference in temperature at which these two thresholds occur. For the majority of values for *T. urticae* in figure 4.3, the temperatures at which T_3 and T_4 occur are quite close to each other.

For all *A. swirskii* groups, the coma recovery threshold temperature was significantly lower than the activity recovery temperature ($F_{11,313} = 29.53$ $P < 0.01$). There was no difference in the temperatures at which the first movement or the first walking step was made by acclimated or non-acclimated adult *A. swirskii*, but both thresholds occurred at significantly lower temperatures than were recorded in either larval group ($P < 0.01$). Adult *A. swirskii* resumed activity for both movement and walking at lower temperatures than the larvae and *T. urticae* adults ($P < 0.05$) and acclimation in the larval groups produced no difference between treatments. Figure 4.6 shows coma recovery and activity recovery for acclimated and non-acclimated larvae; the activity recovery data for all of sample points is incomplete, because even after the arena temperature had returned to the rearing temperature of 25°C, a considerable proportion (57 and 53%; acclimated and non-acclimated respectively) of larvae had still not resumed walking. All larvae were transferred to recovery tiles and returned to the rearing conditions and were observed for 24 hours; 100% of larvae resumed walking within 4 and 24 h after exposure. None of the mites died in the 10 days following exposure to their chill coma threshold. *Tetranychus urticae* did not show

significant differences between chill coma recovery and activity recovery thresholds and also, no acclimation response was recorded.

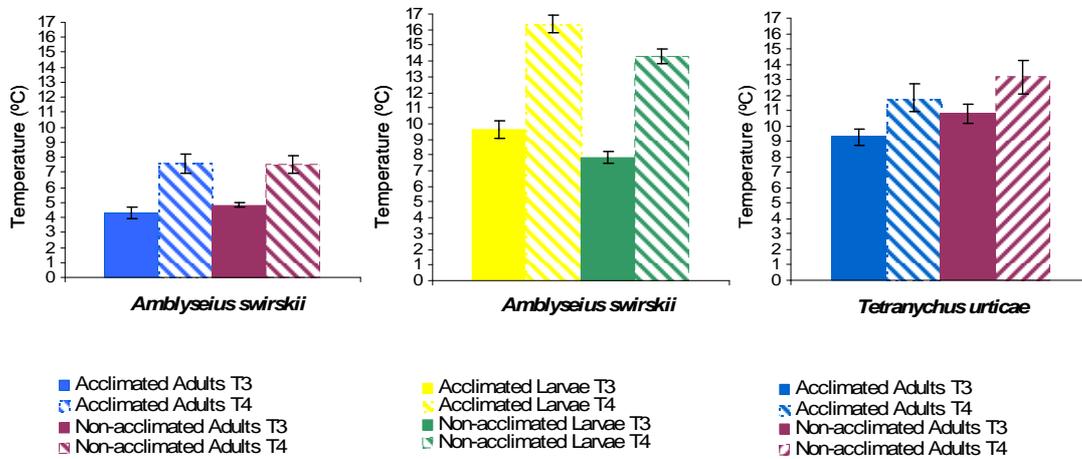


Figure 4.3: Mean threshold temperatures (\pm SE) for acclimated and non-acclimated adult and larval *Amblyseius swirskii* and adult *Tetranychus urticae*: chill coma recovery (T₃) represented by a closed bars and activity recovery (T₄) represented by hatched bars. Different colours distinguish the different treatments.

4.4.3 Walking speed

The mean walking speeds for acclimated and non-acclimated adult *A. swirskii* and *T. urticae* are shown in Figure 4.4. Walking speeds at 5°, 10°, 15°, 20°, 25° and 30°C were recorded as 0, 1.2, 2.1, 3.5, 5.1 and 5.9 mm s⁻¹ and 0, 0.2, 0.4, 0.7, 1.1 and 1.4mm s⁻¹ for non-acclimated adult *A. swirskii* and *T. urticae* respectively. For all mites the walking speed increased with temperature.

Amblyseius swirskii moved fastest at 30°C (P<0.01) and walking speed decreased significantly at each lower temperature (P<0.05). A negative acclimation response was observed for *A. swirskii* at 30° and 25°C; non-acclimated mites walked significantly faster at these temperatures (P<0.05). At no other temperature was there any difference in walking speed between acclimated and non-acclimated mites.

At each experimental temperature, *A. swirskii* moved faster than *T. urticae* ($P < 0.01$) with the exception of acclimated adults at 5°C when walking speeds were 0.31 and 0.16 mm s⁻¹ respectively. An acclimation response was seen in *T. urticae* at 30°C, where acclimated mites walked faster than non-acclimated, but this response was not evident at any other temperature. Walking speed of *T. urticae* at 30°C was faster than at 25°C, which in turn was faster than at 20°C, which was faster than at 10° and 5°C ($P < 0.05$). Walking speeds at 15°, 10° and 5°C did not differ. At 5°C, no walking occurred for non-acclimated mites of either species.

It was not possible to obtain reliable data on the walking speeds of larval mites, as individuals failed to maintain walking movement long enough for an accurate calculation of speed to be made. This result was also apparent following the chill coma recovery experiments where a large proportion (58.3%) of larval mites failed to resume any walking response after recovering from chill coma.

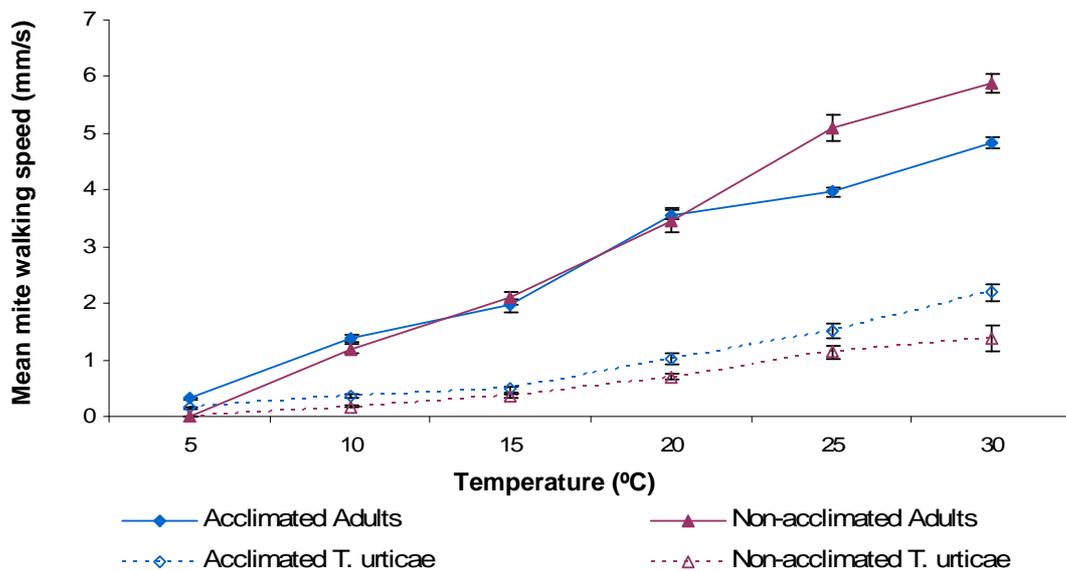


Figure 4.4: Mean (\pm SE) walking speeds (mm/s) of acclimated (blue) and non-acclimated (purple) adult *Amblyseius swirskii* (solid lines) and adult *Tetranychus urticae* (dashed lines) (n=100).

4.4.4 Predation

The mean number of contacts with prey (closed bars), attempts to grasp and handle prey (hatched bars) and time spent engaged with prey (line) are shown in Figure 4.5 for acclimated and non-acclimated adult *A. swirskii*. At each temperature there was no difference between the predatory activity of acclimated and non-acclimated mites. The mean number of times that *A. swirskii* made contact with items of prey were 14.6, 12.4, 8.2, 7.8 and 5.1 at 10°, 15°, 20°, 25° and 30°C respectively and the relationship between these two values is a negative correlation. There was a significant difference between number of contacts made with items of prey between 10° and 20°C, and between 25° and 30°C ($P < 0.05$). At 15°C, the number of contacts with prey was significantly higher than at 25° and 30°C ($P < 0.05$). Attempts to handle prey are lower at 10° and 15°C than at temperatures above 20°C ($P < 0.05$).

When making comparisons between the number of contacts and the number of attempts to attack and handle prey (that is, how many contacts with prey turned into attack events), there are significant differences between these two values at 10° and 15°C ($P < 0.01$), suggesting that *A. swirskii* are less likely to attempt to handle a prey item even when they come into contact with them at these low temperatures. At 20° and 25°C the difference is less, but still significant at $P < 0.05$. At 30°C there was no difference between the number of contacts made and the number of attempts to handle the prey suggesting that *A. swirskii* attempt to handle and attack all of the prey items they come into contact with at this temperature. At temperatures of 15°C and below, *A. swirskii* appeared to actively move away from contact stimulus with mites of both the same species and with their prey. Prey recognition behaviour also appeared to be

reduced at lower temperatures, shown as a delay between making a contact with a mite and the process of prey recognition.

The amount of time spent engaged with and handling prey was significantly higher at 25° and 30°C than at 10°, 15° and 20°C ($P < 0.05$). There were no differences in handling time at 10°, 15° and 20°C.

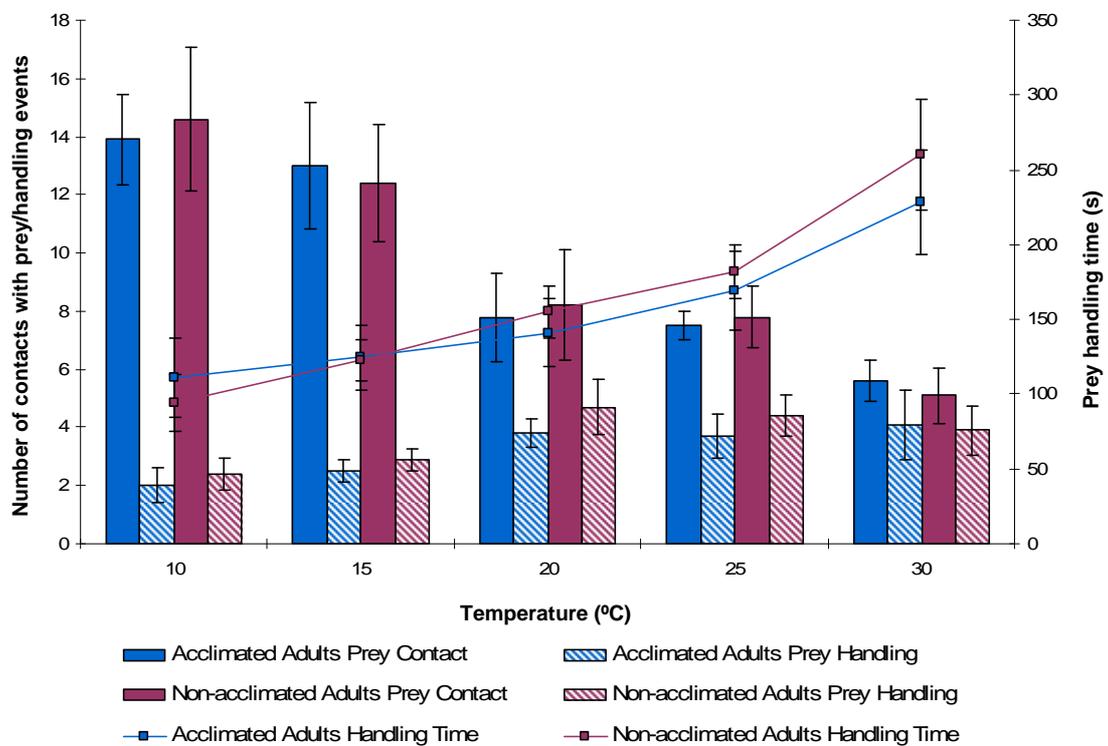


Figure 4.5: Mean (\pm SE) number of contacts between acclimated and non-acclimated adult *Amblyseius swirskii* and their prey (closed bars), mean number of attempts to handle prey items (hatched bars) and mean time in seconds to handle each prey item (line). Error bars on all points indicate the standard error of the mean. (n=30)

4.5 Discussion

The assessment of critical thermal activity thresholds is a first step in understanding the range of conditions under which a predator might be active, and the likely effect that local climatic variation and longer term climate change might have on the species (Jumbam *et al.*, 2008). The present study has found that changes to ambient temperature affects the activity of *Amblyseius swirskii*. The temperature at which insects enter chill coma and the time taken to recover from it are known to be reduced by acclimation in certain species (Mason 2007) and *A. swirskii* appears to follow this rule as for three of the low temperature thresholds; CT_{min} , chill coma and chill coma recovery, acclimated adult *A. swirskii* responded at significantly lower temperatures than non-acclimated adults. Hazell *et al.*, (2008) speculate that thresholds T_1 and T_4 ; the temperature at which walking ceases (CT_{min}) and the temperature at which walking resumes (activity recovery), most likely reflect behavioural response thresholds and as the chill coma threshold of *A. swirskii* was recorded at a significantly lower temperature to that of the last step (CT_{min}) this does suggest that walking is a behavioural response which varies with temperature and is voluntarily halted to reduce metabolic costs at low temperatures. However, non-acclimated adults did continue to walk at temperatures close to their chill coma threshold which may have been in response to the stress of the cold exposure. The minimum chill coma temperature of non-acclimated adult *A. swirskii* was 4.4°C compared to 2.7°C for *T. urticae* and the CT_{min} for both species were 5.0° and 3.3°C respectively. This illustrates *A. swirskii* would enter into cold torpor at temperatures that *T. urticae* would be remain mobile. The mean temperatures at which non-acclimated *A. swirskii* resumed movement (chill coma recovery) and began walking (activity recovery) were

4.8° and 7.6°C respectively. In comparison, *T. urticae* has much higher recovery threshold temperature at 10.8°C and 13.2°C for movement and walking respectively. Therefore, although *A. swirskii* enter cold torpor at higher temperatures than *T. urticae* they also recover and resume activity more quickly which would provide a direct advantage to the predatory mite.

The chill coma threshold temperature for larvae was 3.6°C which is very close to the 3.3°C estimation of the LT_{10} (exposure to temperature to result in 10% mortality) (see Chapter 3). This may provide an explanation for the observation that following the coma recovery experiments and exposure to the chill coma threshold, many individuals failed to begin walking at any temperature up to 25°C and only resumed the ability several hours after being returned to the optimal rearing conditions. CT_{min} occurred at 5.6°C for larval mites which is only 0.6°C above that of the adult *A. swirskii* suggesting that there is very little variation in the activity response to low temperatures within different life stages of the species.

At temperatures below the chill coma threshold, rapid and violent shivering was observed for all treatment groups of *A. swirskii* at temperatures ranging from 0.3° to -2.9°C. Undoubtedly this was due to the frequency of muscle action potentials and resting potentials diminishing with decreasing temperature (Staszak & Mutchmor, 1973b). As the temperature decreases, failure of the muscles to maintain resting potential, disrupts the calcium current across muscle cell membranes, which can trigger a series of bursts of muscle action potentials (Hosler *et al.*, 2000). It is argued that this point is in fact the true chill coma temperature (Staszak & Mutchmor, 1973a) but as muscles in different parts of the insect body can lose the ability to maintain

resting potentials at different times it is often very difficult to pinpoint the exact onset of chill coma using this method. Such a noticeable effect on muscle functionality in this species may be yet more evidence of *A. swirskii*'s inability to tolerate low temperatures.

For the prey species, *T. urticae*, there were no significant difference between either the temperature at which they ceased walking and moving and at the temperatures when they resumed movement and walking. This would suggest that as a prey species, they necessitate the ability to walk as it is beneficial to remain mobile, until otherwise incapable, to avoid predators. Interestingly, it was observed that a number of *T. urticae* flipped over onto their dorsal side during the chill coma experiments. In this position they were unable to right themselves and hence remained immobile. This inability to right oneself has traditionally been used as a measure of the chill coma temperature (Klok & Chown 1997, 2001). However, in this case it would have been a gross underestimate of the true CT_{min} as if disturbed by other mites and rolled back on to their ventral side, all individuals were able to resume walking and active mobility (grooming etc).

The walking speed experiments confirmed observations of mobility of *A. swirskii* compared to *T. urticae* when observed on the rearing stages, that is the predatory mite is a much faster moving species. The predatory nature of *A. swirskii* dictates that it should be an active and highly mobile species but its activity is markedly effected by changes in temperature. At 20°C non-acclimated adult *A. swirskii* can move at almost five times the speed of *T. urticae* (3.47mm s^{-1} compared to 0.69mm s^{-1}). Acclimated

mites of both species were able to illustrate some mobility at 5°C but it was limited when compared to walking speeds at temperatures above 5°C.

The inability to obtain good data for walking speeds and predatory activity for larval *A. swirskii* confirms Swirskii *et al.*, (1967) early research in the developmental biology of *A. swirskii* in that the larvae are a non-feeding and relatively static developmental stage. The data also suggests that walking at speeds for *T. urticae* of over 0.7mm/s requires a minimum temperature requirement of 20°C. This is probably due to the heavily armoured exoskeleton of the mite (Woolley 1988), position of legs underneath the body and a relatively lumbering frame.

Mites of the family Phytoseiidae have been shown to exhibit a type II functional response to prey density (as described by Hollings Disk equation). This means the rate of prey consumption by a predator rises as prey density increases, but eventually levels off at a plateau at which the rate of consumption remains constant regardless of increases in prey density (Sepulveda & Carrillo 2008). Although research has been conducted into the effect of prey density on phytoseiid mites very little work involves the effect of temperature on predation. Temperature appears to have a direct effect on the predatory activity of *A. swirskii* as when temperature increased the number of attempts to handle prey items when contacted also increased. Prey handling time also increased at the higher temperatures indicating that *A. swirskii* are more willing to spend more time feeding from each prey item. These results partially correspond with research into the predatory ability of *Scolothrips takahashii* (Priesner) (Thysanoptera: Thripidae), a predator of the hawthorn spider mite *Tetranychus viennensis* (Zacher) (Acarina: Tetranychidae) where attack rate increased linearly with temperature yet

interestingly only in the females who exhibited the most voracious predatory ability while attack rate in males was independent of temperature. However, handling time of *S. takahashii* decreased with increasing temperature. In the present study only adult females were used to (i) correspond with experimental methods in the previous experiments on cold tolerance and (ii) due to the fact that females are considerably more active as predators (C. Allen personal observations).

Perhaps at higher temperatures the *A. swirskii* have increased nervous and muscular control to enable them to grasp and subdue prey without risking injury or expelling excessive energy reserves in the process. As a result of spending more time engaged with individual prey items, the number of contacts made during the observed period are reduced because the mites' free time in active foraging mode is reduced. At lower temperatures, *A. swirskii* were observed to move away from collisions with both mites from their own species and of the prey species. This unwillingness to engage with prey items is depicted in the data. At higher temperatures mites portrayed a more robust behaviour toward contacts with other items. Time was given over to detecting that item and either moving away (generally in the case of same-species collisions) or making an attempt to engage. This apparent preference to feed at higher temperatures is most likely due to increased metabolism at these temperatures. In contrast mean daily and total prey consumption of *Panonychus ulmi* (Koch) (Acari, Tetranychidae) by adults of the predatory mite *Typhlodromus pyri* (Scheuten) (Acari, Phytoseiidae) decreased significantly as the temperature was increased from 25° to 30°C (Sengonca *et al.*, 2003). A similar response is seen in Skirvin and Fenlon (2003) where results showed that more *T. urticae* are eaten by *Phytoseiulus persimilis* (Athias-Henriot) (Acari : Phytoseiidae) as the temperature increases from 15° to 25°C, but the number of prey eaten then declines at 30°C.

To further research the predatory ability of *A. swirskii*, investigations into preference of prey life stage could be conducted. The ladybird *Stethorus punctillum* (Weise) (Coleoptera: Coccinellidae) shows a distinct preference for either *T. urticae* eggs, nymphs or adults depending on its own developmental stage. Perhaps counter-intuitively the adult *S. punctillum* show a significant preference to *T. urticae* eggs whereas the larval stages show no preference over life stage (Ragkou *et al.*, 2004). Perhaps this may be due to the energy expense of subduing an adult prey item would be considerably more than that required to find and consume a stationary egg.

The experiments carried out in this study were not intended to show the true regulative power of *A. swirskii* as a predator of *T. urticae* and they were not an attempt to determine the functional response (the change in prey number killed per individual predator per unit of time) as described by Solomon (1949). What the data of the present study is hoped to provide is an extension to information on the efficacy, behaviour and physiology of a biological control agent at various temperatures within an ecologically relevant range. This could provide growers with a wealth of information to make a good decision on which biological control agent would be most suited to the particular conditions of their growing environment. Walking speed data at different temperatures could also be extended into studies of dispersal potential of escaping individuals from the glasshouse environment. CT_{min} and chill coma recovery data can also be used as broad indicators of cold tolerance as they play an important role in determining species range distributions (Andrewartha & Birch 1954), and can determine whether an invasive species will become established (Ward & Masters

2007). Low temperature activity thresholds will also help to shed light on how species populations and distributions may be altered by climate change (Walther *et al.*, 2002). With regard to the dispersal ability of *A. swirskii*, a period of acclimation did not significantly increase the mobility at lower temperatures and even at 5°C when only acclimated mites were able to walk their speed did not differ significantly from that of non-acclimated. Principally the cold tolerance of *A. swirskii* demonstrated in Chapter 3 would ensure that this species would find survival for extended periods of time outside of the glasshouse in a UK winter climate almost impossible and therefore dispersal and activity thresholds in an outdoor environment should not be applicable. However dispersal from the glasshouse during summer months can not be discounted and assuming *A. swirskii* could find food and mates reproduction and population development has been shown to be likely. However the voltinism of *A. swirskii* would be relatively short lived and tightly constrained to the high summer months therefore any effect on the ecosystem outside of the glasshouse environment would likely be transient. The application of most use for this data would be the matching of *A. swirskii* as a biological control agent, to specific target prey pest species and optimal conditions in the glasshouse.

CHAPTER 5

Thermal biology of *Phytoseiulus longipes*

5.1 Introduction

Phytoseiulus longipes is a Phytoseiid mite originating from South America and South Africa with its geographical range being entirely restricted to the southern hemisphere (Takahashi & Chant, 1993; Moraes *et al.* 2004). Strains from Argentina (Herrero *et al.*, 1990), Chile (Gonzalez & Schuster 1962) and Brazil (Furtado *et al.*, 2006) are being targeted as a potential biological control agents. It is a specialist predator of the two spotted spider mite *Tetranychus urticae* and performs all of its life functions exclusively within spider mite colonies (McMurty & Croft 1997). At the present time *P. longipes* is commercially developed as a biocontrol agent

At the present time, it appears that there have been few if any studies on the cold tolerance of *Phytoseiulus longipes* or its suitability for use as a biological control agent in the UK. Knowledge of the cold tolerance of *P. longipes* can be used as an indicator of its establishment potential in the UK and similar cool temperate climates (Hatherly *et al.*, 2004), as discussed for *A. swirskii* in Chapter 3. A licence for its release as a biological control agent for use in UK glasshouses against the phytophagous pest mite *Tetranychus urticae* would be dependent on an assessment of its ability to survive UK winter conditions and therefore the likelihood that it could establish wild populations outside of the glasshouse environment. This chapter describes a series of experiments on a strain of *P. longipes* originally collected in

Chile to determine the effects of temperature on its development, cold hardiness under laboratory conditions, ability to diapause and winter field survival.

5.2 Aims

The aims of this chapter were to:

1. Determine the developmental threshold of *P. longipes* and predict its potential voltinism in the UK.
2. Investigate the cold hardiness of *P. longipes* under laboratory conditions.
3. Investigate conditions that might induce a diapause response.
4. Assess survival in the field during winter and compare with laboratory data.

5.3 Methods

The majority of methods and experimental designs employed in the experiments with *P. longipes* are the same as those described in Chapter 3 for the studies on *A. swirskii*. Where differences occur, they are described in this chapter. The rearing conditions for *P. longipes* were considerably different to those used for *A. swirskii* and relevant details are given in Chapter 2. For the same reasons, it was also necessary to modify the set up of some experiments with *P. longipes* and this information is described in the relevant sections of this chapter.

5.3.1 Developmental time

The developmental times of *P. longipes* were studied in incubators at 10°, 12 °, 15 °, 18°, 20°, 23°, 25°, 27°, 30°, 32° and 35°C, 18:6 LD cycle and 80-90% relative humidity. The experimental set up was similar to that used for *A. swirskii* with the exception that moist filter paper provided the base for each arena and a small square of cucumber leaf (1cm²) was placed underside up into each arena. Each tile contained 20 arenas and therefore three tiles were placed at each temperature. For *P. longipes* it was essential to provide *T. urticae* as prey, without which, the predator is unable to complete its development. *P. longipes* are specialist predators of *T. urticae* and their reproduction and development are dependent on them (Ashihara *et al.*, 1978, Badii & McMurty, 1984). Feeding with *T. urticae* causes the arenas to ‘dirty’ with debris, which makes it more difficult to see the nymphal cast skins shed at each moult. As this was unavoidable, during each inspection, it was necessary to clear the debris from each arena and carefully check for signs of the moulted cuticle.

The developmental times of the first 50 mites to complete their development at each temperature were recorded, log transformed and the differences between temperatures and life stages were analysed by Two-way ANOVA and then by pairwise comparisons using Tukey’s honest significant difference (HSD) method.

The data were also analysed by a simple linear regression between temperature and rate of development (Draper & Smith 1981) from which the developmental threshold temperature was extrapolated. The day degree requirement per generation was determined by taking the reciprocal of the slope (Campbell *et al.*, 1974). These data were used in conjunction with temperature recordings taken by the School of Geography, Earth and Environmental Sciences at the University of Birmingham from

which annual number of day degrees was calculated, which in turn, provided an estimate of the number of generations per year of *P. longipes* that was possible under outdoor conditions.

5.3.2 Diapause

Ability to enter diapause was investigated in the same way as for *A. swirskii* described in Chapter 3. The rearing temperature (28°C) was lowered to 18°C and the light regime changed to 6:18 LD. The experimental rearing system used for *P. longipes* differed from that of *A. swirskii* as described in 2.2.3. Thus, individual arenas contained a moist filter paper base supporting a square of cucumber leaf upon which the *P. longipes* female was placed together with *T. urticae* as prey, which were fed to the predatory mites by brushing onto the arenas once a day. Mites were considered to have entered diapause if they did not oviposit when in the diapause regime but then resumed oviposition when returned to the original rearing conditions, after a lag period of 4 days (Jolly 2000). Eggs laid by the first generation females under the diapause conditions were again kept in the same regime and reared to adulthood (becoming the second generation) and monitored for oviposition. Data were analysed by One-way ANOVA.

5.3.3 SCP

Supercooling points (SCP) of acclimated and non-acclimated adult mites were measured at the University of Birmingham using PicoLog data acquisition software.

The SCPs of acclimated and non-acclimated larval *P. longipes* were measured by a differential scanning calorimeter (DSC) at the British Antarctic Survey, Cambridge. A

full description of this method is given in section 3.3.3. The data were analysed by One way ANOVA.

5.3.4 Lower lethal temperatures

The lower lethal temperatures of acclimated and non-acclimated adult and larval *P. longipes* were assessed by the same method as described for *A. swirskii* in section 3.3.4. The mites were cooled at $0.5^{\circ}\text{C min}^{-1}$ from 20°C to a range of temperatures between 5° and -20°C . Acclimated mites were cooled from 10°C (the acclimation temperature) to the same range of temperatures. Mites were held at the required exposure temperature for 5 min and then re-warmed to 20°C at the same rate. A control sample of 50 mites was tested to check for any deleterious effects of the Beem capsules or vibrations of the alcohol bath. After the required exposures, the mites were then removed from the capsules and placed onto individual recovery 'leaf' arenas with food and water provided. Recovery tiles were held at 20°C 18:6 LD and survival was assessed immediately after exposure and then after a further 24 and 48h. All surviving individuals were transferred to arenas with a moisture source, cucumber leaf and *T. urticae* and held at 28°C 18:6 LD. The arenas were monitored to determine if the larvae could develop through to adulthood and the adult females could oviposit as normal.

The results were log transformed and analysed by Probit (Finney 1971) to estimate the lethal temperatures at which 10, 50 and 90% of the sample populations were killed (LTemp_{10, 50, 90}). The analyses were run in MINITAB v 14.0.

5.3.5 Lower lethal time

Lower lethal time experiments were run at 5°, 0° and -5°C to assess the survival of acclimated and non-acclimated adult and larval *P. longipes* in longer exposures at less severe temperatures. The experimental set up, temperatures and regimes for the LTime experiments was the same as for those described in section 3.3.5. Five replicates of 10 individuals from each age and treatment group were taken from the three exposure temperatures at a variety of time intervals so as to determine the progress in mortality with increasing periods of exposure. A control population of 50 adults and 50 larval *P. longipes* was placed at 5°C on separate rearing arenas with water and food provided. The mites were monitored every day to ascertain the maximum survival time for individuals when food and water were provided.

The results were assessed in MINITAB v 14.0 using Probit (Finney 1971) to estimate the lethal times at which 10, 50 and 90% of the sample populations were killed (LTemp_{10, 50, 90}) at 5°, 0° and -5°C.

5.3.6 Field exposures

Four treatment groups were studied (adults and larvae, acclimated and non-acclimated) and exposed to outdoor winter temperatures in much the same way as previously described for *A. swirskii*. Because of the problems of rearing large numbers of *P. longipes* for field experiments and because provision of food was not seen to significantly alter field survival in preliminary experiments, all treatment groups were provided with food; thus in these experiments, the observed mortality could be attributed to the temperatures experienced rather than starvation. Adult mites used in the field exposures were all female and had moulted from the

deutonymph stage no more than 48 h prior to being placed into the field and the larvae were less than 12 h old.

Ten mites from each of the four treatment groups were placed into specialised 'field test vials' consisting of an 8cm diameter Petri dish containing a 1cm layer of agar (2%) on top of which a cucumber leaf was placed, underside facing up. A sample of *T. urticae* adults was brushed on to the leaf and then left for 24 h at 28°C, 18:6 LD for the adults to produce webbing and oviposit. The agar provided a moisture source for the leaf and the mites. A 4 cm diameter hole was cut out of the lid of each Petri dish and covered with 75µm muslin for ventilation. The mites were exposed in groups of ten in each vial as preliminary observations indicated that there was no cannibalism among *P. longipes* in the same life stage (C. Allen unpublished observations).

Sufficient vials to allow 4 replicates of 10 individuals of each treatment group to be sampled at 10 time intervals were secured in plastic boxes and placed in the field location as previously described. The boxes were placed in a sheltered location and protected from direct sunlight and the effects of the wind. At each sampling interval, 4 vials from each treatment group were brought in the laboratory and survival assessed using the same methods as described for *A. swirskii*.

Two field experiments were carried out: the first from 15th December 2007 to 18th January 2008, and the second from 25th January to 7th April 2008. By running the field trails from December until early April it was envisaged that a range of temperatures would be experienced, and the exposures would give some indication as

to whether escapes from glasshouses at different times in winter were more or less likely to survive.

The mortality data of field populations were transformed, tested for normality and analysed using a General Linear Model and pairwise comparisons were made between treatments using Tukey's HSD. A control sample of 40 non-acclimated adult and 40 non-acclimated larval mites were placed in field test vials and kept in an incubator at 28°C, 18:6 LD for 1 week to test for any deleterious effects of the experimental set up.

Short term field exposures were carried out to illustrate outdoor survival over 1 and 2 week periods throughout the winter months of 2007 and the spring and summer period of 2008. Short term field experiments may highlight any temperature regimes where 100% mortality occurs in all groups of *P. longipes* and also conversely, which temperature regimes supported 100% survival.

Ten mites, from each of the 4 groups (acclimated and non-acclimated adults and larvae), were placed into field test vials as previously described. *T. urticae* was provided as food in all vials because the mini trials were extended into the spring and summer season to investigate survival at warmer temperature regimes, it is assumed that lack of food at these temperatures will have a deleterious effect and contribute to mortality rates. 2 boxes, each containing 40 vials of each mite group and a data logger were put out in a sheltered field location at the University of Birmingham. After each week of exposure, 4 replicates of 10 samples for each group were brought in and the percentage mortality was assessed. The data from each week of the short term field exposures were collated and analysed by Probit to estimate the temperatures required to cause 10, 50 and 90% field mortality of *P. longipes*.

5.4 Results

5.4.1 Developmental time

The proportion of individual eggs that hatched at each temperature are shown in Figure 5.1, indicating that a good level of survival occurs at temperatures between 15° and 32°C. At 10°C, 23% of individuals hatched after 12-15 days, but none of these moulted to protonymphs and all were dead by day 35. At 12°C, 45% of individuals hatched after 7-20 days, but again, none moulted to protonymphs and all were dead by day 40. At 15°C, 30% of individuals hatched, 20% of which moulted to protonymph, but all died by day 7. The data for all of these temperatures were omitted from the analysis because they were incomplete. At 32°C, the egg hatch rate was significantly lower than at 15°, 18°, 20°, 23°, 25°, 27° and 30°C and developmental time from egg to adult was longer at 32° than at 30°C; these data were therefore also omitted from the analysis to estimate the developmental threshold.

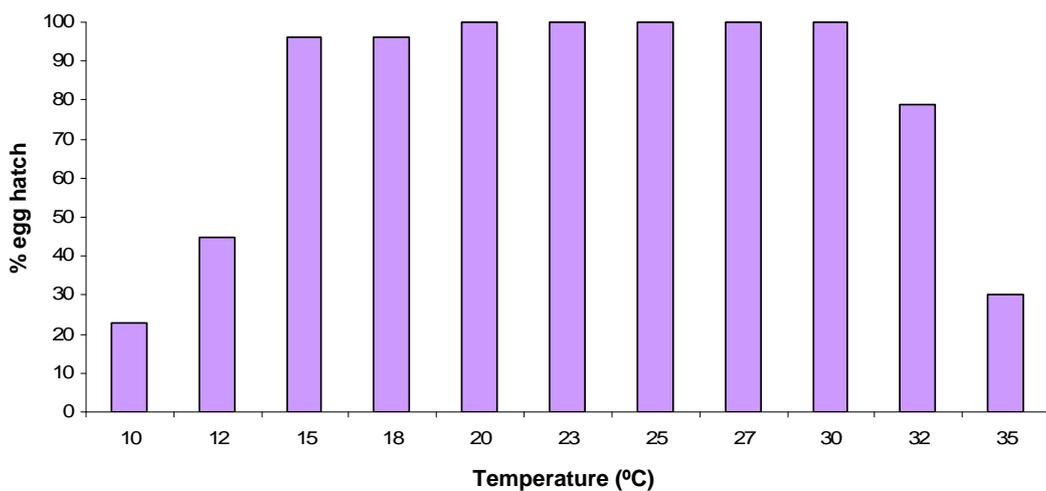


Figure 5.1: Percentage egg hatch of *Phytoseiulus longipes* at temperatures ranging from 10° to 35°C (n = 80).

The developmental times for each life stage of *P. longipes* at different temperatures are shown in Table 5.1. Between 15 and 30°C, the time taken to complete development to the adult stage decreased with temperature. Total developmental time, from egg to adult, at 15°C was significantly longer than at all other temperatures ($P<0.01$). Similarly the time to mature to the adult stage at 18°C was longer than at 20°C ($P<0.01$), at 20° than 23°C ($P<0.01$), at 23° than 25°C ($P<0.01$) and at 25° than at 27° and 30°C ($P<0.01$). Development was significantly faster at 30° than at 32° and 27°C ($P<0.05$ and $P<0.01$ respectively). The optimum temperature for development from egg hatch, through all three immature stages to the adult form was therefore 30°C.

When comparing developmental rate at each life stage the time to egg hatch and time spent as larvae were significantly longer at 15°C and 18°C ($P<0.01$) than at all other higher temperatures, and shorter at 30° ($P<0.05$) than at 27° and 32°C. There were no significant differences between time to egg hatch and time spent as larvae at 25°, 27° and 32°C. In general, all developmental stages were significantly ($P<0.05$) shortened as the temperature increased from 15°C to 30°C. In all cases, 30°C was the optimum temperature for development to the subsequent life cycle stage. For all developmental stages, the time spent at that stage was not significantly different at 27° and 32°C.

When comparing the time spent at each life stage, the larval stage was significantly shorter at temperatures between 15° and 27°C than all other life stages. The deutonymph and egg stage were significantly longer than all other stages at temperatures below 25°C ($P<0.01$). At temperatures close to the optimum (30°C), the developmental time for each life stage became far less varied; however, the deutonymph was always the longest life stage ($P<0.05$).

Table 5.1: Effect of temperature on the mean developmental time (days \pm SE) at each life stage of *Phytoseiulus longipes* (n=50).

Temperature (°C)	Egg hatch	Larvae	Protonymph	Deutonymph	Total time egg-adult
15	6.2 \pm 0.13	1.7 \pm 0.06	3.7 \pm 0.11	4.6 \pm 0.13	16.1 \pm 0.19
18	3.9 \pm 0.10	1.3 \pm 0.07	2.3 \pm 0.06	3.2 \pm 0.09	10.7 \pm 0.15
20	3.1 \pm 0.11	1.0 \pm 0.08	2.1 \pm 0.05	2.3 \pm 0.06	8.5 \pm 0.18
23	2.1 \pm 0.10	0.9 \pm 0.04	1.5 \pm 0.07	2.0 \pm 0.07	6.4 \pm 0.13
25	1.7 \pm 0.07	0.7 \pm 0.04	1.4 \pm 0.07	1.5 \pm 0.09	5.3 \pm 0.16
27	1.0 \pm 0.06	0.6 \pm 0.04	0.9 \pm 0.07	1.2 \pm 0.05	3.7 \pm 0.11
30	0.6 \pm 0.04	0.6 \pm 0.03	1.0 \pm 0.03	1.5 \pm 0.07	3.6 \pm 0.11
32	1.6 \pm 0.07	0.7 \pm 0.06	1.0 \pm 0.06	1.4 \pm 0.09	4.0 \pm 0.20

The developmental rate of *P. longipes* is plotted against temperature in Figure 5.2. The temperature at which development can first occur is the lower developmental threshold. When estimated by simple linear regression, the threshold temperature for *P. longipes* is 12.1°C and the developmental rate (1/days) forms an approximately linear relationship with temperature between 12 and 27°C. *P. longipes* requires a thermal budget (K) of 57 degree days above the threshold temperature to complete development from egg to adult. This value is calculated from the linear regression and is the reciprocal of the slope of the line (1/0.0173). The high R² value indicates that approximately 80% of the variation is accounted for in this relationship.

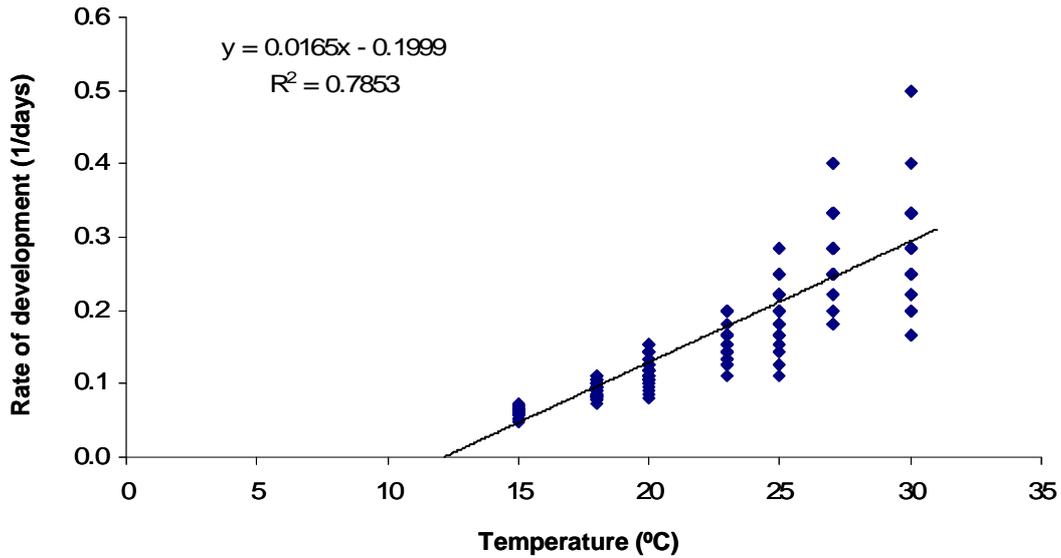


Figure 5.2: Rate of egg to adult development of *Phytoseiulus longipes* (1/days) at a range of temperatures between 15° and 30°C. Line fitted by simple linear regression $y = 0.0165x - 0.1999$ ($R^2 = 0.7853$).

The number of day degrees available each year to *P. longipes* from 1987-2007 were calculated from temperature records as shown in Table 5.2, together with the theoretical number of generations that *P. longipes* would be able to complete each year based on the number of available day degrees. Summer months and winter months have been distinguished from each other to show the potential for population growth within each season. Between the beginning of April and the end of September the mean theoretical number of generations was determined to be 7.5 compared with 0.25 during the winter period.

The estimates of developmental threshold and voltinism indicate that, in reality, from 1987-2007 *P. longipes* would be able to complete an average of 7 generations per year in the Midlands region of the UK. Development and consequently population

increase would be confined to the summer months only (April-September) of each year. Temperature data from 1987-2007 show that temperatures above the developmental threshold of *P. longipes* (12.1°C) are reached only between the beginning of May and middle of September (Figure 5.3). During this time, between 4 and 10 generations could be expected to occur. *P. longipes* would then be required to survive over 6 months of the year at temperatures below its developmental threshold and some exposures as low as -7°C.

Table 5.2: Theoretical number of generations of *Phytoseiulus longipes* each year in summer (between April and September) and winter (between October and March) from 1987-2007 in Birmingham, UK, based on simple linear regression estimation of the developmental threshold (bracketed values refer to the actual number of possible generations).

Year	Available °d per year	Max. no of generations per year	Available °d Apr-Sept	Max. no of generations Apr-Sept	Available °d Oct-Mar	Max. no of generations Oct-Mar
1987	334.4	5.5 (5)	327.9	5.4 (5)	6.5	0.11 (0)
1988	294.9	4.9 (4)	289.1	4.8 (4)	5.8	0.10 (0)
1989	555.2	9.2 (9)	544.7	9.0 (9)	10.5	0.17 (0)
1990	523.6	8.6 (8)	488.4	8.1 (8)	35.2	0.58 (0)
1991	471.0	7.8 (7)	461.1	7.6 (7)	9.9	0.16 (0)
1992	452.3	7.5 (7)	452.3	7.5 (7)	0.0	0.00 (0)
1993	322.7	5.3 (5)	322.3	5.3 (5)	0.4	0.01 (0)
1994	426.7	7.0 (7)	414.9	6.8 (6)	11.8	0.19 (0)
1995	621.1	10.2 (10)	580.3	9.6 (9)	40.8	0.67 (0)
1996	422.6	7.0 (7)	408.3	6.7 (6)	14.3	0.24 (0)
1997	516.6	8.5 (8)	491.2	8.1 (8)	25.4	0.42 (0)
1998	412.2	6.8 (6)	397.1	6.6 (6)	15.1	0.25 (0)
1999	474.9	7.8 (7)	463.5	7.6 (7)	11.4	0.19 (0)
2000	433.5	7.2 (7)	428.9	7.1 (7)	4.6	0.08 (0)
2001	487.6	8.0 (8)	448.9	7.4 (7)	38.7	0.64 (0)
2002	431.1	7.1 (7)	420.9	6.9 (6)	10.2	0.17 (0)
2003	574.8	9.5 (9)	565.3	9.3 (9)	9.5	0.16 (0)
2004	518.9	8.6 (8)	512.2	8.5 (8)	6.7	0.11 (0)
2005	512.4	8.5 (8)	481.4	7.9 (7)	31.0	0.51 (0)
2006	649.0	10.7 (10)	628.9	10.4 (10)	20.1	0.33 (0)
2007	389.7	6.4 (6)	377.8	6.2 (6)	11.9	0.20 (0)
Mean	467.9	7.7 (7)	452.6	7.5 (7)	15.2	0.25 (0)

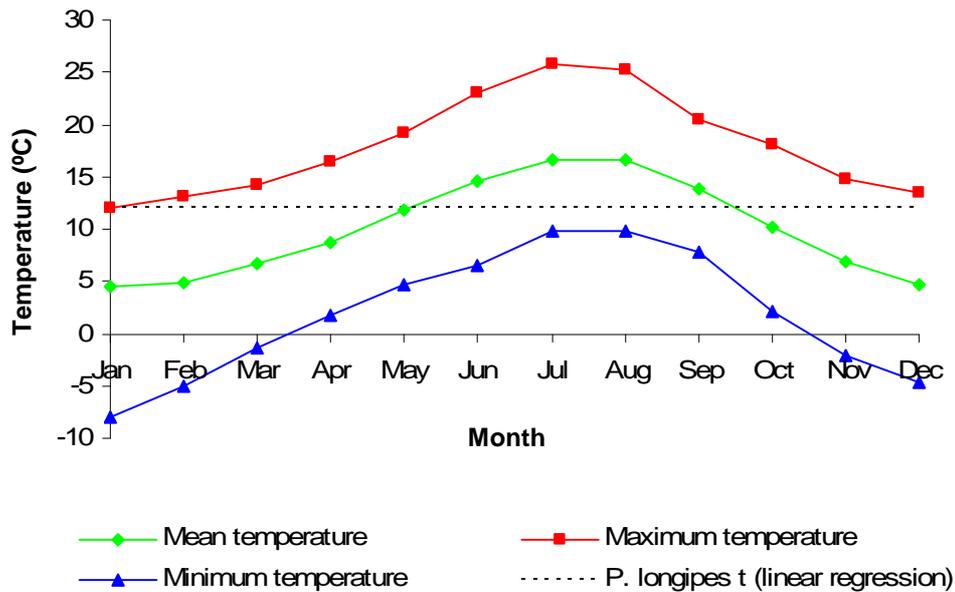


Figure 5.3: Average, maximum, mean and minimum temperatures recorded for each month from 1987-2007 in Birmingham, UK. *Phytoseiulus longipes* developmental threshold (t), as calculated by simple linear regression shown as a dotted line.

5.4.2 Diapause

The number of eggs laid per female under the control and diapause inducing conditions are shown in Figure 5.4. The total mean numbers (\pm SE) of eggs laid per female were 23.0 ± 0.60 , 21.8 ± 0.35 , and 20.2 ± 0.42 for the control, first and second generation diapause populations respectively. Statistical analysis showed that the numbers of eggs laid in the control population differed significantly to that laid by the second generation under diapausing conditions ($F_{2,117} = 7.69$, $P < 0.05$). There was no difference between the number of eggs laid by the first and second generations of ‘diapause’ mites nor between the control and first generation diapause populations.

The number of eggs laid in the control population peaked on day 5 and then decreased slowly to day 18 when oviposition had ceased. For the first generation diapause population, egg laying peaked on day 16, two days after being transferred back to the original rearing conditions (28°C, 18:6 LD). Oviposition did occur from day 1 and

100% of the females were ovipositing by day 7 in the diapause inducing conditions. Egg laying declined rapidly from its peak at day 16 to zero at day 23. With the second generation females, slightly higher egg production was seen over the first six days compared with the first generation females. Oviposition peaked on day 17, three days after being transferred back to the original rearing temperatures and again, rapidly decreased to zero by day 22. There was no difference in the levels of oviposition during the first three days of the experiment between the control and the second generation diapause population.

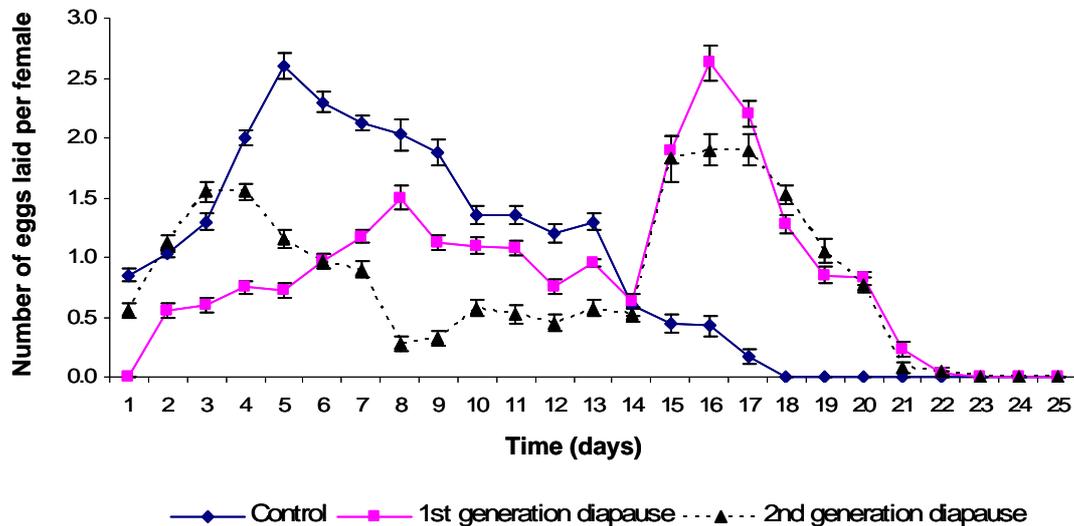


Figure 5.4: Mean number of eggs laid (\pm SE) per female *Phytoseiulus longipes* per day under control conditions (28°C, 18:6 LD) and for first and second generation females reared under diapause inducing conditions (18°C, 6:18 LD) (n=40).

5.4.3 SCP

The mean and range of SCPs of mites from each of the four treatment groups is shown in Table 5.3. All individuals tested were found to be dead after freezing. The mean larval SCPs were significantly lower than the adults ($F_{3,93} = 84.3$ $P < 0.001$).

There were no significant differences between the SCPs of acclimated and non-acclimated adult and larval *P. longipes*.

Table 5.3: Mean (\pm SE) and range of supercooling points of non-acclimated and acclimated adult and larval *Phytoseiulus longipes*.

Treatment group	n	Mean \pm SE ($^{\circ}$ C)	Range ($^{\circ}$ C)
Acclimated adults	29	-23.1 \pm 0.15	-21.5 to -24.4
Non-acclimated adults	28	-22.5 \pm 0.14	-20.8 to -23.6
Acclimated larvae	20	-26.9 \pm 0.30	-25.2 to -29.5
Non-acclimated larvae	20	-27.5 \pm 0.53	-19.4 to -30.3

5.4.4 Lower lethal temperatures

There was no adult or larval mortality in the control samples after confinement to the Beem capsules and exposure at 20 $^{\circ}$ C for 75 min, indicating that the experimental set did not produce any deleterious effects. The lethal temperatures for 10, 50 and 90% mortality of acclimated and non-acclimated adult and larval *P. longipes* are shown in Figure 5.5, where non-overlapping fiducial limits are indicative of significant differences in the data to the level of 5% (StatsDirect, 2008).

A significant difference in survival was observed between adult and larval treatment groups at the 10 and 50% mortality levels, with adults surviving to lower temperatures of -4.3 $^{\circ}$ and -11.1 $^{\circ}$ C for 10 and 50% mortality compared to 3.2 $^{\circ}$ and -8 $^{\circ}$ C respectively for larval mites. At 90% mortality, acclimated adults survived at lower temperatures (-17.5 $^{\circ}$ C vs. -15.5, -14.8 and -15.1) than all three other groups. Acclimation increased survival at significantly lower temperatures, but only for the adult group at the 50% mortality level.

All of the larvae recovered alive from the Beem capsules and transferred to recovery arenas continued to develop through to the adult stage and then to mate and reproduce.

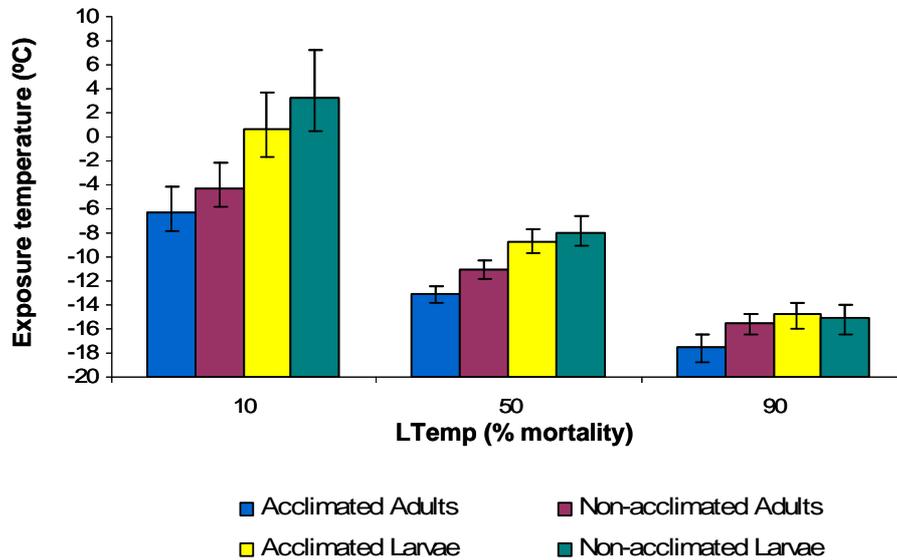


Figure 5.5: LTemp₁₀, ₅₀ and _{90%} mortality (\pm fiducial limits) of acclimated and non-acclimated adult and larval *Phytoseiulus longipes* after cooling at $0.5^{\circ}\text{C min}^{-1}$ to the desired exposure temperature (n=50).

5.4.5 Lower lethal time

The lower lethal times for 10, 50 and 90% mortality (LTime₁₀, ₅₀, ₉₀) of acclimated and non-acclimated adult and larval *P. longipes* at -5° , 0° and 5°C are shown in Figure 5.6. The LTime₅₀ for non-acclimated adults and larvae were 3.7 and 1.3 days, 6.9 and 4.7days, 34.9 and 14.3 days for -5° , 0° and 5°C respectively.

At -5°C survival times varied between a maximum of 7.3 and minimum of 3.6 days for acclimated adults and acclimated larvae respectively. At 10, 50 and 90% there were significant differences in mortality between life stages with adults surviving longer in all three cases. There were no differences in mortality rates between acclimated and non-acclimated treatments for either adults or larvae at any of the

mortality intervals (shown by overlapping fiducial limits bars). Acclimated larvae were consistently the least cold hardy group.

At 0°C there was no difference between acclimation treatments in the adult groups but non-acclimated larvae survived for significantly longer than acclimated larvae at 50 and 95% mortality levels. Adults survived longer than larvae at all levels with the exception of overlapping fiducial limits between adults and non-acclimated larvae at 10% mortality.

P. longipes exposed to 5°C survived the for the longest period of time (42.4 days) compared to the experiments run at 0° and -5°C. Again, adults survived much longer than the larval groups and at 10 and 50% mortality, non-acclimated adult mites survived significantly longer than any other group (25.6 and 34.9 days). At 90% mortality, the acclimation treatment resulted in no differences in adult survival. In general, non-acclimated larvae were more cold hardy than acclimated larvae at all three mortality levels with a significant difference occurring at 50% mortality ($p < 0.05$). The maximum survival time for individuals exposed at 5°C was 42.4 days and 100% mortality occurred in the fed 5°C control population by day 44. These similar data points suggest that mortality recorded during the LTime experiments was due to the cold stress and not to desiccation or starvation.

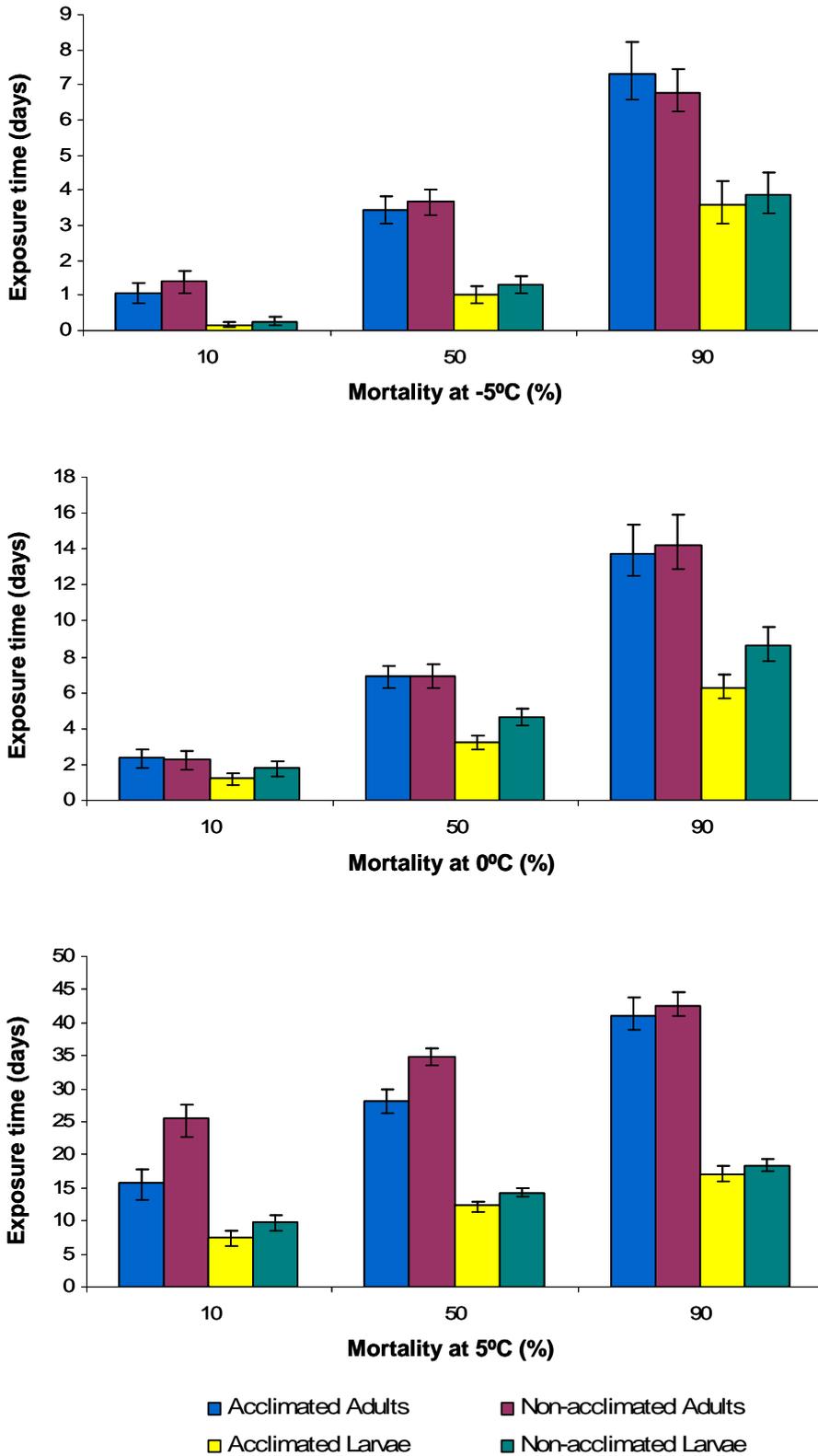


Figure 5.6: LTime₁₀, ₅₀ and _{90%} mortality (\pm fiducial limits) of acclimated and non-acclimated adult and larval *Phytoseiulus longipes* at -5° (A), 0° (B) and 5°C (C) (NB: different scales of axis) (n=50).

5.4.6 Field exposures

Field mortality rates of non-acclimated and acclimated, adult and larval *P. longipes* recorded in the first field exposure (December-January 2007-2008) are shown in Figure 5.7. The corresponding field temperatures for the period are shown in Figure 5.8. Mortality of all treatment groups increased with duration of outdoor exposure. The average daily temperature recorded during this time was 3.8°C, with a maximum of 12.7°C and a minimum of -1.5°C. Mortality levels at day 1 were not significantly different to those of day 4. Between days 4 and 10 larval mortality levels increased rapidly from below 5% to above 82.5%. For the adult treatment groups, mortality levels increased relatively slowly and steadily throughout the exposure period. 100% mortality of both larval groups was recorded after 21 days of exposure. However, during the entire 34 day trial, adult mortality reached 70% and 52.5% for acclimated and non-acclimated treatments respectively. The results show a marked population decline in both larval groups at day 6 following two overnight periods when there were sub-zero temperatures. However, this sub-zero exposure, although no doubt contributing to adult mortality, did not produce in a sudden increase in mortality as observed with the larvae. Mortality in the larval groups was comparatively more rapid than in the adult groups. From day 10 onwards, there was a significant difference between adult and larval survival on each sampling day. At all sampling intervals there was no difference in the rate of decreasing survival of acclimated and non-acclimated larval mites. Only after 27 days of exposure was a significant difference between survival of adult treatment groups apparent, with non-acclimated adults surviving significantly higher numbers than acclimated mites at days 27 and 34 ($P < 0.05$ and $P < 0.01$ respectively).

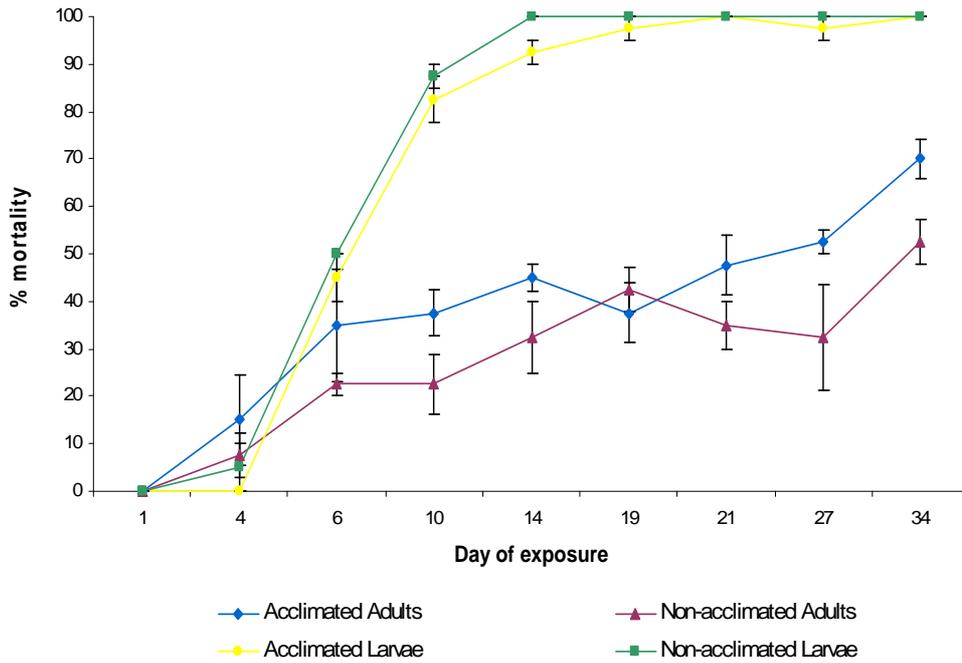


Figure 5.7: Field mortality of acclimated and non-acclimated, adult and larval *Phytoseiulus longipes* from 15 December 2007 to 18 January 2008 (n=40).

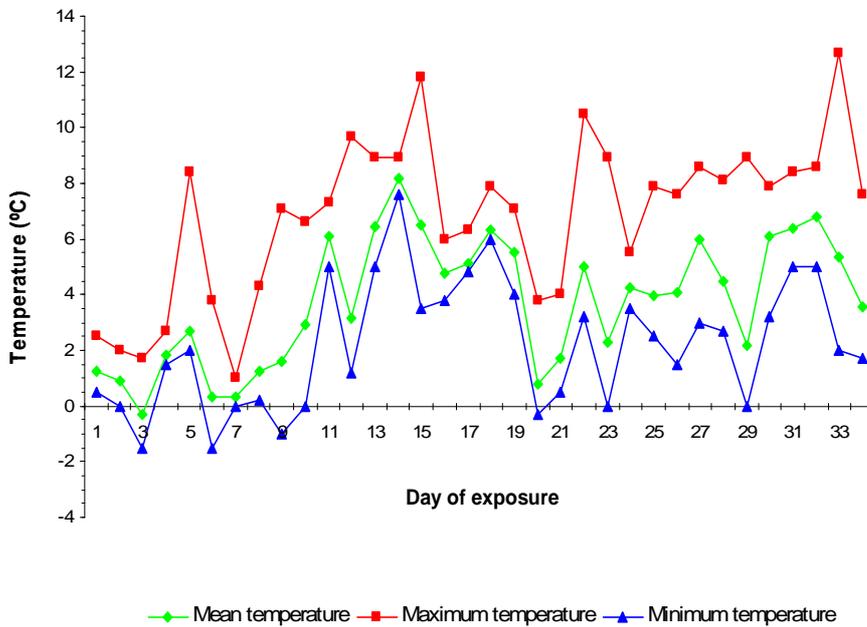


Figure 5.8: Mean, maximum and minimum outdoor temperatures experienced by *Phytoseiulus longipes* in the field from 15 December 2007 to 18 January 2008.

Field mortality rates of acclimated and non-acclimated adults and larvae recorded in the late winter field experiment (January to April 2008) are illustrated in Figure 5.9. The average daily temperatures recorded during the field exposures are shown in Figure 5.10. The average temperature throughout the trial was 6.3°C and temperatures fluctuated between a maximum of 16.6°C and minimum of -2.5°C. Levels of mortality in this trial reached 100% in all treatment groups after 73 days of exposure to winter field temperatures. Mortality levels for all groups on days 4 and 10 were not significantly different from each other. Mortality increased significantly at each sampling interval from day 10 to day 24 across all treatment groups ($P < 0.05$ for all observations). Mortality across all groups showed no difference in mortality levels between days 24 and 31, 31 and 46, and 46 and 59. Significantly higher mortality was recorded on day 73 than day 59 for the adult groups. At all sampling intervals from day 17 onwards, adults survived in significantly higher numbers than the larval groups ($P < 0.05$), with the exception of day 73. After 59 days of outdoor exposure non-acclimated adults had significantly lower ($P < 0.01$) mortality rates than all other treatment groups. The acclimation treatments in the larval groups did not increase survival at any of the sampling intervals. Data from day 6 show increasing mortality levels across all groups. Temperatures during this field exposure declined to below 0°C only on days 8, 23 and 24. No effect on mortality can be seen from the sub-zero exposure periods on day 8 and by day 23, mortality was increasing steadily in all groups.

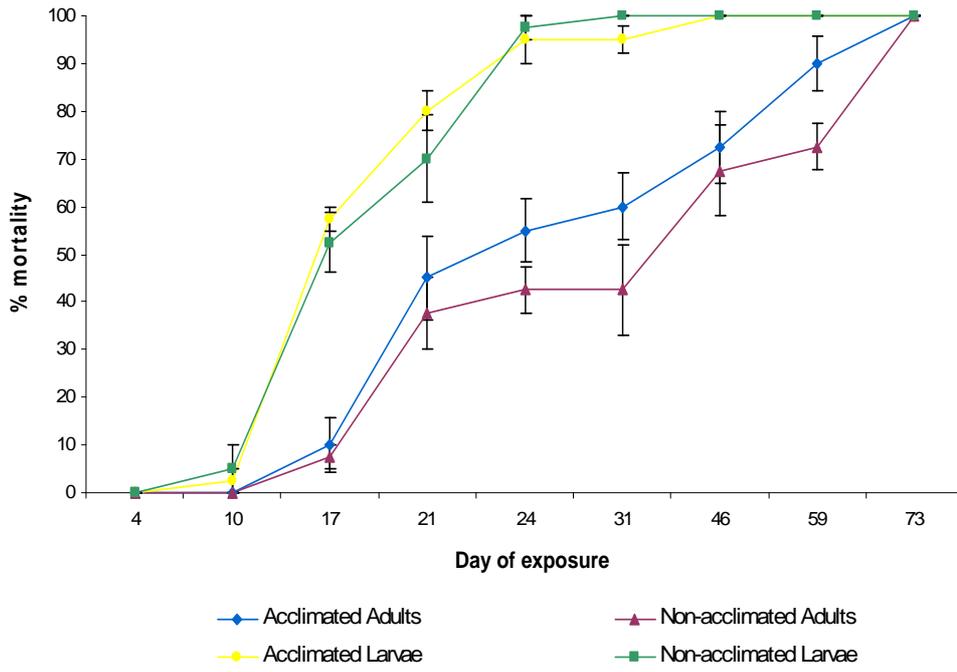


Figure 5.9: Field mortality of acclimated and non-acclimated, adult and larval *Phytoseiulus longipes* from 25 January 2008 to 7 April 2008 (n=40).

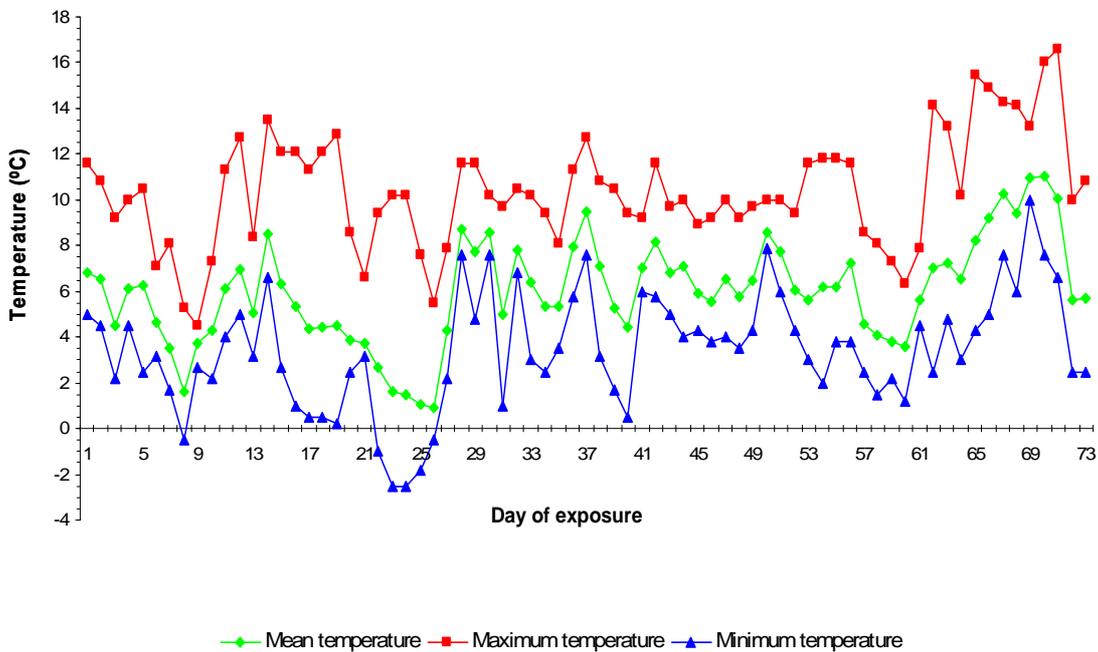


Figure 5.10: Mean, maximum and minimum outdoor temperatures experienced by *Phytoseiulus longipes* in the field from 25 January 2008 to 7 April 2008.

Mortality data from the short term field trials are shown in Table 5.4. Where mortality did occur, an increase was recorded in week 2 compared to week 1 for all of the field trials. Mortality levels were higher in larvae than adults for all of the experimental weeks. Sub-zero temperatures affected larvae more than adults; the two periods with minimum temperatures of -0.3° and -2.5°C resulted in larval mortalities of 75% and higher. By comparison, adult mortality at these same intervals was 30% or less. At minimum temperatures above 5.1°C there was 48.5% or higher survival of *P. longipes* larvae and 77.5% or higher survival of adult mites. In the May 2008 two week field trial acclimated adults produced a total of 20 eggs and 13 larvae and non-acclimated adults produced 3 eggs, 5 larvae and 1 protonymph. After two weeks of exposure to June temperatures, 47% of the surviving acclimated larvae had moulted to a nymphal stage and 59% of the surviving non-acclimated larvae had also moulted to the eight-legged nymphal stage.

Table 5.4: Mean % mortality (\pm SE) of acclimated (AA) and non-acclimated (NA) adult and larval (AL and NL) *Phytoseiulus longipes* after 7 and 14 days of outdoor temperature exposure at intervals between December 2007 and June 2008 (n=40). Also displayed are the mean, maximum and minimum temperatures for each 7 day period and the mean temperature of the total 14 day period.

Date into field	Mite treatment group	Mean % mortality (\pm SE)		Mean, max and min temperatures ($^{\circ}$ C)		Mean temperature ($^{\circ}$ C) days 1-14
		7 days	14 days	days 1-7	days 8-14	
29/12/07	AA	7.7 \pm 4.8	22.5 \pm 4.8	4.0, 10.5, -0.3	4.2, 8.9, 0.0	4.1, 10.5, -0.3
	NA	15.0 \pm 6.5	27.5 \pm 2.5			
	AL	40.0 \pm 10.0	75.0 \pm 9.6			
	NL	37.5 \pm 10.3	85.0 \pm 2.9			
04/02/08	AA	10.0 \pm 4.1	20.0 \pm 9.1	6.3, 13.5, 1.0	3.5, 12.9, -2.5	4.9, 13.5, -2.5
	NA	17.5 \pm 6.3	30.0 \pm 8.2			
	AL	32.5 \pm 8.5	77.5 \pm 4.8			
	NL	32.5 \pm 10.3	85.0 \pm 2.9			
11/03/08	AA	2.5 \pm 2.5	5.0 \pm 2.9	6.7, 11.8, 2.0	5.0, 11.6, 1.5	5.9, 11.8, 1.5
	NA	0	7.5 \pm 4.8			
	AL	20.0 \pm 10.8	35.0 \pm 8.7			
	NL	27.5 \pm 6.3	35.0 \pm 11.9			
31/03/08	AA	0	2.5 \pm 2.5	9.7, 16.6, 2.5	10.9, 16.5, 3.3	10.3, 16.6, 2.5
	NA	2.5 \pm 2.5	5.0 \pm 5.0			
	AL	17.5 \pm 6.3	17.5 \pm 4.8			
	NL	22.5 \pm 11.1	25.0 \pm 5.0			
02/05/08	AA	0	5.0 \pm 2.9	16.9, 28.0, 8.6	18.2, 32.3, 10.0	17.5, 32.3, 8.6
	NA	0	10.0 \pm 7.1			
	AL	10.0 \pm 4.1	12.5 \pm 2.5			
	NL	12.5 \pm 7.5	12.0 \pm 6.3			
06/06/08	AA	0	0	19.1, 32.3, 11.0	15.9, 25.8, 8.6	17.5, 32.3, 8.6
	NA	0	0			
	AL	2.5 \pm 2.5	2.5 \pm 2.5			
	NL	0	2.5 \pm 2.5			

The mortality data from the 6 mini-field trials were collated and analysed in relation to the mean and minimum temperatures experienced by the mites over the exposure period. The results of the Probit analysis are shown in Figure 5.11. Significant differences between the temperatures at which 10, 50 and 90% mortality occurred in each group are indicated by non-overlapping fiducial limits. Mean and minimum temperatures required to cause 50 and 90% field mortality were significantly lower for adult *P. longipes* than for the larval groups. No differences were observed between acclimated and non-acclimated groups with the exception of the minimum temperature required to kill 50% of non-acclimated larvae being lower at -1.3°

compared to -0.31° for acclimated larvae ($P < 0.05$). 50% field mortality of non-acclimated adults occurred at significantly lower minimum temperatures than in acclimated adults -7.4° compared to -6.3°C . Mean and minimum field temperatures required to kill 90% of a population were significantly lower for non-acclimated adults compared with acclimated adults ($P < 0.05$). At this mortality level, the mean and minimum temperatures required to cause 90% mortality of non-acclimated mites were -13.3° and -14.5°C respectively.

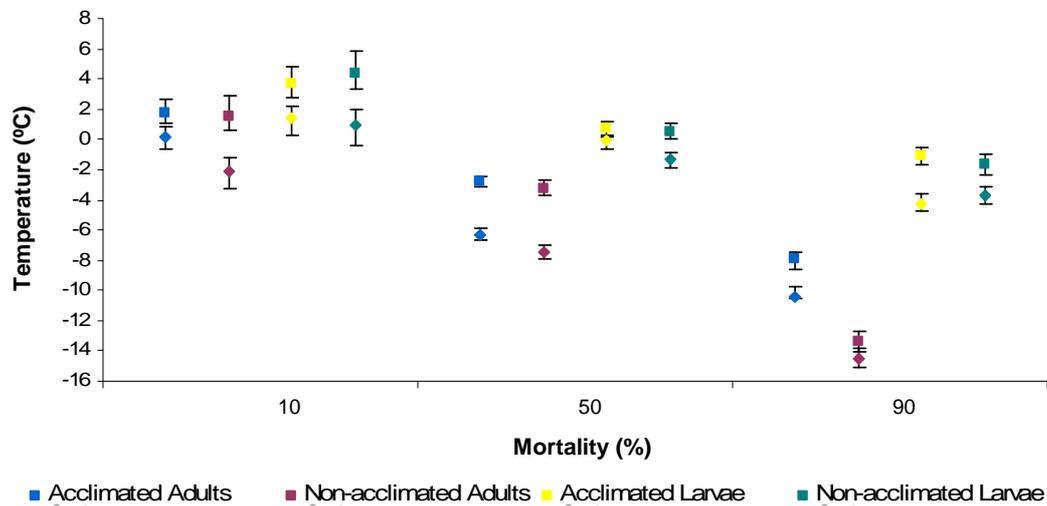


Figure 5.11: Mean (■) and minimum (◆) temperatures (\pm 95% fiducial limits) predicted to cause 10, 50 and 90% field mortality of acclimated and non-acclimated adult and larval *Phytoseiulus longipes*. Data collated from the short term field trials and analysed by Probit ($n=480$).

5.5 Discussion

This Discussion will focus mainly on the data described in this Chapter on the low temperature biology of *Phytoseiulus longipes*. Comparisons will be made with *Amblyseius swirskii* and other non-native (to the UK) biological control agents, and discussed in a wider context in the General Discussion (Chapter 7).

The optimal developmental temperature for *P. longipes* was approximately 30°C and at this temperature mean egg to adult development occurred in 3.6 days (± 0.11). In comparison to other previously studied Phytoseiid mites this is considerably shorter than the 6.1 days for *A. swirskii*, 6.3 days for *T. montdorensis* (Hatherly *et al.*, 2004) and 8.1 days for *N. californicus* (Hart *et al.*, 2002a). Development at 32°C is significantly slower than at 30°C and does not occur beyond the larval stage at temperatures below 12°C. The optimal developmental temperature for *P. longipes* is higher than that of *A. swirskii* (27°C).

P. longipes responds to an increase in rearing temperature by increasing its rate of developmental at each developmental stage. The time spent as the deutonymph stage was the longest at all temperatures ($P < 0.05$) and this may be attributable to this life stage spending time accumulating energy reserves for maturation of reproductive organs and production of gametes before moulting to the adult stage (Woolley 1988).

The developmental data between 15° and 30°C is often linear (Higley *et al.*, 1997), but outside of this range, usually becomes more curvilinear (Lamb 1992; Wagner *et al.*, 1984). *P. longipes* can tolerate temperatures up to 38°C if humidity is high (90%),

but can also survive at low relative humidities (down to 40%) at 21°C (Henn *et al.*, 1995). However, normal developmental is unlikely to be maintained at humidities outside of this preferred range (Yvonne Van Houten, Koppert, personal communication). A simple linear regression analysis of the developmental data accounted for approximately 80% of the variability in the relationship, with an estimated thermal budget of 57 day degrees (°d) per generation and a lower developmental threshold temperature of 12.1°C. When considered in relation to the developmental times at different temperatures, this temperature appears to be accurate as there was no development beyond the larval stage at 12°C and below. The developmental threshold for *P. longipes* is therefore very similar to that of *A. swirskii* (11.9°C) but in comparison with other biological control agents it appears to be relatively high. Thus, the developmental threshold temperatures of *T. montdorensis* and *N. californicus* are 10.7° and 9.9°C respectively, but the thermal budget per generation of these two species (105.3 °d and 123.5 °d respectively) are both higher than for *P. longipes* (Hatherly *et al.*, 2004; Hart *et al.*, 2002a). The day degree requirement for *P. longipes* is low in comparison to *A. swirskii*, but this might be expected as *P. longipes* has the greatest capacity for development and population growth of all Phytoseiid mites (Zhang 1995). The relatively high developmental threshold may suggest a lack of cold tolerance; however when considered alongside its potential voltinism, at temperatures above this threshold, development will progress more rapidly in any other closely related species.

Predictions of annual voltinism were made by using the estimated thermal budget requirement and developmental threshold of *P. longipes*. Based on temperature data collated over a 20 year period, *P. longipes* is capable of completing a maximum of

10.7, a minimum of 5.3 and an average of 7.7 'theoretical' generations per year. In comparison with *A. swirskii* where the maximum annual voltinism was estimated to 6.8 generations per year, the fecundity and developmental potential of *P. longipes* is higher. However, *P. longipes* is similar to *A. swirskii* in that the population increase would be constrained solely to the summer months (April to September), as the mean number of day degrees available during the winter period is only 15.2°d. When looking more closely at the temperature data, it is more likely that *P. longipes* development and population increase would occur from the beginning of May to the middle of September, when temperatures rose above the developmental threshold of 12.1°C, and during this time, approximately 10 generations could be completed; and the mite would then have to survive for over 6 months of the year at temperatures below its developmental threshold and endure exposures of sub-zero temperatures during the winter months.

P. longipes does not have the ability to diapause under conditions which have been shown to induce diapause in other species of mite (Jolly 2000). Oviposition levels in the first generation of mites reared under diapause-inducing conditions was similar to that of the control population, but lower in the second generation of 'diapause' females compared with the control. Oviposition occurred and continued in both 'diapause populations' from day one and rapidly increased immediately after transfer back to the normal rearing conditions, peaking after three days in this regime. As egg production was continuous during the diapause-inducing conditions for both the first and second generations, and as none of the females were observed to have a pale and flattened appearance (typical of diapausing mites), it can be concluded that *P. longipes* lacks the ability to enter a diapause state (Morewood & Gilkeson, 1991;

Overmeer 1985a; Veerman 1992). The fewer eggs laid in the first 7 days when maintained under the 'diapause' conditions is most likely attributable to the difficulty of maintaining high humidity levels (that are necessary for successful oviposition) at 18° compared with 28°C. Also, in similarity to the findings of the *A. swirskii* diapause experiments, many more successful predation events were observed after transfer back to the original 'non-diapause' rearing conditions, suggesting that the reduced egg numbers were due in part to the lower temperature of the diapause regime.

The laboratory low temperature experiments indicate that *P. longipes* is unable to survive freezing but it does possess a greater level of cold hardiness than other mites. The mean supercooling points for acclimated and non-acclimated adult and larval *P. longipes* were -23.1 ± 0.2 , -22.5 ± 0.14 , -26.9 ± 0.3 and -27.5 ± 0.5 °C respectively. As with *A. swirskii*, larval mites survived at significantly lower temperatures than the adults, but there was no difference in survival between acclimated and non-acclimated treatments. The range of SCPs for adult *P. longipes* was -20.8 to -24.4 °C and -19.4 to -30.3 °C. The range of adult SCPs for *P. longipes* is narrower than for *A. swirskii*, suggesting there is less biological variation in the sample of *P. longipes*. The *P. longipes* adult SCPs are comparable to those of *T. montdorensis*, *N. californicus*, *P. persimilis* and *A. cucumeris*, which have mean SCPs -24.1 °, -21.6 °, -22.5 ° and -20.7 °C respectively (Hatherly *et al.*, 2004; Hart *et al.*, 2002a; Morewood 1992). In comparison with studies on *P. persimilis* (Morewood 1992), the SCPs of *P. longipes* and *A. swirskii* do not appear related to the body mass of these mites.

The lethal temperature experiments show that *P. longipes* exhibits considerable pre-freeze mortality as all individuals died at temperatures above the mean SCP. Lethal

temperature data often provides a more reliable estimate of cold tolerance than SCPs as the temperatures experienced during the experiments are more representative of natural winter exposures. Across all of the life stages and treatment groups of *P. longipes*, the range of temperatures at which 10, 50 and 90% of the entire sample populations died were 3.2° to -6.4°C, -8.0° to -13.1°C, -15.1° to -17.5°C respectively. The greatest significant difference was between acclimated adults and non-acclimated larvae ($P < 0.05$). Adults survived significantly lower temperatures than larvae at all three mortality interval but a period of acclimation did not increase survival within any life stage. The LTemp₅₀ for non-acclimated adult *P. longipes* was -11.1°C with the 10 to 90% mortality range between -4.3° and -15.5°C. The equivalent values for *A. swirskii* were -4.5° and -0.8° to -6.9°C; it is clear that *P. longipes* is more cold hardy than *A. swirskii*. The 10 to 90% temperature range of non-acclimated adult *P. longipes* is also wider than that of *T. montdorensis* (-5.6° to -8.1°C; Hatherly *et al.*, 2004) and. Non-acclimated adult *N. californicus* can survive at -15.1°C (Hart *et al.*, 2002a), this temperature being only 7°C above their SCP, approximately the same difference as in *P. longipes* whereas 50% of *A. swirskii* die 11°C above their SCP. However, *T. montdorensis* has a SCP up to 16°C lower than the LTemp₅₀, and first instar nymphs of the aphid *Myzus persicae* have an LTemp₅₀ of -8°C and a SCP of -27°C (Bale *et al.*, 1988), which provides further evidence for that supercooling points in isolation, are not a reliable indicator of cold tolerance. All of the *P. longipes* larvae recovered alive from Beem capsules and transferred to recovery arenas developed to the adult stage, mated and reproduced; thus mites are able to resume development within a few days of cold stress exposure. The LTemp data concurs that *P. longipes* ability to supercool is primarily a physical characteristic rather than an adaptation for survival of exposure to subzero temperatures (Morewood 1992).

Lethal time experiments are the best representation of naturally occurring cold stress because they integrate cumulative mortality over longer periods of time. For non-acclimated *P. longipes* adults, 10% survival times at 5°, 0° and -5° were 42.4, 14.2 and 6.8 days respectively. In comparison with other 'cold hardy' Phytoseiid mites, *P. longipes* is more cold hardy than *T. montdorensis* (which can survive for 22 days at 5°C), but less cold hardy than *N. californicus* (3 months at 5°C; Hatherly *et al.* 2004; Hart *et al.* 2002a). However, *P. longipes* is also more cold hardy than non-acclimated *A. swirskii* adults which can survive for 4.8 days, 3.8 days and 17.9 minutes at 5°, 0° and -5°C respectively.

The range of lethal times for all treatment groups of *P. longipes* at the 50% mortality level were between 12.3 and 34.9 days, 3.3 and 6.9 days and 1.0 and 3.7 days respectively when 5°, 0° and -5°C. The limited range in the data suggest that there is little variation in the cold tolerance ability of *P. longipes* and most individuals can survive for a reasonably time at 5°C without feeding. At all temperatures, adults survived significantly longer than larvae and a period of acclimation at 10°C did not in general increase survival in either life stage. At all three exposure temperatures, lowest survival was observed in acclimated larvae at all mortality intervals. In effect, the acclimation regime was itself deleterious for the larvae, resulting in comparatively higher mortality in the experiments.

It can be concluded from the laboratory data and comparisons with other mites that have been used as biologically control agents that *P. longipes* has a level of cold tolerance that is greater than some species (*A. swirskii* and *T. montdorensis*) but less than others (*N. californicus*).

The objective of the field exposures was to determine the ability of *P. longipes* to survive outside of a glasshouse environment. The laboratory data indicated that *P. longipes* was more cold tolerant than *A. swirskii* and its survival under naturally fluctuating winter temperatures (that are difficult to simulate in the laboratory) was unknown. As overwintering is likely to occur in sheltered locations such as leaf litter, under bark or building crevices as shown by other Phytoseiid mites (Broufas *et al.*, 2002; Veerman 1992), the field trials were conducted in concealed boxes, sheltered from the effects of wind and direct sunlight, to provide a more realistic assessment of likely survival outside of a glasshouse.

In general, a period of acclimation prior to placing in the field did not confer any increase in survival and in the more prolonged exposure to outdoor winter conditions, acclimation appeared to be disadvantageous; this may be attributable to the extension of deleterious effects that were first accrued during the acclimation period at 10°C. In both field trials, acclimated adults survived less well than non-acclimated adults. but this difference was not always statistically significant. Survival of acclimated and non-acclimated larvae was similar in both trials and consistently lower than that of adult *P. longipes*.

Sub-zero field temperatures appear to have little immediate effect on mortality of *P. longipes*; on none of the three occasions in the second trial when the temperature fell below 0°C was there any sudden or marked change in survival. By contrast, field survival of *A. swirskii* declined on the first day of the first winter field trial when the mites experienced a temperature below 0°C.

Maximum winter field survival time of *P. longipes* was 73 days in the second winter field trial (January to April 2008); however, in the first field trial, when the last samples were collected from the field after an exposure period of 34 days, 47.5 and 30% of non-acclimated and acclimated adults were still alive, but all of the larvae had died. The minimum temperature during the first trial (December to January 2007 - 2008) was -1.5°, the maximum 12.7° and the mean 3.2°C. During the second trial, the mean and maximum temperatures were higher (6.3° and 16.6°C). This may explain the extended survival during the second trial; however the minimum temperature was -2.5°C. This may indicate that in *P. longipes*, any cryo-injury that occurs during brief exposure to sub-zero temperatures can be repaired at higher temperatures, but this ability is absent in *A. swirskii*.

In the larval groups, initial population decline was much more rapid than in the adult groups, with high mortality occurring over the first 10 and 21 days of exposure in the first and second trials. In comparison with other Phytoseiid mites the maximum field survival of *P. longipes* (73 days) is longer than *T. montdorensis* (35 days; Hatherly *et al.*, 2005) but less than that of unfed non-diapausing *N. californicus* (100 days; Hart *et al.*, 2002a). However, in the *N. californicus* field trial the mites did not experience temperatures below 0°C. It is possible that after 73 days in the field, some of *P. longipes* mortality may be attributable to old age. It does though appear that *P. longipes* is a Phytoseiid mite with level of cold tolerance that is intermediate between other species .

The short term field exposures ran from December 2007 to June 2008 and provide further information on the effect of different temperature regimes on the survival and

voltinism of *P. longipes*. The data show that fluctuating low temperatures and exposure to sub-zero temperatures are not lethal across all treatment groups. When the mean temperature is relatively high and overnight temperatures fall below 0°, this exerts considerably more stress on larvae than adults. The combined data from the short term field exposures (Figure 5.11) indicate that a combination of mean temperatures over time and minimum temperatures contribute to mortality. However, it appears that when considered alongside milder daytime conditions, *P. longipes* is not only able to withstand some sub-zero temperatures (albeit not very low temperatures), but also able to resume development and oviposition when conditions are more favourable. This is illustrated by the fact that during the May and June 2007 mini-field trials, both non-acclimated and acclimated adult females began to oviposit and larvae from both groups began to develop to the nymphal stages in June. It is very difficult to identify Phytoseiid mites as either protonymph or deutonymph without careful observation of moulting and the presence of a cast skin. For this reason it is not possible to state categorically whether larvae had moulted once to the protonymph stage, or twice to the deutonymph stage, but the potential to do so must be taken into account when considering a risk assessment for *P. longipes*.

Prolonged low average temperatures alongside sub-zero minimum temperatures produces the greatest level of mortality yet sub-zero temperatures followed by milder averages do not result in as high levels of mortality as seen in *A. swirskii*. Perhaps this is due to the deleterious effects of the cold only accumulating very slowly and the effect of this is paused each time the average temperature returns to milder conditions.

This is corroborated by the LTemp₅₀ data which illustrates that survival at -5°C is for several days for *P. longipes* compared to minutes for *A. swirskii*.

When considering the cold hardiness and establishment potential of *P. longipes* in UK, the mite falls into a ‘marginal’ or ‘medium risk. Category (Bale et al., 2009).

Figure 5.12 shows the relationship between LTime₅₀ at 5°C and maximum field survival time in days for nine non-native biological control agents with *P. longipes* added to the graph.

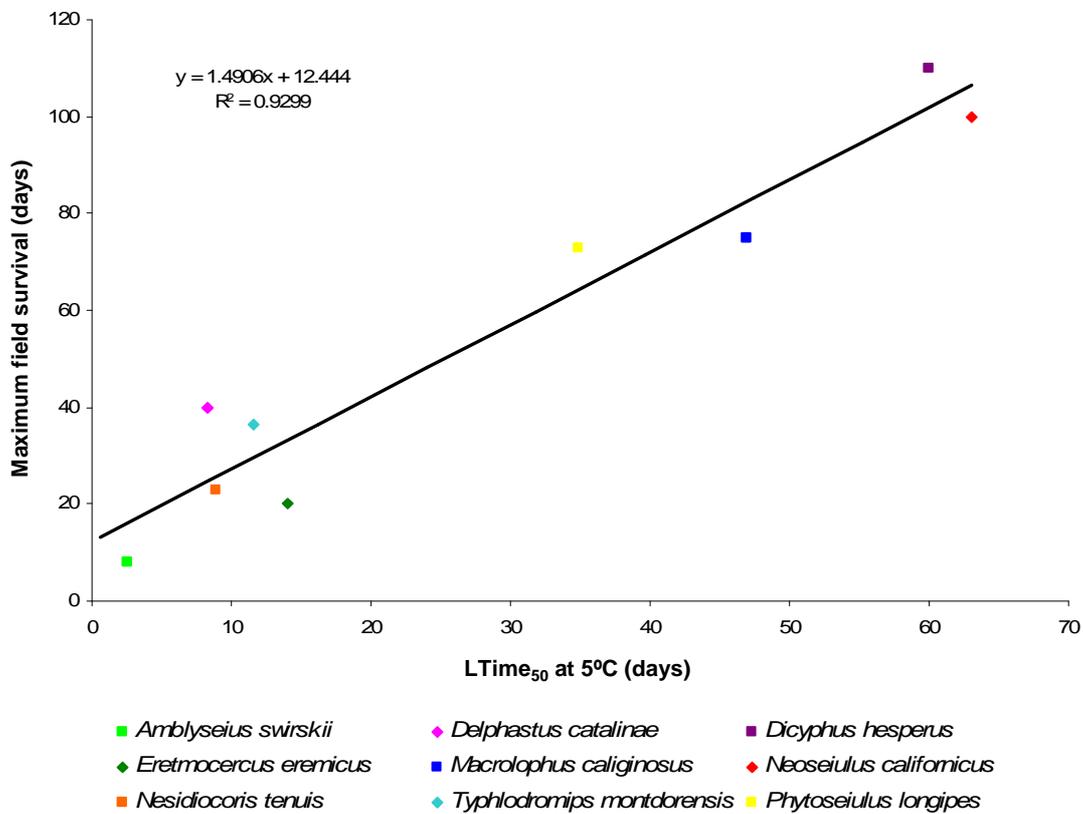


Figure 5.12: Relationship between maximum field survival (days) and LTime₅₀ at 5°C (days) for nine non-native biological control agents (expanded on from Hatherly *et al.*, 2005). Sources of data: *Amblyseius swirskii* (C. Allen, present study), *Delphastus catalinae* (Tullett 2002), *Dicyphus hesperus* (Hatherly *et al.*, 2008), *Eretmocercus eremicus* (Tullett *et al.*, 2004), *Macrolophus caliginosus* (Hart *et al.*, 2002b), *Neoseiulus californicus* (Hart *et al.*, 2002a), *Typhlodromips montdorensis* (Hatherly *et al.*, 2004)

The $LTime_{50}$ at 5°C in combination with the maximum field survival time suggests that *P. longipes* is less cold hardy than *M. caliginosus*, *N. californicus* or *Dicyphus hesperus* but more cold hardy than *T. montdorensis* and *A. swirskii* (Figure 5.12). It is difficult to suggest that *P. longipes* could definitely establish outdoors in the UK, but *N. californicus* has done so (Jolly 2000) and *M. caliginosus* has been observed outside of glasshouses in winter.

In summary, laboratory data show that *P. longipes* is unable to, exhibits extensive pre-freeze mortality and but is able survive through cold exposure for longer periods of time than *A. swirskii*. Prolonged winter field exposures can cause high mortality but population decline is progressive rather than rapid, especially for adults. The mite appears unable to acclimate, but is capable of high population growth during the summer months. Overall, *P. longipes* can not be regarded as a 'safe' biological control agent for glasshouse release in the UK, but nor is it a species where establishment seems certain to occur.

This work has played a direct role in the risk assessment for the release of *P. longipes* and in the decision on whether to market this predatory Phytoseiid mite for use a biological control agent in UK glasshouses.

CHAPTER 6

Low temperature activity thresholds of *Phytoseiulus longipes*

6.1 Introduction

In broad terms, temperature exerts two main types of effects on insects and mites. Firstly, it influences ‘rate-based’ processes such as development, reproduction and activity; secondly, it can prove lethal, either in acute or longer term chronic exposures. In the context of biological control, the effect of low temperature on mortality is the basis for predicting the likelihood of establishment of non-native species in different climatic zones (van Lenteren *et al.*, 2006). The value to biological control companies, of investigating other thermal tolerance traits such as the CT_{min} and chill coma recovery temperature has yet to be evaluated, but it would seem that knowledge of the relative thermal thresholds of control agents and their prey or hosts is likely to be beneficial for the biological control industry and for effective pest management. The experiments described in this Chapter investigated the effect of changes in temperature on *Phytoseiulus longipes* quantified as the temperatures at which walking ceases (CT_{min}), all coordinated body movements cease (chill coma), movement of appendages resumes (chill coma recovery), and finally, coordinated walking occurs (activity recovery). As with *A. swirskii* (Chapter 4), studies were also carried out on the effects of temperature on walking speed in relation to a prey species *Tetranychus urticae*, and on predator-prey interactions between the two species.

6.2 Aims

The aims of this chapter were to:

1. Determine the CT_{min} and the recovery temperature of *P. longipes*.
2. Investigate the effect of temperature on walking speeds of *P. longipes* and make comparisons with those of *T. urticae*.
3. Establish the effect of temperature on the predator-prey interactions in a potential *P. longipes*-*T. urticae* ecosystem

6.3 Methods

Investigations into low temperature activity thresholds were carried out on four mite treatment groups: acclimated and non-acclimated adults and larval *P. longipes*. These data were compared directly to the corresponding values of activity thresholds of acclimated and non-acclimated *T. urticae* adults (see Chapter 4). In the case of the predation experiments, only adult *P. longipes* were used as the larvae are a non-feeding life stage. The acclimation treatment was the same as previously described for the cold tolerance experiments (i.e. 3 days at 10°C). *P. longipes* were reared using methods described previously in Chapter 2.

The investigations used the same experimental set up as described in Chapter 4 (and in Hazel *et al.*, 2008) and which have proved extremely successful at studying very small terrestrial arthropods. All experiments were conducted within a small temperature controlled aluminium block (see Figure 4.1), which allowed direct control of the temperature experienced by the mites. The arena was continuously

monitored throughout the experiments by video capture equipment and recordings were subsequently observed and analysed. For all experiments, the temperature within the arena was initially set at 28°C, the rearing temperature for non-acclimated mites or at 10°C for acclimated treatment groups. Temperatures in all experiments were reduced slowly at a rate of 0.1°C min⁻¹ to reflect environmentally realistic temperature change.

The data concerning *T. urticae* within this chapter, with the exception of the predation experiments, are purely replicates of those shown in Chapter 4 but for completeness and ease of reference the figures have been renumbered and are shown again in this Chapter.

6.3.1 CT_{min} and chill coma

The temperature was reduced from the rearing temperature to 10°C at a rate of 0.5°C min⁻¹ and was then further lowered from 10°C to -5°C at 0.1°C min⁻¹. Mite behaviour was recorded continuously during the experiment and the video footage was reversed before playback (using StudioPlayer, Studio86Designs, Lutterworth, U.K.). The temperatures at which each mite stopped walking (CT_{min}) and then moved an appendage (leg or antennae) for the last time (chill coma) were recorded. Acclimated and non-acclimated adult and larval mites of *P. longipes* were tested and the CT_{min} and chill coma temperatures were recorded for each mite. Data were tested for distribution and analysed by ANOVA and Tukey's HSD using MINITAB v 14.0.

6.3.2 *Chill coma recovery and activity recovery*

Using a new sample of mites, the arena temperature was lowered from the rearing temperature to 10°C at a rate of 0.5°C min⁻¹ and then further lowered from 10°C to 1°C at a rate of 0.1°C min⁻¹ to 1°C below the previously recorded minimum chill coma temperature. After a hold period of 15 min, the mites were heated back up to 28°C at a rate of 0.1°C min⁻¹. The experiment was recorded and the recovery phase was played back as previously described. The observed temperatures at which the mites began to move an appendage for the first time (chill coma recovery) and then to walk spontaneously (activity recovery) were recorded. Data were tested for distribution and analysed by ANOVA and Tukey's HSD using MINITAB v 14.0.

6.3.3 *Walking speed*

Walking speeds of adult and larval acclimated and non-acclimated *P. longipes* were measured at a range of temperatures. The mites were held for 10 min at each test temperature: 30°, 25°, 20°, 15°, 10° and 5°C. Non-acclimated mites were exposed to the highest temperature first and then the arena was progressively cooled to the lowest temperature. Acclimated mites were first exposed at 10°C and then cooled to 5°C, after which they were warmed back up to each higher temperature. This method was chosen so as not to remove any acclimation response that had been acquired with the 10°C treatment. Mite behaviour and movement was recorded during the entire experiment and video footage was examined at each temperature interval to measure the distance covered per unit time from which the walking speeds of the mites could be calculated. The walking speed of 100 mites from each treatment at each temperature was recorded. The mean walking speeds of the different treatment groups

of *P. longipes* were compared against each other and to that of *T. urticae* by one-way ANOVA and Tukey's HSD.

6.3.4 Predation

Interactions between predatory mites (*P. longipes*) and their prey (*T. urticae*) were observed at a range of temperatures, the experimental methods for which were conducted in entirely the same manner as for *A. swirskii* in Chapter 4. The predatory ability of ten adult *P. longipes* were tested at 30°, 25°, 20°, 15°, 10° and 5°C. Both acclimated and non-acclimated *P. longipes* were tested, and for the experiments with acclimated predators, *T. urticae* were also subjected to the same pre-exposure acclimation. After each experiment, the arena was cleaned and new mites, both predator and prey, were introduced for the next experiment. *P. longipes* were starved for 12 h prior to the experiments. Observations were made on (i) the frequency with which a predatory mite made contact with a prey mite, (ii) the number of attempts made to handle and attack a prey item, and if successful, (iii) how long the predatory mite remained engaged with its prey. Each experiment was repeated three times at each temperature and the data analysed by one way ANOVA.

6.4 Results

6.4.1 CT_{min} and chill coma

The temperatures at which the last mite in each sample of acclimated and non-acclimated adults and acclimated and non-acclimated larval *P. longipes* ceased

walking (CT_{min}) were 1.3°, 2.4°, 3.3° and 5.0°C respectively. The equivalent mean values for these groups were 2.8°, 4.3°, 4.8° and 14.9°C. The lowest chill coma temperatures for individual mites in the four treatment groups were 0.8°, 1.5°, 2.3° and 3.6°C respectively, and the mean values were 1.8°, 2.8°, 3.4° and 7.9° (shown in Table 6.1). Figure 6.1 illustrates the mean CT_{min} and chill coma temperatures for each of the mites sampled for acclimated and non-acclimated adult and larval *P. longipes* and adult *T. urticae* respectively. All graphs show the CT_{min} occurring at a higher temperature than chill coma and give some indication of the difference in temperature at which these two thresholds occur.

Table 6.1: Mean (\pm SE) temperatures (°C) at which walking stops (CT_{min}), movement stops (chill coma), movement starts (chill coma recovery) and walking starts (activity recovery) for four treatment groups of *Phytoseiulus longipes* and adult *Tetranychus urticae* (n=30)

Mite treatment group	Mean (\pm SE) activity threshold temperatures (°C)			
	CT_{min}	Chill coma	Coma recovery	Activity recovery
Acclimated Adults	2.8 \pm 0.12	1.8 \pm 0.14	3.3 \pm 0.19	4.7 \pm 0.40
Non-acclimated Adults	4.3 \pm 0.39	2.8 \pm 0.16	5.6 \pm 0.25	7.6 \pm 0.50
Acclimated Larvae	4.8 \pm 0.43	3.4 \pm 0.22	4.7 \pm 0.19	10.9 \pm 0.94
Non-acclimated Larvae	14.9 \pm 0.97	7.9 \pm 0.53	9.7 \pm 0.59	19.7 \pm 0.53
Acclimated Adult <i>T. urticae</i>	4.3 \pm 0.43	2.8 \pm 0.21	11.8 \pm 0.93	9.3 \pm 0.52
Non-acclimated Adult <i>T. urticae</i>	5.5 \pm 0.39	4.4 \pm 0.26	13.2 \pm 1.12	10.8 \pm 0.59

The mean chill coma threshold was recorded at a significantly lower temperature than the mean CT_{min} for only the non-acclimated larval group ($P < 0.05$), indicating that mites in the other three groups (acclimated and non-acclimated adults and acclimated larvae) continued walking behaviour until close to their chill coma threshold temperature. For both thermal indices, CT_{min} and chill coma, there was no difference

in the temperatures of the thresholds displayed by acclimated and non-acclimated mites of either life stage.

Adult *P. longipes* had a lower mean CT_{min} and chill coma temperature than larval groups ($P < 0.05$), but these values did not differ significantly from those of adult *T. urticae*. Mean CT_{min} and chill coma temperatures of larval *P. longipes* occurred at higher temperatures than those of *T. urticae*. At temperatures below the chill coma threshold, rapid and violent shivering was observed for both treatment groups of larval *P. longipes* at temperatures ranging from 0.9° to -4.7°C. This shivering response was not observed in adult *P. longipes* at temperatures down to -5°C.

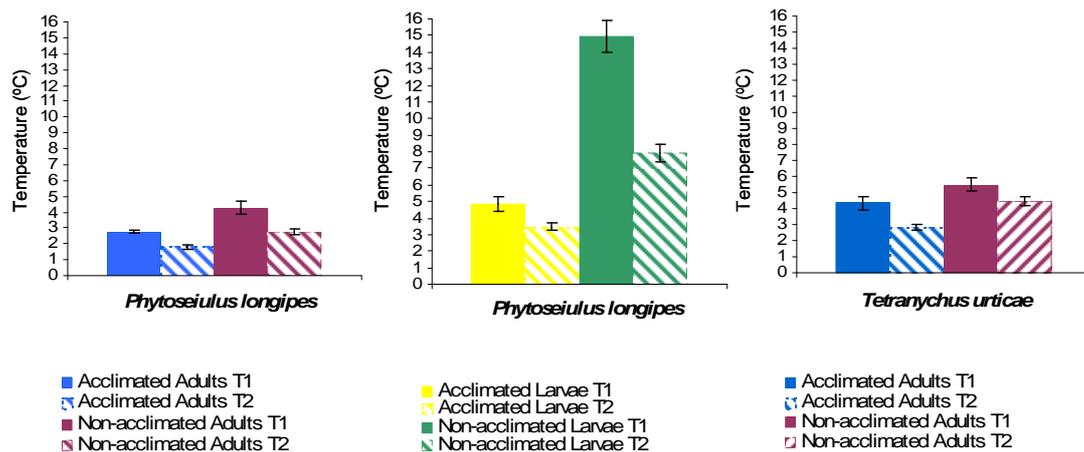


Figure 6.1: Mean threshold temperatures (\pm SE) for acclimated and non-acclimated adult and larval *Phytoseiulus longipes* and adult *Tetranychus urticae*: CT_{min} (T_1) represented by a closed bars and chill coma (T_2) represented by hatched bars. Different colours distinguish the different treatments.

6.4.2 Chill coma recovery and activity recovery

The temperatures at which the first mite from those sampled, began to recover from chill coma were 1.1°, 2.2°, 3.0° and 3.6°C for acclimated and non-acclimated adult and larval *P. longipes* respectively. Subsequently the temperature thresholds for resumption of walking activity for the first mite of each treatment group were 2.7°,

2.7°, 4.8° and 15.3°C. The mean temperatures at which mites began to recover from chill coma were 3.3°, 5.6°, 4.7° and 9.7°C for the first movement and 4.7°, 7.6°, 10.9° and 19.7°C for the first walking step (activity threshold) for acclimated and non-acclimated adults and larval mites respectively (see Table 6.1).

Figures 6.2 illustrates the mean chill coma recovery and activity recovery thresholds of acclimated and non-acclimated adult and larval *P. longipes* and adult *T. urticae* respectively. All three figures show walking activity occurring at a higher temperature than the coma recovery and gives a general indication of the difference in temperature at which these two thresholds occur.

It was only with the larval groups of *P. longipes* that the coma recovery temperature differed significantly from the activity recovery temperature ($P < 0.05$ for acclimated larvae and $P < 0.01$ for non-acclimated larvae). There was no difference in the temperatures at which the first movement (coma recovery) or the first walking step (activity recovery) was made by acclimated or non-acclimated adult *P. longipes*, but both adult thresholds occurred at significantly lower temperatures than were recorded in either larval group ($P < 0.01$). Adult *P. longipes* also resumed activity for both movement and walking at lower temperatures than *T. urticae* adults ($P < 0.05$).

A period of acclimation resulted in lower threshold temperatures for both chill coma recovery and activity recovery in the acclimated larval group compared to the non-acclimated group ($P < 0.05$). The walking activity threshold for larval *P. longipes* is incomplete because some individuals failed to resume walking during the experimental period even after arena conditions had returned to 28°C. The proportion

of larval mites that failed to walk during the activity recovery threshold experiments were 33% and 57% for acclimated and non-acclimated mites respectively. However, appendage movement (chill coma recovery) was recorded in 100% of mites sampled. Following the recovery experiments, all larvae were transferred to recovery tiles and returned to their normal rearing conditions (28°C, 18:6 LD) and were observed for 24 h; 100% of larvae resumed walking within 1 and 15 h after exposure. None of the mites died in the 10 days following exposure to their chill coma temperature.

T. urticae did not show any differences between chill coma recovery and activity recovery thresholds and also, no acclimation response was observed.

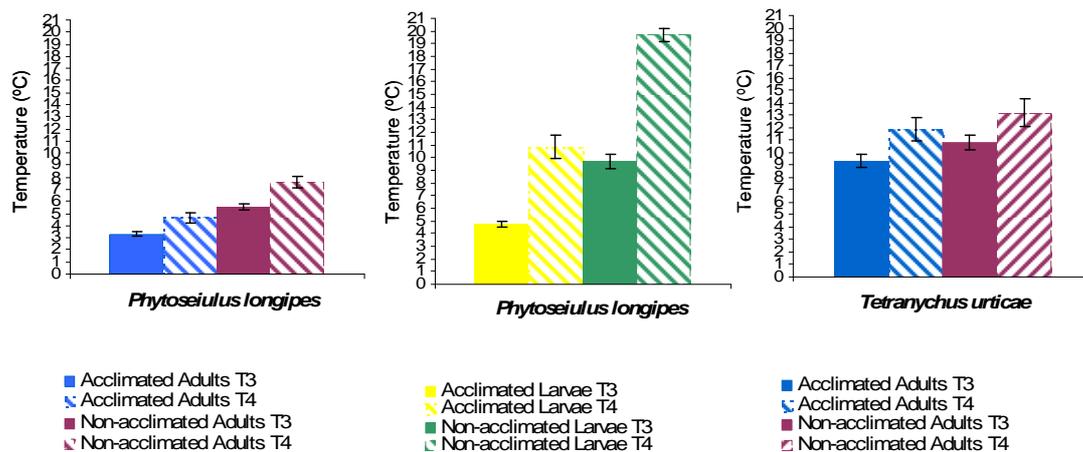


Figure 6.2: Mean threshold temperatures (\pm SE) for acclimated and non-acclimated adult and larval *Phytoseiulus longipes* and adult *Tetranychus urticae*: chill coma recovery (T_3) represented by a closed bars and activity recovery (T_4) represented by hatched bars. Different colours distinguish the different treatments.

6.4.3 Walking speed

The mean walking speeds for acclimated and non-acclimated adult *P. longipes* and *T. urticae* are shown in Figure 6.3. Mean walking speeds at 5°, 10°, 15°, 20°, 25° and

30°C were 0, 0.3, 1.0, 2.8, 4.9, 5.4 mm s⁻¹ for non-acclimated adult *P. longipes* compared with 0, 0.2, 0.4, 0.7, 1.1 and 1.4mm s⁻¹ for *T. urticae*. *P. longipes* moved fastest at 30°C (P<0.01) and walking speed decreased significantly at each lower temperature (P<0.05). Acclimated adults walked faster than non-acclimated adults at 30°, 25°, 10° and 5°C (P<0.05); there was no difference in walking speeds at 15°C.

At each experimental temperature, acclimated *P. longipes* moved faster than acclimated *T. urticae* (P<0.01). At 30°C *P. longipes* moved more than three times the speed of *T. urticae* (6.8 compared to 2.2mm s⁻¹ respectively). The only temperature at which an acclimation response was seen in *T. urticae* was 30°C, where acclimated mites walked faster than non-acclimated. Walking speed of *T. urticae* decreased with a decrease in temperature in a curvilinear relationship. There were significant differences between speeds at 30°, 25°, 20° and 5°C (P<0.05). Walking speeds at 15°, 10° and 5°C did not differ. At 5°C, no walking occurred for non-acclimated mites of either species.

Reliable data on the walking speeds of larval mites was not possible to obtain as individuals failed to maintain walking movement long enough for an accurate calculation of speed to be made. This result was also apparent following the chill coma recovery experiments where a large proportion (45% of larval mites sampled) failed to resume any walking response after recovering from chill coma.

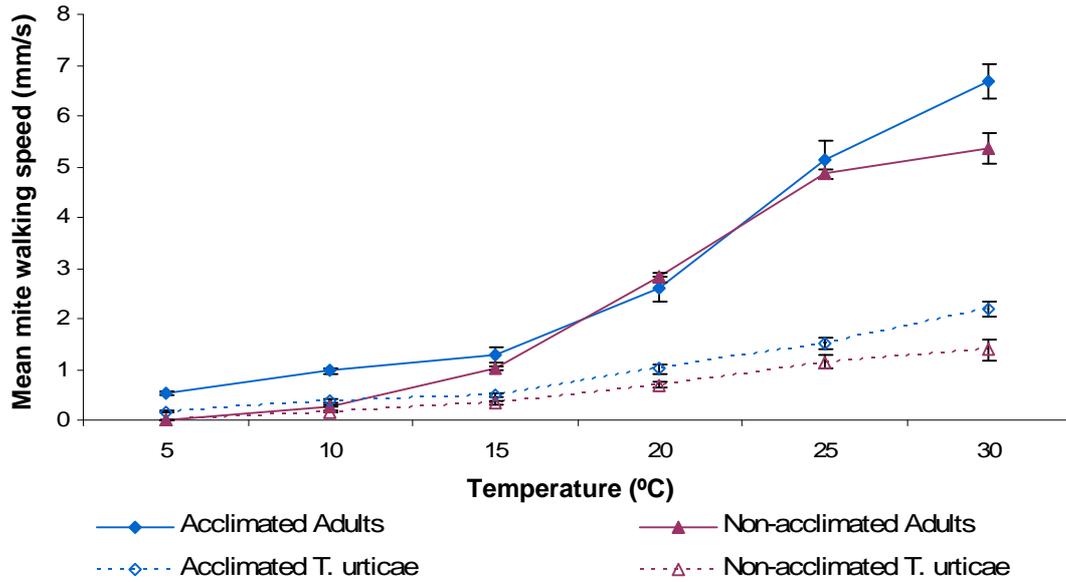


Figure 6.3: Mean (\pm SE) walking speeds (mm/s) of acclimated (blue) and non-acclimated (purple) adult *Phytoseiulus longipes* (solid line) and adult *Tetranychus urticae* (dashed line). (n=100)

6.4.4 Predation

The mean number of contacts with prey, attempts to grasp and handle prey and time spent engaged with prey are shown in Figure 6.4 for acclimated and non-acclimated adult *P. longipes*. At each temperature there was no difference between the frequency of contacts made between prey and predators of acclimated and non-acclimated *P. longipes*. The mean number of times that non-acclimated *P. longipes* made contact with items of prey were 5.3, 7.9, 7.0, 8.4 and 6.8 at 10°, 15°, 20°, 25° and 30°C respectively. At 10°C, the number of contacts were less than at all other temperatures ($P < 0.01$), and also, the number of contacts made at 30°C were significantly lower than at 12°, 20° and 25°C ($P < 0.05$). Contacts made with items of prey increased between 10° and 15°C increased ($P < 0.05$). At 15°, 20° and 25°C there were no differences in the number of contacts with prey. At lower temperatures, *P. longipes* appeared to spend more time investigating items that it came into contact with (including prey and

same species mites and the OecoTak® barrier) than at higher temperatures, even though this behaviour was not considered to be prey/attack handling.

There was a significant difference between number of attack attempts between at 10° and 15°C with non-acclimated mites attempting to handle more prey items that they came into contact with than acclimated mites ($P < 0.01$). Number of handling attempts appears to follow a non-linear distribution where the optimal number of prey handling attempts was 7.0 and 7.8 *T. urticae* (for acclimated and non-acclimated mites respectively) within the 30 min experimental period at 25°C, with similar to the values recorded at 20°C.

There were significant differences at 10° and 15°C between the contacts with prey and the number of attack attempts for both treatment groups ($P < 0.01$). This suggests that at these temperatures fewer contacts with prey result in an attack response from *P. longipes*. At 20°, 25° and 30°C there was no significant difference between the number of contacts made and the number of attempts to handle the prey, suggesting that *P. longipes* attempt to handle and attack all of the prey items they come into contact with at this temperature. Prey recognition behaviour also appeared to be much slower at lower temperatures, as shown by the time delay between making a contact with a mite and any attack response.

The amount of time spent engaged with and handling prey increased with temperature and was significantly longer at 30°C than at 25°C ($P < 0.01$), longer at 25° than 20°C ($P < 0.01$) and longer at 20° than at 15°C ($P < 0.05$). There was no difference between handling time at 15° and 10°C. As handling time increased at higher temperatures this

would result in a reduced level of contact with new prey items and therefore a reduced the number of attacks. This can be seen for both acclimated and non-acclimated *P. longipes* at 30°C in Figure 6.4. Handling time for acclimated mites was lower than for non-acclimated at 30° and 25°C ($P < 0.05$), but no differences resulting from acclimation were observed at any other temperature.

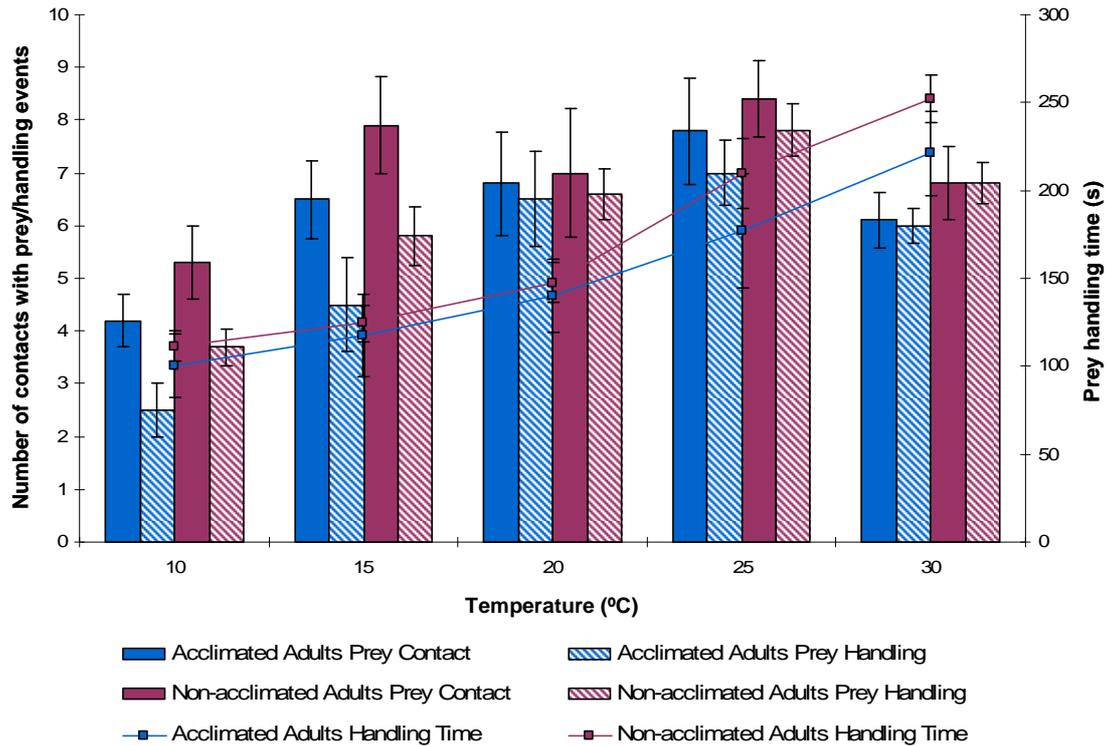


Figure 6.4: Mean (\pm SE) number of contacts between acclimated and non-acclimated adult *Phytoseiulus longipes* and their prey (closed bars), mean number of attempts to handle prey items (hatched bars) and mean time (in seconds) to handle each prey item (line). Error bars on all points indicate the standard error of the mean.

6.5 Discussion

As discussed in Chapter 4, the assessment of thermal activity thresholds to identify the range of conditions under which different species are active has potential to lead to a better understanding of the efficacy of predatory biological control agents. Activity at low temperatures can also be used to infer possible dispersal ability of

control agents outside of a glasshouse environment. As part of an environmental risk assessment of candidate agents, this measure would provide useful information on local dispersal ability in the immediate surroundings of the glasshouse and those further a field. Also, these data can be integrated into studies on the likely effects that local climatic variation and longer term climate change might have species distributions (Jumbam *et al.*, 2008).

The present study has been shown that temperature affects the physiological and behavioural activity of *P. longipes* and that this relationship is not always increase linearly with temperature. *P. longipes* adults respond to a period of acclimation by significantly lowering the temperature at which they are active for all four of the threshold indices compared to the larval and *T. urticae* treatment groups. This suggests that *P. longipes* conforms with the generalised rule described by Mason (2007), that the temperatures at which insects enter chill coma and recover from it can be reduced by acclimation.

There was more variation in the CT_{min} and activity recovery temperatures (T_1 and T_4 respectively) than in the chill coma and coma recovery thresholds (T_2 and T_3 respectively), particularly in the larval groups. These observations support the view of Hazell *et al.*, (2008) that the temperature at which walking ceases (CT_{min}) and the temperature at which walking resumes (activity recovery) most likely reflect behavioural response thresholds. The chill coma threshold of *P. longipes* was significantly lower than the CT_{min} , which also suggests that walking is a behavioural response to low temperatures and perhaps voluntarily halted to save energy in species such as predatory mites.

The thermal activity thresholds of *P. longipes* are lower than *T. urticae* as illustrated by CT_{min} temperatures of 2.4° and 3.3°C for non-acclimated adult *P. longipes* and *T. urticae* respectively. Also, the chill coma threshold for non-acclimated *P. longipes* was lower than that of the same treatment in *T. urticae* (1.5°C compared with 2.7°C respectively). In similarity to the response of *T. urticae* to low temperatures, adult *P. longipes* have mean chill coma and CT_{min} temperatures close to the lowest value of the sampled population. This suggests that as temperatures decrease, adults will remain mobile and active for as long as possible until the effects of the cold on physiological functions causes them to cease movement. These data also suggest that *P. longipes* would remain active at temperatures at which *T. urticae* would become immobile, giving the predatory mite an advantage.

The mean temperatures at which non-acclimated *P. longipes* resumed movement (chill coma recovery) and began walking (activity recovery) were 5.6° and 7.8°C respectively. In comparison, *T. urticae* has much higher recovery threshold temperatures of 10.8°C and 13.2°C for movement and walking respectively. Therefore, again *P. longipes* would also exit from chill coma at lower temperatures than *T. urticae* and thus be able to actively seek prey when *T. urticae* would still be immobile. It seems likely that the advantage conveyed to a predator that is active at temperatures at which their prey have limited or on mobility would contribute to the efficacy of *P. longipes* as a control agent of *T. urticae*.

The chill coma temperature for larval *P. longipes* was the same as that recorded for *A. swirskii* (3.6°C), which for *P. longipes* is <0.5°C above the LTemp₁₀ (3.2°C) (exposure temperature that results in 10% mortality) (see Chapter 5). This may

explain why in the coma recovery experiments and exposure to the chill coma threshold, many individuals failed to begin walking at any temperature up to 28°C and only resumed the ability several hours after being returned to the optimal rearing conditions. The lowest recorded CT_{min} occurred at 5.0°C for non-acclimated larval mites compared with 2.4°C for non-acclimated adult *P. longipes*, suggesting that adults are more active at low temperatures and more cold tolerant than the larval stage in this species.

At temperatures below the chill coma threshold, rapid and violent shivering was observed for both larval groups of *P. longipes* at temperatures ranging from 0.9° to -4.7°C. These rapid muscle spasms are indicative of the failure of muscles to maintain the resting potential and disruptions in calcium channels (Staszak & Mutchmor 1973b). The same shivering response was not observed in adult *P. longipes* at temperatures down to -5°C. It may therefore be argued that the adults had not entered true chill coma as defined by Staszak & Mutchmor, 1973a. However, as chill coma has been described in more recent work (Hazell *et al.*, 2008) as the temperature at which all coordinated movement ceases and as the current study more sophisticated equipment to monitor small movements of mite appendages, it seems that estimates of chill coma presented in this Chapter are accurate. The relative inactivity of larval *P. longipes* even in favourable conditions highlights the greater accuracy that can be gained by observations of small-scale movements rather than a gross descriptor such as the temperature at which the organism cannot walk. Also, observations made in real time with the naked eye are laborious and subject to error.

The walking speed experiments confirmed casual observations of the relative mobility of *P. longipes* compared with *T. urticae* during routine culturing i.e. the predator is a faster moving species. At 20°C non-acclimated adult *P. longipes* walk at approximately four times the speed of *T. urticae* (2.8mm compared with 0.69mm s⁻¹). Acclimated mites of both species showed some mobility at 5°C but it was very limited when compared with walking speeds at temperatures above 5°C.

The larvae of *P. longipes* do not feed and do not require food to moult to the protonymph stage (Takahashi and Chant 1992), hence the relative inactivity of this stage is to be expected. Consequently, it was not possible to obtain any data for walking speeds and predatory activity of larval *P. longipes*. The data also suggests that at walking speeds of over 0.7 mm s⁻¹ *T. urticae* requires a minimum temperature of 20°C, for reason discussed in Chapter 4.

There has been little work previous work on the effects of temperature on predation by phytoseiid mites (Sepulveda & Carrillo 2008). Temperature appears to have a direct effect on the predatory activity of *P. longipes* as when temperature increased, the number of attempts to handle contacted prey items also increased. The proportion of contacts with prey that resulted in an attack increased with temperature; in general, the predatory ability of *P. longipes* showed a curved relationship, with an optimum efficiency at 25°C. Other studies on predatory Phytoseiid mites used as biocontrol agents (*Neoseiulus californicus* and *Phytoseiulus persimilis*) showed similar responses, with a specific optimum temperature above which, rates of predation declined; for example, *N. californicus* prey consumption was highest at 25°C and lower at both 20° and 30°C (Kustutan & Cakmak 2009), and the mean daily

consumption of prey by adult *P. persimilis* females was 11.85, 20.64, and 15.41 at 20°, 25° and 30°C respectively (Kazak 2008). A further example includes the mean daily and total prey consumption of *Panonychus ulmi* (Koch) (Acari, Tetranychidae) by adults of the predatory mite *Typhlodromus pyri* (Scheuten) (Acari, Phytoseiidae) which also decreased significantly as the temperature was increased from 25° to 30°C (Sengonca *et al.*, 2003).

There is a greater difference in predatory activity of acclimated and non-acclimated *P. longipes* at 10° and 15°C than at any other experimental temperature. There was also a negative acclimation response with non-acclimated mites being most active in finding and attacking prey at these lower temperatures. This may be due to a deleterious effect of the acclimation regime, or because metabolism has been reduced to a level that non-acclimated mites will not experience during such a short period at low temperatures. At the higher temperatures this difference between acclimated and non-acclimated mites is lost suggesting that the slowing down of metabolism as a consequence of exposure to low temperatures takes a longer time than the increase in metabolism when an acclimated mite is returned to more favourable conditions. This would serve well as an evolutionary function, as once out of unfavourable conditions it would be beneficial to resume predation as rapidly as possible, whereas when entering unfavourable conditions, continued predation even for a short time period could convey a selective advantage to the individual.

The time spent handling prey (and it can be assumed feeding) increased at higher temperatures indicating that *P. longipes* spends more time feeding from each prey item at higher temperatures. This apparent preference to feed for longer at higher

temperatures may be attributable to an increase in metabolism. As a result of spending more time engaged with individual prey items, the number of contacts made during the observation period (30 min) is reduced i.e. because *P. longipes* spends more time feeding, the time spent actively foraging is correspondingly reduced. At lower temperatures, *P. longipes* were observed to remain stationary following contacts with other mites of the same species and with their prey. This apparent slowing of reactions and inability to recognise items as prey or otherwise could be explained by the decreased neuromuscular control at lower temperatures. At higher temperatures mites showed a more attacking behaviour toward contacts with other mites.

Further research on the predatory ability of *P. longipes* could investigate preferences for different prey life stages. For example, it was observed that *N. californicus* consumed more eggs and larvae than nymphs and adults of *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae), suggesting that the energy cost to attack and subdue an egg or larvae is less than that for an adult (Kustutan & Cakmak 2009). Also, the ladybird *Stethorus punctillum* (Weise) (Coleoptera: Coccinellidae) showed a preference for *T. urticae* eggs, nymphs or adults depending on its own developmental stage. Perhaps counter-intuitively, adult *S. punctillum* showed a preference for *T. urticae* eggs, whereas the larval stages had no preference for different life stages (Ragkou *et al.*, 2004). Another research direction could be to investigate the feeding capacity of *P. longipes* and link this to its efficacy as a biological control agent down at the level of individual organism. (For example how many prey items can individual *P. longipes* consume before becoming satiated?) As activity levels and foraging success of ectotherms are dependent on both extrinsic (e.g., ambient temperature) and intrinsic factors (e.g., hunger level) (Subach *et al.*, 2009), total prey consumption per

unit time could be investigated and this data then used to determine the density of control agents to release in any given size of glasshouse environment.

The experiments in this Chapter were not intended to estimate the pest suppressive power of *P. longipes* as a predator of *T. urticae* nor to determine its functional response (Solomon 1949). Rather, the data provide information on the efficacy, behaviour and physiology of *P. longipes* at various temperatures within an ecologically relevant range. With regard to the dispersal ability of *P. longipes*, a period of acclimation significantly increased mobility at lower temperatures and based on equivalent data for *A. swirskii* described in Chapter 4, it seems that *P. longipes* is active at lower temperatures. If escapes from a glasshouse occurred during summer then *P. longipes* could sustain a population over several generations provided that food and mates could be found. Also, *P. longipes* can survive for considerable periods of time outside of glasshouses in a UK winter climate (see Chapter 5) though no development or reproduction occurred during this time. For this reason, whilst *P. longipes* is more cold hardy than *A. swirskii* and is active at lower temperatures, permanent outdoor establishment seems unlikely. This area is discussed in more detail in Chapter 7. In summary, the most useful application of data presented in this Chapter would be the matching of *P. longipes* as a biological control agent to specific target prey and optimal conditions for its use as a predator in glasshouse environments.

CHAPTER 7

General Discussion

Biological control by natural enemies has been in operation for hundreds of years, though the control of the scale insect *Icerya purchasae* in California in the 1880s (Waage & Greathead 1988) is widely regarded as the first successful scheme in the modern era; biocontrol therefore predates the use of chemical pesticides in agriculture and horticultural practices by more than 50 years. More than 5000 introductions of exotic natural enemies have taken place worldwide in the past 100 years (van Lenteren *et al.*, 2003) and up until the 1990s, the safety of biological control was rarely questioned. Researchers and growers alike assumed a natural control would have no harmful side effects. However, some of the methods employed in the first forms of environmental risk assessment of biological control agents lacked scientific rigour; for example climate matching was used to assess establishment potential rather than a direct assessment of cold tolerance. It was assumed that an exotic species used as a control agent in a cooler climate would be unable to survive outside the protected glasshouse environment, and thus unable to establish wild populations. Until quite recently the non-target affects of non-native species introductions have rarely been considered (Babendreier *et al.*, 2006). In contrast to the detailed risk assessment procedures for weed biocontrol, the systems used for invertebrate biological control agents (IBCA) are less structured, or in some countries, completely absent (van Lenteren *et al.*, 2006). Following retrospective analyses of previous releases where the introduced species has managed to establish in the non-native environment, there is now a recognition of the need to assess

establishment potential and risks for non-target species as part of the evaluation of a biological control programme.

The gradual withdrawal of insecticides without replacement compounds and uncertainties surrounding the use of genetically modified crops, has provided an opportunity and a need for biological control to be more successfully exploited. It would probably take less time to identify and assess a new control agent than it would to engineer a resistance trait in a crop, and for many horticultural crops, there have been no major developments in genetic engineering (J. Bale, personal communication).

Whilst there have been very few negative effects of biological control (van Lenteren *et al.*, 2006), the releases that cause problems are widely publicised, and rightly so. It is important to protect our native flora and fauna and at the same time encourage safe and successful biological.

There is strict regulation of non-native releases in Canada, USA, New Zealand and Australia, and in some European countries, but regulation across Europe is patchy. For example, non-native control agents are regulated in Switzerland but not in neighbouring France and Italy. Invertebrates have no knowledge of international country boundaries and non-native species are commonly dispersed around the world via the movement of people and freight, and accidental introductions vastly outnumber those intentionally introduced (Pimentel *et al.*, 2001). Currently there is a move towards a more stringent and consistent regulatory framework in Europe and

this requires appropriate methods to provide more ecological relevant information and thus increase the accuracy of risk assessment.

Even with these environmental concerns, the use of biological control is likely to increase in the future, so it is important to devise effective environmental risk assessments (ERAs) which do not compromise environmental safety but also, do not put excessive financial burden on small companies. An ERA for a potential glasshouse biological control agent usually follows a series of assessments in sequence as shown in Figure 1.1. In northern Europe and temperate regions, the ERA would normally start with an assessment of cold tolerance as an indicator of establishment potential. However, in the warmer climates of southern Europe where conditions are favourable for year round development and reproduction, there no such thermal constraints and the ERA would focus on host range and dispersal.

In the UK release of non-native biological control agents is regulated under the Wildlife and Countryside Act 1981 by DEFRA. Whilst it is acknowledged that all introductions carry a risk of effects on the native environment, regulatory measures must be technically justified and not based on a biased viewpoint or a protectionist barrier to trade (McNamara 1997; Gray et al. 1998). The work undertaken in this study contributes to the risk assessment currently operating in the UK for non-native biocontrol agents intended for release in glasshouses.. Assessment of cold tolerance and overwintering ability therefore formed a large part of the programme of research.

This general discussion will focus on the three main areas of research undertaken in the project and discuss the results obtained in a wider context: firstly the establishment potential of *Amblyseius swirskii* in the UK; secondly the establishment

potential of *Phytoseiulus longipes* in the UK; and thirdly, the comparative thermal thresholds of *A. swirskii*, *P. longipes* and *Tetranychus urticae*. Some of the data obtained during this study has already been used by the CASE partner (Koppert) to seek a release licence in the UK for *A. swirskii*. Some final comments will therefore be made on the value of risk assessment protocols in current biological control programmes and for future applications.

7.1 Establishment potential of *Amblyseius swirskii* in the UK

Amblyseius swirskii is a generalist predator of several phytophagous pest species including whitefly, thrips and Tetranychid mites (Teich 1966, Swirskii *et al.*, 1967, Ragusa & Swirskii 1975, Hoda *et al.*, 1986). It originates from the warm climates of Israel and the Eastern Mediterranean area and has been targeted by biological control companies as an almost ideal ‘super’ control agent due to its polyphagous nature and simplicity to rear artificially in large numbers. It has been found to be an effective biological control agent on sweet pepper, cucumber, aubergine, strawberry and some cut flowers. It has been commercially available since 1995 (Arthurs *et al.*, 2009) and a successful licence application for glasshouse release in the UK was made in 2006.

In the current study, estimates of the developmental threshold indicate that between 1987 and 2007 *A. swirskii* could produce an average of four generations per year outdoors in the Midlands area of the UK. This development is most likely to be confined to the summer months between the start of May and middle of September. Between the middle of September and early May, no complete generation would be possible. Thus, to establish a wild population outdoors in the UK, the mite would

have to overwinter for over seven months of the year at temperatures well below its developmental threshold. *Amblyseius swirskii* has a developmental threshold temperature of 11.9°C and requires 101 degree days to complete development from egg to adult. At the optimal temperature of 27°C, *A. swirskii* takes 6.1 days to complete its development from egg to adult. Levels of egg hatch above 87% occur only at temperatures between 15° and 30°C.

Laboratory studies concluded that *A. swirskii* has no diapausing ability as oviposition continued in 100% of the sample population when reared under a regime known to induce diapause in other Phytoseiid mites. Oviposition rates were reduced when mites were reared under diapause-inducing conditions and continued for a short while increased when mites were returned to normal rearing conditions. There was no change in appearance in females placed in the diapause-inducing regime.

Investigation into the SCPs of *A. swirskii* showed that the species was freeze intolerant with substantial pre-freeze mortality. SCPs were distributed over a relatively wide range of temperatures (-13 to -28°C) and larval mites had consistently lower SCPs than adults. The data obtained from the lethal temperature and lethal time experiments showed that high levels of mortality occurred during exposures to sub-zero temperatures. The LTemp₅₀ ranged from -1.0° to -4.7°C for all treatment groups and the temperatures required for 10 to 90% mortality of the sampled mites was between 4.1° and -7.1°C. When held at 5°C the LTime₅₀ ranged from 1.1 to 3.9 days for non-acclimated larvae and acclimated adults respectively. At 0°C the LTime₅₀ ranged from 0.6 to 2.0 days and at -5°C 50% survival time was 13.7 minutes. In

combination, these data strongly suggest that *A. swirskii* is weakly cold tolerant and unlikely to survive outdoors through a UK winter.

The two winter field experiments confirmed this finding as a maximum survival time of only 8 days was recorded in the first field trial and 12 days in the second. During these times the mean field temperature was 2.3° and 3.2°C respectively and throughout the entire field exposure investigations outdoor temperature did not fall below -3.2°C. When temperatures did fall below zero during the first 48 h of the first field trial, mortality levels were between 20 and 92.5%. Following further sub-zero exposures in the subsequent few days, 100% mortality was recorded by day 8. These results concur with the laboratory data as the $LTime_{50}$ at 0°C was a maximum of 2 days and sub-zero temperatures proved to be lethal in a matter of minutes.

Acclimation appeared to provide a small advantage in laboratory and field exposures and adults survived for longer and at lower temperatures than larvae in every test. However, it must be noted that the acclimation period used previously by other researchers (seven days at 10°C) was found to cause both lethal and sub-lethal effects and had to be reduced to 3 days at 10°C for *A. swirskii* .

In comparison with studies on previously released biological control agents (Hatherly *et al.*, 2004, 2005; Hart *et al.*, 2002a), *A. swirskii* has an extremely weak level of cold tolerance with no possibility of permanent outdoor survival. Furthermore, it is difficult to envisage that currently predicted levels of climate warming would change climatic conditions in a UK sufficiently for *A. swirskii* to survive through winter and establish outdoor populations. It can therefore be concluded that *A. swirskii* is a ‘safe’

candidate for release as a biological control agent in the UK and in other northern European countries with similar a winter climate (see figure 7.1).

As a result of the data obtained during this study, *A. swirskii* was granted a licence for release into UK glasshouses in 2006 (ACRE, 2007).

7.2 Establishment potential of *Phytoseiulus longipes* in the UK

Phytoseiulus longipes is a Phytoseiid mite originating from South America that is under consideration as a potential biological control agent against *T. urticae*. It is a specialist predator of *T. urticae* (McMurty & Croft, 1997) and has been selected for possible use as a control agent because of its ability to associate very closely with *T. urticae* colonies. *Phytoseiulus longipes* has a delicate long-limbed body structure that enables it to move relatively unhampered through heavily webbed infestations and on leaves with hairs (Furtado *et al.*, 2006). It has not yet been commercialised and remains subject to investigations into target prey and ease of mass rearing.

In the present study the developmental threshold of *P. longipes* was estimated to be 12.1°C with a the thermal budget of 57 day degrees. Using this data it was estimated that *P. longipes* could produce an average of seven generations per year outdoors in UK based on average temperatures between 1987 and 2007. In a relatively warm year, *P. longipes* can produce up to 10 generations. All of the development, reproduction and thus population growth would be constrained to the summer months of May to September as average temperatures outside of this period were below the developmental threshold. Total development time from egg to adult was relatively fast

(3.6 days) at the optimal temperature of 30°C which is almost twice as fast as *A. swirskii*'s optimal developmental rate. Percentage egg hatch of *P. longipes* reached over 96% at temperatures between 15° and 30°C.

No ability to diapause was detected in *P. longipes* as all females continued to oviposit when maintained under diapause inducing conditions. As with *A. swirskii*, egg production was lower under the diapause-inducing conditions but this may have been attributable to the reduced relative humidity with this diapause regime. Following transfer back to optimal rearing conditions, *P. longipes* continued to oviposit and their metabolic rate increased, as observed by increased motility and feeding and also higher oviposition rates. There was no change in appearance of females under the diapausing conditions, as occurs in other mites when in diapause.

The cold tolerance of *P. longipes* was measured by SCPs, lethal temperature and lethal time experiments. The mites are killed by freezing but also show substantial pre-freeze mortality. The mean SCPs, particularly of adults, were considerably lower than those of *A. swirskii*. When exposed to sub-zero temperatures for brief periods of time, high mortality (LTemp₉₀) was not recorded until temperatures were lowered to between -15.1° and -17.5°C. Temperatures which resulted in 50% mortality of the sample populations were in the range of -8.0° and -13.1°C. It would be unusual for temperatures to fall to this level in a UK winter, except in very cold winters. When subjected to longer exposures at the more ecologically relevant temperatures of -5°, 0° and 5°C, maximum survival times for 90% of the sample were 7.3, 14.2 and 42.4 days respectively. These results suggested that *P. longipes* could survive for a maximum of 6-7 weeks under winter field conditions. In combination, these results suggest that *P. longipes* is more cold hardy than *A. swirskii*, but with no ability to diapause or to

develop and reproduce during in winter, is still unlikely to establish outside of a glasshouse environment.

Long term field trials corroborated the laboratory cold tolerance data for *P. longipes*; maximum field survival time 73 days. When working with a new species with unknown level of cold tolerance and acclimation ability and with only one winter season in which to collect field data, it is difficult to determine the appropriate sampling interval in relation to the total number of samples placed in the field. Also, the *P. longipes* field trials were set up before the laboratory assessment of cold tolerance had been completed. In retrospect, the sampling intervals used in the first field trial of the present study were too short with the result the final samples were brought in from the field before 100% mortality had occurred. However, although 30 to 50% adult survival was still apparent in the final sample of the December-January field trial (after 34 days), the second trial (January-April) did reach 100% mortality in all groups. In this case though, some of the later mortality may have been attributable to 'old age'. No reproduction or larval development occurred during the long term winter field trials. During the short field trials, some development and reproduction was observed, but only in the May and June exposures. These results support the predictions of potential voltinism of *P. longipes* based on the developmental threshold, thermal budget and average daily temperatures in the UK.

Acclimation did not confer any advantage to this species in either laboratory or field cold tolerance. Although *P. longipes* can survive for relatively long periods of time outdoors and short term exposure to sub-zero temperatures, this is not indicative of establishment potential. The mite has a relatively high developmental threshold which

would restrict development and reproduction to the summer months after which it would have to survive for over 7 months of the year in an overwintering state. Some localised survival and reproduction may occur close to glasshouses but this would be transient and permanent establishment seems highly unlikely. As with *a. swirskii*, it is difficult to envisage that climate change would alter this conclusion (Schermer *et al.*, 2000; Feehan *et al.*, 2009). By way of a comparison, *N. californicus* was not only able to survive for 3 months in the field, but could also reproduce during outdoor winter conditions, and the diapausing strain survived throughout the winter (Jolly 2000).

P. longipes is more cold tolerant than *A. swirskii* but not as cold tolerant as *N. californicus* or *M. caliginosus* (Figure 7.1). It is interesting to consider whether it is now possible to predict winter survival and establishment on the basis of laboratory data alone i.e. survival at 5°C. This may be possible if the species exhibited cold tolerance at or below the level of *A. swirskii* (see Figure 7.1). But for species that fall into an intermediate area on the graph, field trials would be the only accurate way of assessing establishment potential. Whilst *P. longipes* is without doubt more cold tolerant than *A. swirskii* and falls in this mid-range of risk, permanent establishment would require winter survival and both reproduction and development to occur in the field, and on the basis of present information, this is not possible. There may therefore be a role for *P. longipes* as a glasshouse biological control agent in the UK.

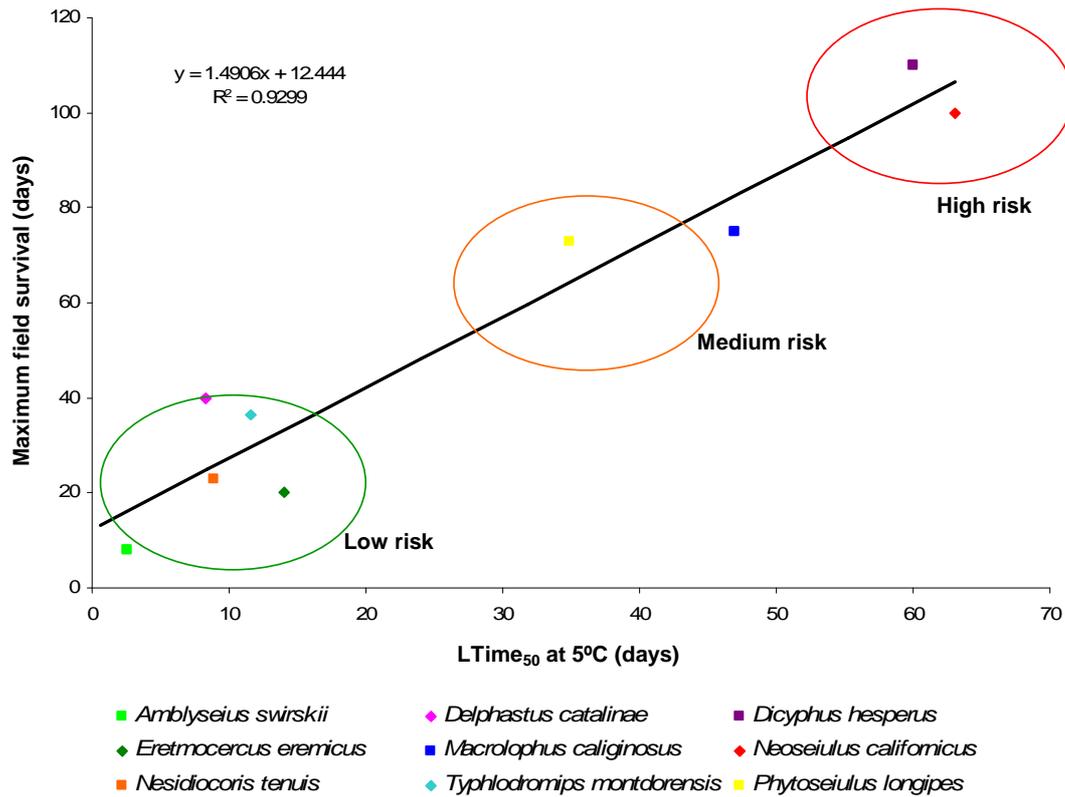


Figure 7.1: Correlation between $LTime_{50}$ at 5°C (days) and the maximum field survival time (days) for nine biological control agents intended for use in non-native countries (Adapted from Hughes *et al* 2009).

7.3 Comparative thermal thresholds of *Amblyseius swirskii*, *Phytoseiulus longipes* and *Tetranychus urticae*

The experiments described in this study are amongst the first to attempt to quantify the activity thresholds of biological control agents and their target prey, and to investigate their behaviour at ecologically relevant temperatures. Measurement of the CT_{min} and activity recovery threshold temperature of a biological control agent and comparison with its target prey species may provide valuable information on the

activity threshold and dynamics of the predator-prey interaction (Croft & Croft 1996; Dixon 2000).

Over a period of time, a list of the activity thresholds (CT_{\min} and activity recovery temperature) of a range of biological control agents could be produced that would enable companies and growers to match agents not only to target species, but also to the growing conditions of the crop. This could improve the efficacy of control whilst being more cost effective with regard to energy consumption in glasshouses.

In this study the mean CT_{\min} of *A. swirskii*, *P. longipes* and *T. urticae* were 6.2°, 4.3° and 5.5°C respectively. From this data it can be concluded that *P. longipes* were still able to walk when its target prey had become immobile, but *A. swirskii* would not be an effective predator at such low temperatures. The activity recovery thresholds for the three species were 7.8°, 7.6° and 13.2°C respectively. On this index, the two predatory mites were similar, and both had recovered the ability to walk well before *T. urticae*. It would seem sensible to suggest that in terms of efficacy, the most important thresholds are the CT_{\min} and the activity recovery temperature as these dictate whether the species will be active and predate successfully. On this measure, it can be concluded that *P. longipes* would be the most effective predator under a fluctuating temperature regime, as it retains the ability at a lower temperature and recovers from coma sooner than *T. urticae*. Knowledge of chill coma temperatures, would be important only if the control agents experienced such conditions and entry into chill coma proved to be damaging and irreversible.

Measurement of walking speeds showed that at temperatures above 10°C *A. swirskii* was the fastest moving predator and could travel almost five times faster than its prey. This suggest that if a glasshouse was held at an optimal temperature for *A. swirskii* the mite would come into contact with more prey in a given time period than *P. longipes* and reduce prey numbers more rapidly. Such knowledge would be useful if it was necessary to achieve a rapid reduction in pest numbers.

The predation interaction experiments made observations of (i) the number of contacts made between a predator and prey, (ii) the number of attempts the predator made at attacking a prey item and (iii) the handling time of a predatory attack. This data must be interpreted carefully if it is to be use in control situations. The proportion of contacts made with prey which then converted into an attack, increased with temperature for both predatory mites and reached a maximum at 30°C. The time spent handling an item of prey increased with temperature for both *A. swirskii* and *P. longipes* and there was little difference between the mean times at 30°C (252 and 260 s respectively).

Perhaps counterintuitively, the number of contacts made with prey items decreased with temperature and this was true for both *A. swirskii* and *P. longipes*. However, this observation can be explained by the fact that observations were made for a total of only 30 min; hence, the more time a predator spends handling each item of prey, the less time it will have to make new contacts. At lower temperatures, the number of contacts made with prey items by *A. swirskii* was significantly higher than for *P. longipes*. However, as the walking speed of *A. swirskii* was faster at all temperatures it seems apparent that they were less inclined to undergo prey recognition behaviour

than *P. longipes* at these temperatures, evidenced by *A. swirskii* immediately moving away from most contacts and *P. longipes* confronting more items it came into contact with. It can be concluded that at these lower temperatures the behaviour of *A. swirskii* may be related more to physiological stress and avoidance strategies than predatory ability (Zilhal *et al.*, 2007; Kim *et al.*, 2009).

In conclusion, the thermal activity experiments indicate that *P. longipes* would still be active at temperatures when its target prey had become immobile, can move faster at all temperatures compared with its prey, and can convert more predation opportunities (i.e. contacts with prey) into actual predation events at all temperatures when compared with *A. swirskii*.

It is apparent from the data acquired in this study that the separate predatory events can not be considered in isolation and do not relate linearly to temperature. The number of contacts made between a predator and its prey at different temperatures is not necessarily the most useful data to record if the contacts do not result in a definable attack on the prey item. Attempts at attack and handling of prey items is a more robust measure of predatory ability; and even though it can be difficult to quantify the damage to prey, it can be assumed that once a predator becomes engaged with a prey item for longer than a few seconds, then some damage will have been inflicted even if the prey has not been immediately killed. Further studies could be done using the same method to identify the optimum temperature at which predators engage and attack prey but do not then spend extended periods of time handling the item to maximise predator efficiency. This study has developed a robust method for

observing predatory behaviour at ecologically relevant temperatures and could be further refined to improve the selection and use of different control agents.

There is no doubt that biological control is an important technology and one that has an even greater role to play in pest management in the future. Demands for increases in world food production will continue and it may prove more cost effective to select and release biocontrol agents than to develop a GM crop targeted at a single pest (Bale *et al.*, 2008), though GM and biocontrol can be used in combination (Sanvido *et al.*, 2007; Romeis *et al.*, 2008). However, regulatory controls for the release of non-native biological control agents have to be sound, evidence-based and affordable by an industry that does not have large research and development budgets at their disposal (REBECA, 2008).

In summary, the work presented in this thesis is an example of research that is directly relevant to both the biological control industry and to regulatory authorities in the selection of safe and effective control agents. It also provides an opportunity to match specific agents with particular pests and growing conditions, with the possibility of reducing costs for growers. Overall, it is hoped that this work will contribute toward the development of a suite of well researched methodologies to promote safe biological control for the mutual benefit of the industry, growers, consumers and the wider environment.

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