1 Introduction

Temperature is one of the most important abiotic factors affecting life on earth. Temperature extremes are a major threat to life, affecting both the distribution and abundance of species. This is especially true for insects which are ectothermic and have a limited ability to regulate body temperatures above or below ambient. Ectotherms are consequently greatly influenced by environmental conditions (Walther *et al*., 2002), with winter temperatures often determining the limit to species' distributions (Bale, 1993b).

Temperature can affect insects in numerous ways, influencing reproduction, longevity, and development (Parish & Bale, 1993; Hutchinson & Bale, 1994), the latter of which may result in morphological abnormalities (Bale *et al*., 1989). At low temperature extremes the probability of survival is dependent upon the interaction between temperature and duration of exposure and the cold hardiness of the individual (Bale, 1987). Bale (1987) defines cold hardiness as the attributes of an individual that prevent deleterious effects when exposed to low temperatures. However, the survival of the insect is of little ecological importance if the exposure results in the individual's failure to reproduce and contribute to the next generation (Bale, 1987).

Insects are regarded as the most successful taxa, with species distributed from tropical to polar climates. At present, approximately 10^6 insect species have been described, representing over half of all known species, although the total number of insect species on the Earth is estimated to lie in the region of 10^7 to 10^8 (Pimm *et al*., 1995; Thomas, 2005). A major factor contributing to insect success is the development of a range of mechanisms to overcome the problems associated with temperature extremes.

1.1 Insect survival strategies at low temperatures

1.1.1 Classification of cold hardiness strategies

Insects survive at low temperatures either by an ability to tolerate the internal freezing of their body tissues and fluids or alternatively, to avoid such freezing by supercooling (Bale, 2002). Salt (1961) produced a classification scheme that categorized insects depending on their ability to survive the formation of extracellular ice, categorizing insects as either freeze tolerant or freeze avoiding (Salt, 1961). To determine the species' classification, the insect is cooled at a constant rate, typically 1°C min^{-1} (Baust, 1986), to the temperature of crystallization or 'supercooling point' (SCP). The SCP is detected by the release of heat that occurs when water crystallizes to ice. The insects are then warmed and survival assessed, providing an indication of the cold hardiness of the insect and whether the insect is freeze tolerant or intolerant.

Freeze tolerant insects are those which can survive extracellular, and possibly intracellular, ice formation and in doing so are able to survive temperatures below the supercooling point. The majority of freeze tolerant insects occur in areas where extreme winter conditions are experienced, for example, the Arctic and sub-Arctic regions of America, Scandinavia, Russia and China (Bale, 1996). More recent work has suggested that freeze tolerance, in addition to being a strategy for surviving extreme Arctic conditions, is advantageous to surviving the milder conditions of the southern hemisphere where unpredictable cold spells can occur throughout the year (Sinclair *et al*., 2003; Sinclair & Chown, 2005). Sinclair *et al*., (2003) propose that freeze tolerance evolved in parallel in the two hemispheres, allowing survival during seasonal periods of extreme cold winters in the northern hemisphere and in the southern hemisphere, survival during unpredictable, intermittent cold spells. Freeze tolerance is achieved via the production of three main chemical compounds: ice nucleating agents (INAs), antifreeze proteins (AFPs) and polyhydroxy alcohols (polyols) and sugars, which are generally accumulated in the autumn and winter months.

Freeze intolerant or freeze avoiding insects as they are sometimes described, in contrast to freeze tolerant insects, are unable to survive extracellular ice formation and must actively avoid freezing via supercooling. The process of supercooling maintains the body tissues and fluids at a temperature below which freezing would occur and is achieved using polyols and AFPs. It is thought that most temperate insect species fall into this category (Bale, 1991).

The key difference, therefore, between freeze tolerant and freeze avoiding insects is the role of INAs. Within freeze tolerant insects INAs are synthesized and activated to allow for controlled extracellular freezing, whereas the converse is true in freeze avoiding insects where INAs are removed or masked to prevent freezing (Zachariassen, 1985). Unlike INAs, both freeze tolerant and freeze avoiding insects contain AFPs, polyols and sugars, although the role of these chemicals differs between the two strategies (Figure 1). In freeze tolerant species polyols, such as glucose, increase the insect's supercooling capacity and aid in the cryoprotection of tissue before INAs have become fully active and AFPs play a role in the prevention of recrystallization when body temperatures begin to rise at the end of winter (Bale, 1996). Polyols, in freeze avoiding insects, also enable insects to supercool to temperatures below which freezing would normally occur. The AFPs then act to stabilize the supercooled state (Zachariassen, 1985; Bale 1996).
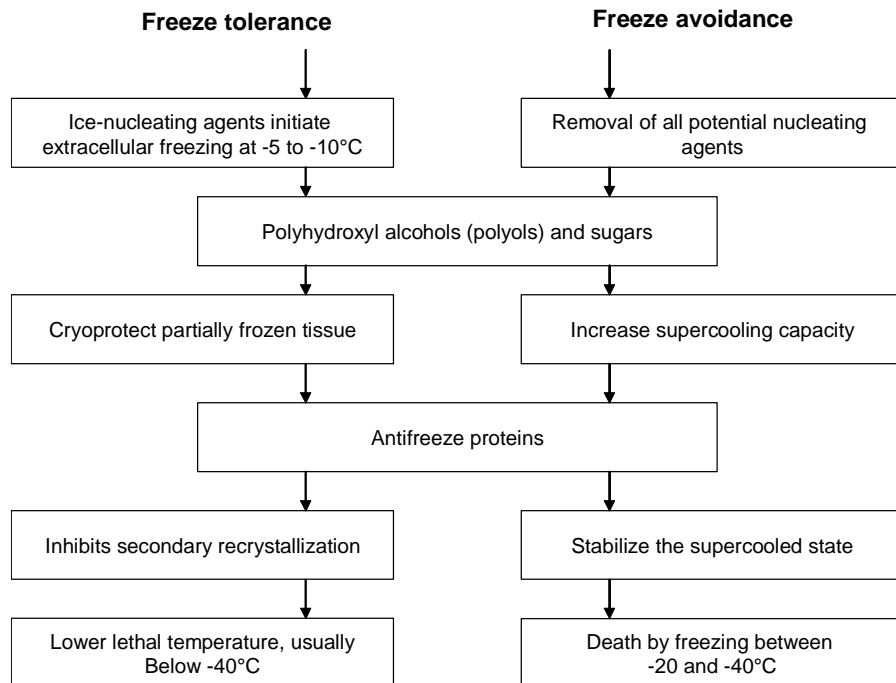
Figure 1. The biochemical mechanisms adopted by freeze tolerant and freeze avoiding insects (Bale 1996, 2002).

The classification scheme assumes that the only threat to insects at low temperature is freezing, although many insects in reality die at temperatures higher than their supercooling point, with death resulting from chill-related factors as opposed to actual freezing. The classification therefore incorrectly assumes that supercooling points are the lower limit of insect survival, when in fact they represent the lower limit to the insect's ability to supercool (Bale, 1996). The grain aphid, *Sitobion avenae*, is one such insect that experiences extensive pre-freeze mortality. This led Knight (1987) to the conclusion that supercooling points can be ecologically misleading because, in nature, *S. avenae* would die before freezing occurred. Similar pre-freeze mortality was evident in the peach-potato aphid *Myzus persicae* (Bale *et al*., 1988). The cause of such pre-freeze mortality is unknown, although possible reasons

include protein denaturation, membrane disruption, alterations to lipid fluidity or the decoupling of metabolic processes (Ring, 1980; Knight *et al.*, 1986). The classification of insects as freeze intolerant was thus considered inappropriate due to failure of such insects to survive to temperatures as low as the supercooling point, leading to the production of a new classification scheme by Bale (1996).

The new classification scheme has retained the categories of freeze tolerance and freeze avoidance, although the criterion for freeze avoidance has been modified. Modification of the scheme has enabled incorporation of the knowledge that the effects of low temperature, i.e. chilling, and freezing are not synonymous, with death occurring as a consequence of short or prolonged exposure to low temperatures in the absence of freezing in many insects (Bale, 1991). Freeze tolerant insects are the most cold hardy species and withstand extracellular freezing initiated by INAs and include the larvae of the hoverfly *Syrphus ribesii*, which experiences 70% survival at -35°C (Hart & Bale, 1997, 1998). Under this new classification, insects are classified as freeze avoiding if low levels of mortality occur in the absence of freezing. An example of a freeze avoiding insect is the moth *Epirrita autumnata*, the eggs of which have a mean supercooling point of -35.5°C and do not die until freezing occurs (Virtanen *et al.*, 1998). For such freeze avoiding species, SCPs provide a reliable indicator of cold hardiness.

In addition to freeze tolerant and freeze avoiding, three new categories have been proposed: chill tolerant, chill susceptible and opportunistic survival. Chill tolerant insects are those that possess relatively low sub-zero supercooling points, around -20 to -30°C, although experience some mortality at temperatures above the supercooling point, for example, the mite *Alaskozetes antarticus* and the beech weevil *Rhynchaenus fagi*. Overwintering adults of *R. fagi* possess the ability to supercool to low sub-zero temperatures, with a reported mean SCP of -25°C in mid-winter (Bale, 1991). However, 74% of the overwintering population were killed following 50 days at -15°C; a temperature 10°C above the mean SCP for the species (Bale, 1991). The SCP therefore becomes increasingly unreliable as an indicator of cold hardiness. Chill susceptible insects, including many aphid species such as *M. persicae* and *S. avenae*, also have the ability to supercool to low sub-zero temperatures, although experience relatively high levels of mortality at temperatures well above the supercooling

point in very brief exposures of a few minutes or hours. For chill susceptible insects, mortality is unrelated to their SCP and as a consequence SCPs do not provide reliable indicators regarding the cold tolerance of such species. The final category, opportunistic survival, includes all insects unable to survive below the threshold temperature required for normal metabolic activity, such as the housefly *Musca domestica*. In a study population of *M. domestica* pupae, 90% were killed within 4 days when held at 0° (Coulson & Bale, 1990). Such species actively seek out sheltered overwintering sites to avoid unfavourable conditions. Once again, the cold tolerance of the species is unrelated to their SCP.

More recently, Sinclair (1999) has suggested that the category of freeze tolerance, as with freeze intolerance, forms a separate continuum and can be subdivided according to insect SCPs and lower lethal temperatures (LLT). The resultant subcategories include partially freeze tolerant insects which can survive a small amount of body water freezing but will die when their body temperature reaches equilibrium with the environment, moderately freeze tolerant, whereby the insects die less than 10°C below their SCP, strongly freeze tolerant, whereby insects have LLTs 20°C or more below their SCP and finally, freeze tolerant insects that possess very low SCPs and can survive below these temperatures (Sinclair, 1999).

1.1.2 Survival mechanisms at low temperatures

Mechanisms to survive at unfavourable low temperatures can include both chemical (briefly mentioned previously) and behavioural responses. These mechanisms act either to increase the cold hardiness of the individual or to prevent exposure to the unfavourable conditions.

1.1.2.1 Chemical mechanisms

Chemical mechanisms act to increase the cold hardiness of the insect and include the use of INAs, polyols and sugars, and AFPs. INAs are proteins produced in the autumn by freeze tolerant insects in preparation for winter and were first described in the beetle *Eleodes blanchardi* by Zachariassen and Hammel (1976) and later purified in the hornet *Vespula*

*maculat*a (Duman & Patterson, 1978). Containing a high proportion of hydrophilic animo acids, mainly glutamate and glutamine, INAs are believed to function by their ability to hydrogen bond with water molecules, thus creating organised embryo ice crystals (Duman, 2001). INAs act to initiate protective ice formation in extracellular areas at high sub-zero temperatures. In doing so, water is drawn out of cells via an osmotic gradient, concentrating cell contents and thus preventing potentially damaging intracellular freezing (Figure 2). In contrast to freeze tolerant insects, insects classified as freeze avoiding expel or mask INAs in autumn as opposed to producing them (Zachariassen, 1985).
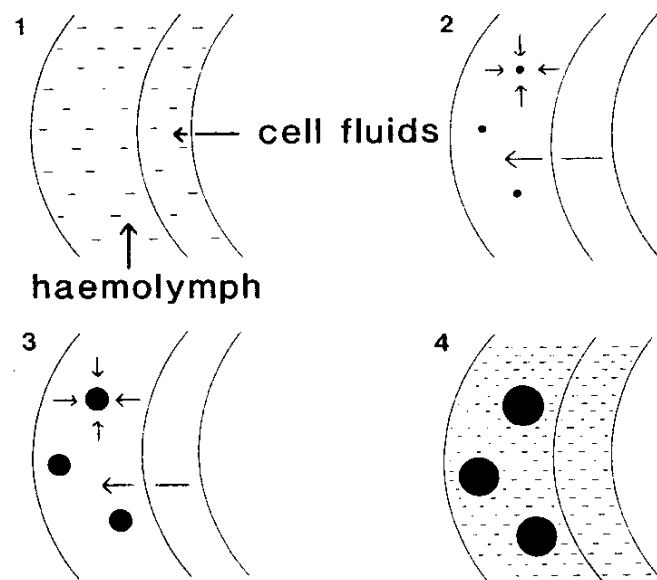
Figure 2. The activity of ice nucleating agents allowing for protective extracellular ice formation at sub-zero temperatures (Bale 1996). 1. Haemolymph and cell fluids are in osmotic equilibrium. 2. INAs initiate freezing in the haemolymph at sub-zero temperatures. 3. Growth in extracellular ice results in water being drawn from the cell into the haemolymph via an osmotic gradient. 4. Protective extracellular freezing within the haemolymph. Cellular fluids become concentrated preventing dangerous intracellular freezing and thus protecting the cell.

Polyols and sugars are found in both freeze tolerant and freeze avoiding species, with concentrations typically increasing in autumn months. Examples of polyols and sugars include glycerol, sorbitol, threitol, erythritol, fructose, sucrose and trehalose (Bale, 2002), although glycerol is reported to be the most common and effective polyol due to its low molecular weight, allowing movement readily through cell membranes, its high solubility and low toxicity (Salt, 1957). In freeze tolerant insects the polyols act to protect partially frozen tissue and in freeze avoiding insects have an antifreeze function (Duman, 1982). Tissue protection in freeze tolerant species is brought about by the polyols preventing the potential causes of freeze damage, for example, mechanical damage, electrolyte imbalance and recrystallization (Bale, 2002). This is achieved via increasing the binding capacity of water, which in turn decreases the rate of ice formation, stabilizing protein structure, buffering electrolytes and decreasing transmembrane water flux (Baust, 1982; Bale, 2002). Glycerol further acts as a solvent, keeping potentially harmful salts in solution as they become more concentrated during ice formation (Miller & Smith, 1975).

Also found in both freeze tolerant and freeze avoiding insects are AFPs which lower the freezing point of fluids in relation to the melting point, creating a thermal hysteresis (Zachariassen & Husby, 1982). AFPs are adsorbed onto the surface of embryonic ice crystals, depressing the nucleation temperatures and thus preventing increased ice crystal growth (Duman & DeVries, 1972). Bale (2002) suggests that the AFPs offer protection to freeze tolerant insects in autumn months prior to INA production.

The process of vitrification, rather than freezing, can further aid insect survival at sub zero temperatures (Duman *et al*., 1991) and provides an alternative strategy for managing internal water at sub zero temperatures (Hawes & Bale, 2007). Vitrification is the process by which body water is supercooled and forms a glass-like, amorphous structure without the occurrence of crystallization. In doing do, the haemolymph is prevented from becoming too concentrated and thus protects against enzyme denaturation and maintains cell hydration. Vitrification has been reported in larvae of the gall fly *Eurosta solidaginis* (Wasylyk *et al*., 1988) and the Alaskan beetle *Cucujus clavipes* (Bennett *et al*., 2005).

The restructuring of cell membranes aids insect cold acclimation and assists in the protection of membranes from perturbation. In ectotherms, cold acclimation commonly acts to increase membrane fluidity via modification of membrane lipids by increasing the proportion of unsaturated fatty acids (Los & Murata, 2004). This is achieved by increasing the proportion of *cis* unsaturated and long chain fatty acids and increasing the phosphatidylethanolamine content relative to phosphatidylcholine (Los & Murata, 2004; Lee *et al*., 2006). Changes to the levels of unsaturated fatty acids maintain cell membranes in a liquid phase, preventing transition to a gel phase, which is considered a major cause of cold injury in insects in the absence of freezing (Drobnis *et al*., 1993; Clark & Worland, 2008). Other reported changes to membrane structure include increased proportions of glycophosphoethanolamines in relation to glycerophosphocholines, decreased proportions of plasmalogens in relation to diacyl-glycerophospholipids, and changes to the cholesterol content, all believed to function in the protection of the cell membrane (Hazel, 1989; 1995).

Additionally, insects can enter diapause, a period of suspended growth and development, to endure unfavourable conditions. Facultative and obligatory diapause exists with facultative diapause being induced by changes in the environment and obligatory diapause being genetically programmed, occurring at specific times of the year, independent of environmental conditions (Bale & Hayward, 2010). In temperate and Polar Regions, day length provides the main cue for facultative diapause, with short day lengths providing a reliable cue for the onset of winter and is termed photoperiodism (Denlinger, 2002; Bale & Hayward, 2010). Declining temperatures, in combination with day length cues, also contribute to inducing diapause, although temperature alone does not induce diapause (Bale & Hayward, 2010).

Diapause enables growth and development to be coordinated with favourable environmental conditions and, in many insects, acts to increase cold tolerance during these unfavourable periods (Denlinger, 2002). Increased cold tolerance is attributed to the synthesis of cryoprotectants such as trehalose, galactose and glucose (Khani *et al*., 2007; Zeng *et al*., 2008), upregulation of heat proteins (Denlinger, 2002; Rinehart *et al*., 2000; 2007) and changes to the lipid cell membrane (Michaud & Denlinger, 2006; Tomčala *et al*., 2006). The larvae of the codling moth *Cydia pomonella*, for example, experience a threefold increase in

trehalose when in full diapause, acting to increase supercooling capacity, survival at low temperature and chilling tolerance (Khani *et al*., 2007a). Additionally, changes to the lipid cell membrane occur, with diapausing larvae of the moth having increased proportions of unsaturated fatty acids, acting to increase membrane fluidity at low temperatures (Khani *et al*., 2007b).

The chemical mechanisms discussed above are seasonal adaptations allowing survival through predictable cold spells of winter months. In addition to seasonal adaptations, short term protection is possible in many insects via rapid cold hardening (RCH). RCH was first described in the flesh fly *Sarcophaga crassipalpis* by Lee *et al*. (1987) who defined the process as a rapid protective mechanism against cold injury in response to changing environmental temperatures on an hourly to daily basis (Lee *et al*., 1987). RCH has since been reported in many insect species including the fruit fly *Drosophila melanogaster* (Czajka & Lee, 1990), the housefly *M*. *domestica* (Coulson & Bale, 1991), the monarch butterfly *Danaus plexippus* (Larsen & Lee, 1994), western flower thrips *Frankliniella occidentalis* (McDonald *et al*., 1997), the migratory locust *Locusta migratoria* (Wang & Kang, 2003), the grain aphid *S*. *avenae* (Powell & Bale, 2004) and a Karoo beetle *Afrinus* sp (Sinclair & Chown, 2006), the majority of which are chill susceptible (Wang *et al*., 2003). RCH is further reported outside of the Class Insecta, although rather limited to the Arthropoda, in the Class Arachnida, for example, in the mites *Euseius finlandicus* (Broufas & Koveos, 2001), *Alaskozetes antarcticus* and *Halozetes belgicae* (Worland & Convey, 2001).

It is suggested that RCH allows chill susceptible insects to survive unpredictable and unfavourable changes in temperature and is especially important when seasonal adaptation is incomplete during spring and autumn months (Coulson & Bale, 1990). Such short term adaptation is considered to be of great importance to aphid species with short generation times (Coulson & Bale, 1990) where not all aphids experience winter conditions and enables a rapid response to sudden changes in temperature (Powell & Bale, 2008). Proposed mechanisms involved in RCH include changes to the composition of phospholipid bilayers, increases in haemolymph osmolalities and the production of polyols (Wang *et al*., 2003).

1.1.2.2 Behavioural mechanisms

Behavioural responses can act to increase the cold hardiness of the insect or to prevent exposure to unfavourable conditions and include the expulsion of gut contents and migration. The cessation of feeding and expulsion of gut contents increases cold hardiness via removal of potential ice nucleators in freeze intolerant species. Insects that adopt this method include most of those that rely on supercooling to aid winter survival such as the beech weevil *R. fagi* (Bale, 1980). In *R. fagi*, the cessation of feeding lowers the supercooling point by approximately 3°C (Bale, 1980). However, a previous study on the grain aphid, *S. avenae*, has revealed that starvation of the insect did not act to lower the supercooling point (Knight, 1987). Aphids feed on phloem sap which is known to be deficient in nucleators (Sømme & Zachariassen, 1981) and high in cryoprotectant carbohydrates (Danks, 1978). The composition of phloem sap thus enables aphids to continue feeding throughout unfavourable low temperatures without compromising the supercooling ability of the individual.

The selection of overwintering sites increases survival by preventing exposure to unfavourable conditions. Migration in search of overwintering sites can occur over varying scales, with the monarch butterfly *D. plexippus* displaying extensive annual migrations over thousands of kilometres from breeding sites in North America to overwintering sites in Mexico (Larsen & Lee, 1994). Conversely, migrations over very small scales are also evident with insects often migrating down into the soil or up host plants to higher air temperatures to avoid ground frosts. *M. persicae* is one such insect that migrates up plants, although this migration also has nutritional advantages as leaves lower down the plant senesce (Harrington & Taylor, 1990). However, it should be noted that aphids such as *M. persicae* may also engage in large scale migrations, although, over which, they have very little control and are commonly transported large distances on low level jet streams (Berry & Taylor, 1968; Zhu *et al*., 2006), giving them the name 'aerial plankton' (Drake & Farrow, 1989).

1.2 Insect survival strategies at high temperatures

The survival of insects at high temperatures has received comparatively less research interest than at low temperatures. As a consequence, the available literature and knowledge base is somewhat poor, although research interest on the subject matter is increasing with the focus being primarily on drosophilid species.

Following exposure to a heat stress, death can occur as a result of protein denaturation, affecting enzyme and substrate binding, and alterations to the fluidity of phospholipid bilayers (Dahlgaard *et al*., 1998; Neven, 2000). In addition, structural damage to DNA, RNA and carbohydrates may arise and critical pH and ion concentrations can become distorted (Neven, 2000).

Heat shock proteins, synthesized following exposure to a heat stress, play an important role in survival at unfavourably high temperatures. In addition to extreme high temperatures, heat shock proteins are induced by a variety of stresses which include low temperature extremes, depletion of cellular energy, and extreme concentrations of ions, osmolytes, gases and toxic substances (Feder & Hofmann, 1999). It should be noted that not all heat shock proteins are induced by stress (Feder & Hofmann, 1999).

Of the heat shock proteins known, proteins belonging to the Hsp70 family are best characterized. Hsp70 proteins are believed to act as molecular chaperones, aiding in the transportation of denatured or non-functional proteins to lysosomes for degradation, to prevent aggregations of denatured proteins by binding with hydrophobic domains (Feder & Hoffmann, 1999; Bahrndorff *et al*., 2009b) or to assist the re-folding of proteins on removal of the heat stress (Sørensen *et al*., 2003; see Neven 2000 for review). In the flesh fly *S. crassipalpis* exposure to a heat stress led to increased thermal tolerance at a previously lethal exposure regime. The newly acquired heat tolerance was found to decline with time, although persisted long after the heat shock proteins had degraded, suggesting that additional mechanisms could be involved in the acquisition of heat tolerance (Yocum & Delinger, 1992).

A trade off exists between the benefits conferred by the upregulation of Hsps enabling survival at unfavourably high temperatures and the associated costs. These costs include

detrimental affects on rates of growth, development and reproduction, and provide an explanation as to why cells remove Hsp70 in the absence of stress (Feder *et al*., 1992; Sørensen *et al*., 2003). Such costs associated with the stress response are suggested to be a consequence of normal cell functions shutting down during the response, toxicity of Hsps70 in high concentrations, or due to requiring large amounts of energy (Feder & Hoffmann, 1999).

1.3 Indices of insect thermal tolerance

A number of indices are commonly adopted in the laboratory to measure insect thermal tolerance. Such indices enable comparisons between species or populations of the same species and allow the effects of acclimation and experimental treatments on thermal tolerance to be determined. The focal indices discussed below include the supercooling point, lethal temperatures and times, critical temperatures and the discriminating temperature which is used in rapid cold hardening studies.

1.3.1 Supercooling point

The supercooling point or crystallization temperature is the lowest temperature before spontaneous freezing of body water occurs. It is determined in the laboratory by cooling the insect at a constant rate until freezing occurs. With careful monitoring of the insect's body temperature, the supercooling point can be readily identified as the exotherm caused by the latent heat of crystallization. Determination of insect supercooling points has previously been used in the classification of insect cold hardiness into the categories of freeze tolerance and freeze avoiding depending on whether the insect can survive following exposure at the supercooling point.

1.3.2 Lethal temperatures and times

The lethal temperature (LT) is the temperature at which a specific percentage of an insect sample population is killed. The LT_{50}, for example, is the temperature at which an experimental population experiences 50% mortality. Insects are cooled or heated at a set rate to a predetermined temperature, held at the temperature for a desired length of time, and then returned to the start temperature, usually at the same rate. Insect survival is assessed following a recovery period. The LT_{50} is estimated by exposing samples to a number of decreasing or increasing temperatures depending on whether cold or heat tolerance is being assessed. From the acquired data set, a graph of insect survival against exposure temperature can be produced which follows a typically sigmoidal dosage-mortality curve. Probit analysis is commonly used to linearise the sigmoidal curve and enables an accurate estimate of the LT_{50} value. Following a similar principle, lethal times are determined by exposing an insect sample to a pre-determined stressful temperature (e.g. 0°C, -5°C, -10°C) for varying lengths of times before re-warming. Insect survival is then assessed and Probit analysis performed.

1.3.3 Critical temperatures

The ability to move at increasingly higher or lower temperatures also provides an indication of the thermal tolerance of an insect. Critical temperatures are estimated by cooling or heating a sample population at a set rate and recording the temperature at which movement of an individual insect ceases or coma is induced, depending on the critical temperature being studied. The temperature at which insects lose the ability to walk is known as the CT_{min} or CT_{max}, referring to the movement threshold at low and high temperature respectively. Additional cooling or heating will eventually result in the insect entering a state of coma, referred to respectively as the chill or heat coma, and is defined as the last twitch of an appendage. In the literature critical temperatures can also refer to the temperature at which an individual insect can no longer right itself after being artificially knocked over. Due to the numerous characterizations of critical temperature values, it is vital that, when using such indices, the adopted definition and experimental procedure be stated clearly to avoid ambiguity. The measurement of critical temperatures perhaps provides more ecologically

relevant information on thermal tolerance since survival is of little importance if the individual is unable to move to find food, a mate or escape predation.

1.3.4. Discriminating temperatures and rapid cold hardening

When assessing an insect's ability to rapidly cold harden, the discriminating temperature is an essential measurement that enables the RCH response to be identified and quantified. The discriminating temperature is the temperature which results in 80-90% mortality in a population sample when transferred directly from the rearing temperature to a particular sub-zero temperature. The discriminating temperature is therefore a reference point to determine changes in mortality following treatment to induce RCH.

1.4 Aphids and the study species *Myzus persicae*

1.4.1 Aphid lifecycles

The majority of aphid species so far studied are considered chill susceptible species due to experiencing high mortality at temperatures above the supercooling point. Aphids can be classified as monoecious or heteroecious, depending on the occurrence of host-alternation during the lifecycle. Monoecious species, such as *S. avenae*, remain on the same herbaceous host species throughout the year, although this life strategy is only possible if the herbaceous host is present throughout the year. Heteroecious species, such as *M. persicae*, alternate between a summer herbaceous host and a winter woody host plant. Aphids that possess the host-alternating lifecycle have a potential nutritional advantage over species with a monoecious lifecycle, although they are required to return to the primary woody host to lay eggs (Shaposhnikov, 1987). Conversely, it could be argued that the monoecious lifecycle is

advantageous because the mortality associated with a migrating stage is avoided. It should, however, be noted that some species of monoecious aphids such as *S. avenae* do migrate.

Many aphid species, although not all, can overwinter as one of two lifecycle types that differ in cold tolerance. The first overwintering strategy used by most aphid species is as a holocyclic (sexual) egg, produced by the mating of an ovipara and a male. The second strategy involves overwintering as an anholocyclic or asexual aphid which arises due to a stable genetic mutation affecting the photoperiodic switch leading to a failure to produce sexual forms in autumn (Moran, 1992; Dixon, 1998). The anholocyclic aphids do not seek out hibernation sites to overwinter, but instead remain active on the underside of leaves as they do for the remainder of the year (Harrington & Taylor, 1990). The holocyclic egg is more cold tolerant than the overwintering anholocyclic aphid (Strathdee *et al.*, 1995), with aphid eggs supercooling to sub-zero temperatures as low as -35°C (Hutchinson & Bale, 1994). Overwintering as an egg therefore increases the chance of surviving through the winter under unfavourable conditions. Although less cold hardy, the anholocyclic aphid can continue to feed and reproduce throughout the winter, albeit at a lower rate than in summer. The two lifecycles represent a trade off between increased probability of winter survival (the holocyclic lifecycle) and increased population size through continuous reproduction and thus greater spring dispersal (the anholocyclic lifecycle).

The proportion of holocyclic and anholocyclic clones in a population is governed by the severity of winter conditions. Consequently, locations experiencing milder winters would be predicted to have increased proportions of anholocyclic clones compared to locations experiencing severe winters (Broadbent & Heathcote, 1955). At more northerly latitudes where low temperature extremes would be experienced more frequently, the proportion of holocyclic clones is proposed to increase (Walters & Dewar, 1986). Understanding the complex interactions between winter severity and overwintering strategy can assist in the prediction of spring population abundance and aid the production of reliable forecasting systems for aphid outbreaks (Walters & Dewar, 1986; Harrington *et al.*, 1990; Cocu *et al.*, 2005).

1.4.2 The study species *Myzus persicae*

*M*yzus *persicae* (Sulzer) (Hemiptera: Aphididae) is a heteroecious aphid host alternating between the primary peach *Prunus persica* host in winter and various herbaceous hosts, belonging to approximately 40 different families, which include brassicas, potatoes and sugar beet, in summer (Blackman & Eastop, 2000). Due to the primary peach host originating from Asia, it is believed that *M. persicae* also originates from the region (Blackman & Eastop, 2000). The aphid can overwinter as both holocyclic eggs and anholocyclic aphids (Figure 3).
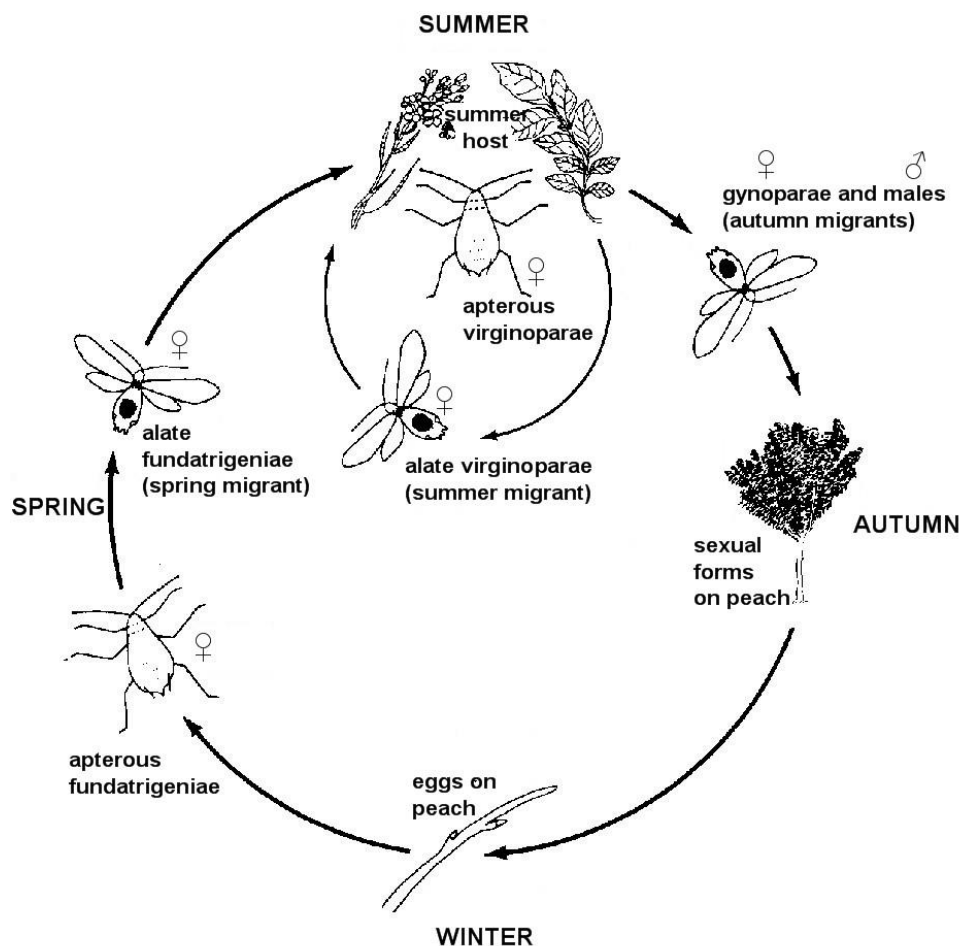

Figure 3. The lifecycle of the peach-potato aphid *Myzus persicae*.

In contrast to the majority of insect species, functional roles, such as sexual reproduction, migration etc., are not allocated to specific stages in the lifecycle such as in Lepidoptera where dispersal is largely restricted to the adult life stage. Due to the short generation times, an aphid, for example in autumn, will not survive through to the following spring and as a consequence specific roles are instead partitioned between generations. Throughout the year a sequence of morphs are produced. Males and gynoparae migrate in winter to the primary peach host. Once on the winter host, the gynoparae produce oviparae via parthenogenesis. The oviparae are sexual, egg laying female forms which mate with the males to produce overwintering eggs. When conditions become favourable in spring, the eggs hatch into apterous (wingless) fundatrices. The fundatrices reproduce parthenogenically for several generations throughout spring, producing apterous fundatrigeniae. Eventually, alate (winged) fundatrigeniae are produced which migrate to the summer, herbaceous host. Throughout the summer, aphids reproduce via parthenogenesis, producing both alate and apterous forms, with the apterous virginoparae producing both males and gynoparae in the autumn in response to decreasing photoperiod in preparation for migration to the winter host. A separate annual cycle co-occurs in which apterous virginoparae fail to produce sexual forms and continue reproducing parthenogenically throughout the year, overwintering as anholocyclic individuals with intermittent alate generations. In some aphid species, including *M. persicae*, a third and fourth lifecycle exists known as the androcyclic and intermediate lifecycle. With the androcyclic lifecycle, anholocyclic clones produce males in addition to virginoparae. Males are also produced in addition to virginoparae in the intermediate lifecycle, although alates have the ability to produce both apterous offspring and sexual female morphs under winter conditions; this is an intermediate of the anholocyclic lifecycle in which only apterous offspring are produced and the holocyclic lifecycle in which sexual female morphs are produced. The third and fourth lifecycle types will not be discussed hereafter.

Some aphid species, including *M. persicae*, are distributed widely across the globe. In Europe, *M. persicae* can be found from northern Scandinavia to southern Spain. With knowledge of the lifecycle and physiology of *M. persicae*, predictions can be made on interpopulational variation along the latitudinal cline. For example, regional adaptations in thermal tolerance and changes to the ratio of anholocyclic to holocyclic clones could be expected. Such knowledge would further allow the level of gene flow between populations to be deduced. Significant variation in thermal tolerance and lifecycle predominance between populations would suggest a lack of gene flow, and would provide the basis for future speciation.

1.5 Geographic variation in thermal tolerance

Geographic variation in thermal tolerance has recently been the focus of research in a number of species from the Phylum Arthropoda, including drosophilid species (Davidson, 1990; Goto & Kimura, 1998; Gibert & Huey, 2001; Gibert *et al*., 2001ab; Bubliy *et al*., 2002; Ayrinhac *et al*., 2004), the common woodlouse *Porcellio laevis* (Castañeda *et al*., 2004, 2005), the pea leafminer *Liriomyza huidobrensis* (Chen & Kang, 2004), the springtail *Orchesella cincta* (Bahrndorff *et al*., 2006, 2009a) and European diving beetles (Calosi *et al*., 2010).

Studies concerning variation in the thermal tolerance of drosophilid species have primarily focused on the differences in lower thermal limits between tropical and temperate populations, both within and between species. In *D. melanogaster*, temperate populations were more resistant to cold and desiccation stress than tropical populations (Davidson, 1990). Similarly, in a comparison of 26 temperate drosophilid species and 48 tropical species, temperate species displayed shorter recovery times following a cold induced coma (Gibert *et al*., 2001b). A limited number of comparative studies have been conducted comparing populations along latitudinal and altitudinal gradients. In *Drosophila subobscura*, the temperature inducing chill coma declined with increasing latitude, indicating that populations from high latitudes were increasingly cold tolerant (Gibert & Huey, 2001). In *D. melanogaster*, both mortality and recovery time following a cold shock decreased at temperate latitudes and mortality decreased and knockdown time increased following a heat

shock at tropical latitudes (Hoffmann *et al*., 2002; Ayrinhac *et al*., 2004). Also within the Order Diptera, cold tolerance of the pea leafminer *L. huidobrensis*, this time measured by lower lethal temperature, was again found to increase with latitude (Chen & Kang, 2004).

Literature regarding geographical variation in thermal tolerance for species outside the Order Diptera is further limited, although research is less biased towards lower thermal limits. Castañeda *et al*. (2004) studied the crustacean *P. laevis* along a 10° latitudinal gradient through Chile. *P. laevis* from low latitudes were found to display lower cold tolerance and higher optimum temperatures and were consequently more adapted towards high temperatures. *P. laevis* from high latitudes had greater cold tolerance and lower optimum temperatures. Likewise, recovery time from chill coma revealed an inverse relationship with latitude (Castañeda *et al*. 2005), suggesting that populations along a latitudinal gradient display local adaptations in thermal physiology.

More recently, Bahrndorff *et al*. (2006, 2009a) studied populations of the springtail *O. cincta* collected along a 2000 km latitudinal gradient throughout Europe from Denmark to Southern Italy. The studies revealed that individuals from high latitudes exhibited the greatest cold shock resistance, but lowest resistance to heat shock. The reverse was true of *O. cincta* from low latitudes, once again suggesting adaptation to local climate. Similar relationships are, in addition to along latitudinal gradients, also evident in altitudinal gradients. Cold tolerance increased with increasing altitude, as indicated by declining values of CT_{min}, in species of scarab dung beetle along an altitudinal gradient in South Africa; dung beetles from high altitude populations could remain active to lower temperatures and avoid entering a state of cold induced torpor for longer (Gaston & Chown, 1999). CT_{max} was revealed to vary less with altitude than CT_{min}, resulting in increased thermal tolerance at high altitude (Gaston & Chown, 1999).

In the majority of insect species studied, upper thermal limits show much less variation with regard to genetic variation and phenotypic plasticity than lower lethal limits, indicating that, in insects, thermal limits are 'decoupled' (Addo-Bediako *et al*., 2000). Put simply, the relationship between upper and lower thermal limits appears not to be a fixed one, with one limit not necessarily moving in accordance with the other. Given that upper lethal limits are

generally less variable than lower lethal limits, insects experiencing lower temperatures (i.e. at high latitude or altitude) are expected to display greater differentials between upper and lower limits due to lower lethal limits being lowered to a greater extent in relation to the upper lethal limit. This decoupling of thermal limits has been attributed to the increased climatic variability associated with increasing latitude, resulting in a need for greater thermal tolerance (Addo-Bediako *et al*., 2000).

1.6 Applications of insect thermal biology

The limited ability of insects to maintain body temperature above or below the environmental temperature results in insects being greatly influenced by the environment (Walther *et al*., 2002). An understanding of insect thermal biology can thus provide valuable information for predictive models for the forecasting of pest outbreaks, the establishment of invasive species, shifts in range patterns, and the potential implications of global climate change.

Over the past century the Earth's climate has warmed by approximately 0.6°C (Easterling *et al*., 2000; Walther *et al*., 2002). However, this trend in rising temperature has not been a steady process and since the mid 1970s parts of the world have experienced a rapid increase to a rate of warming of 0.2°C per decade (Karl *et al*., 2000). Using mid range climate warming scenarios, it is predicted that 15-37% of species and taxa could become extinct by 2050 (Thomas *et al*., 2004). In addition to warming, climate change can encompass changes to climatic variability, atmospheric composition, land cover and land use and can occur over varying temporal and spatial scales (Scherm *et al*., 2000). The impacts of global change are likely to have profound effects on distribution patterns and the pest and invasive status of insect species. It is therefore understandable that knowledge of the variability in insect thermal tolerance and acclimation ability would provide valuable information for such areas.

1.6.1 Forecasting pest outbreaks

The development of a forecasting system for predicting pest outbreaks is of particular importance for aphid species. Aphids feed on the phloem sap of host plants using modified mouthparts known as stylets which penetrate the plant phloem, allowing passive feeding on phloem sap due to the positive pressure within the phloem. By feeding on host plants, aphids can result in the stunting of plant growth, the lowering of crop yields and aid the transmission of viral diseases (Block *et al.*, 1992).

Aphids are vectors in the transmission of approximately 275 plant virus species from 19 genera, equating to more than 50% of all insect transmitted plant viruses, many of which are commercially important (Nault, 1997). The short lifecycles of aphids, in addition to high rates of population increase and dispersal, contribute to the success of aphids as one of the main groups of plant disease vectors (Fereres & Moreno, 2009). Aphid-transmitted viruses can be classified as either stylet-borne or circulative. Stylet-borne viruses, commonly referred to as non-persistent viruses, are short-lived within the aphid, with the vector often remaining infectious for less than 24 hours and include sugarcane mosaic virus and maize dwarf mosaic strain (Slykhuis, 1976). Circulative viruses, referred to as persistent viruses, are those which are ingested into the aphid gut and pass to the saliva via the haemolymph, allowing infection of the plant during subsequent aphid feeding. Once infectious, an aphid can remain so for a long time and such viruses are retained through moulting to later instars and adults. Circulative viruses include barley yellow dwarf virus, wheat yellow dwarf virus, maize leaf fleck virus and beet mild yellowing virus (Russell, 1962; Slykhuis, 1976).

Perhaps the most common and widespread of crop viral diseases is barley yellow dwarf (BYD), caused by barley yellow dwarf viruses (BYDV) belonging to the Luteoviridae family (Edwards, 2001) and transmitted by over 20 species of aphid (Irwin, 1990). Symptoms of infected plants include yellowing of the leaves and stunted growth. In addition to yield loss, BYDV can further be economically damaging through reduction in crop quality. Since the 1970s the disease has become more widespread throughout Britain as a consequence of increased abundance of a major vector, *S. avenae*. However, although widespread, large

scale yield losses in Britain are infrequent (Knight *et al*., 1996). For the subject species *M. persicae*, it is the transmission of the potato viruses that are the most damaging. The potato leaf roll virus (PLRV), also belonging to the Luteoviridae family, is a persistent, circulative virus and has contributed to declining potato productivity over the last century (Radcliffe & Ragsdale, 2002; Chatzivassiliou *et al*., 2008). Perhaps more damaging is the potato virus Y (PVY) belonging to the Potyviridae family. This non-persistent, stylet-borne virus is transmitted by mainly winged aphids (Saucke & Döring, 2004) and it is reported to cause yield losses of up to 80% (Takacs, 2000).

In addition to reducing crop yields and transmitting viral diseases, many pest insects cause extensive damage via defoliation resulting in a direct loss of biomass, but further indirect loss by leaving the host plant susceptible to fungal infection. The majority of insect defoliators belong to the Order Lepidoptera, with examples including the Autumnal moth *E. autumnata*, a major defoliator of mountain birch in Scandinavia (Virtanen *et al*., 1998) and the gypsy moth *Lymantria dispar*, the main defoliator of broadleaved trees in Eastern North America (Kegg, 1971; Kenis *et al*., 2009). Other examples of major insect defoliators outside the Lepidoptera include the sawflies *Neodiprion sertifer* (Eklundh *et al*., 2009), *Perga affinis* (Jordan *et al*., 2002), *Diprion pini* and *Pristiphora abietina* (Dajoz, 2000) to name a few, gall midges of the family Cecidomyiddae (Dajoz, 2000) and a variety of weevils, leaf beetles, and chafers of the Coleoptera (Dajoz, 2000).

The study species *M. persicae* gains its pest status due to the efficacy with which it spreads viral diseases in crop plants. It alone is a vector in the transmission of approximately 100 plant diseases and is consequently a pest of major economic importance (Kennedy *et al*., 1962). The development of effective forecasting systems would decrease the need for prophylactic insecticide spraying, which can result in the death of non-target organisms, contribute to the pollution of freshwater and can lead to increased insecticide resistance amongst target organisms (Block *et al*., 1992).

The lifecycle type prevalent in an aphid population will further have implications for the severity of potential pest outbreaks. As previously discussed, the overwintering anholocyclic clones of pest aphids such as *M. persicae* and *S. avenae* are less cold hardy than

overwintering holocyclic eggs (Strathdee *et al*., 1995) and consequently, their proportions are dependent upon winter (Walters & Dewar, 1986). On arrival of spring conditions, anholocyclic clones respond more quickly to higher temperatures, resulting in increased numbers, an earlier production of the migratory alates and consequent earlier spring migration to the summer host. For *M. persicae*, almost 80% of the variation in the timing of first capture in suction traps, a sampling method by which air is continually sampled at specific heights above ground, can be explained by mean winter temperatures (Harrington *et al*., 1990). Holocyclic clones, originating from the overwintering egg, require several generations before production of migratory alates can commence. In contrast, production of migratory alates from anholocyclic clones can begin as soon as conditions become favourable. A trade-off therefore exists between the two clonal types regarding winter survival and spring migration.

During mild winters, greater survival of anholocyclic clones leads to earlier migration of potentially infectious aphids to the summer host (Figure 4). Transmission of viruses, for example BYDV, is most likely to occur via the progeny of anholocyclic clones as opposed to aphids originating from virus-free holocyclic eggs. BYDV causes the greatest damage when infection of the barley plant occurs at a young age due to mature plants being less susceptible to viral infections (McKirdy and Jones, 1996; McKirdy *et al*., 2002). This is particularly problematic when milder winters lead to a greater prevalence of anholocyclic aphids which are, not only more prone to carrying the virus, but also more likely to infect the younger barley plants through earlier spring migrations. Likewise, large numbers of winged aphids are required to successfully colonise sugar beet and as a consequence, mild winter conditions will have implications for *M. persicae* populations and the spread of beet yellow viruses (BYV) (Williams *et al*, 2000).
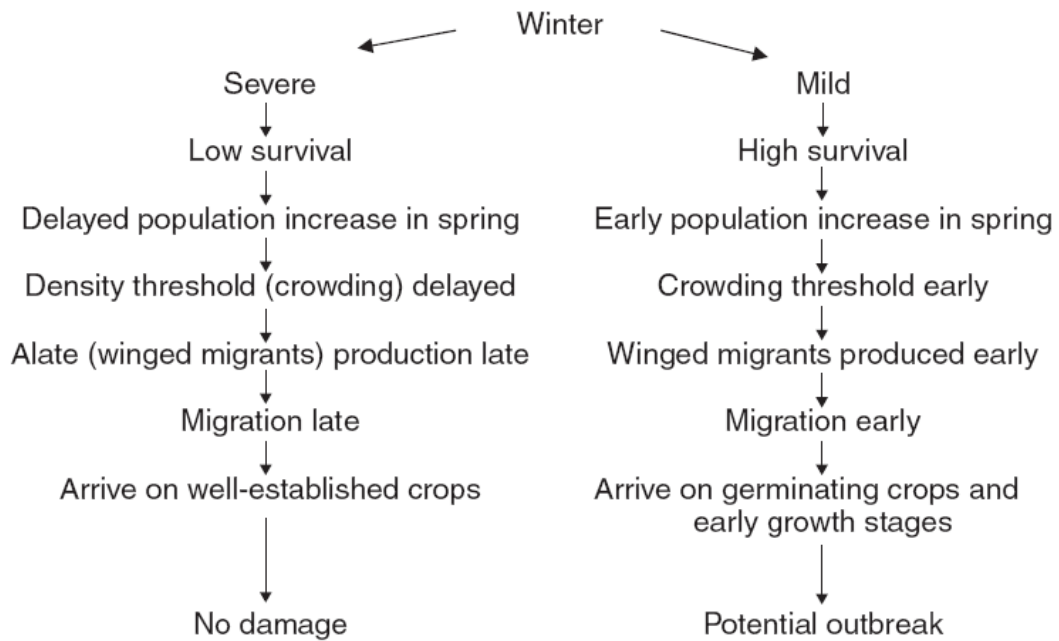
```
                              Winter
                 ←                        →
           Severe                              Mild
              ↓                                 ↓
         Low survival                      High survival
              ↓                                 ↓
  Delayed population increase in spring   Early population increase in spring
              ↓                                 ↓
  Density threshold (crowding) delayed    Crowding threshold early
              ↓                                 ↓
  Alate (winged migrants) production late Winged migrants produced early
              ↓                                 ↓
       Migration late                      Migration early
              ↓                                 ↓
  Arrive on well-established crops    Arrive on germinating crops and
                                           early growth stages
              ↓                                 ↓
         No damage                       Potential outbreak
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Figure 4. The effects of mild and severe winters on spring and summer populations and related damage potential of aphids overwintering as anholocyclic clones (Bale & Hayward, 2010).

Changes in temperature will further impact the number of generations per year, known as voltinism, with voltinism being positively correlated with temperature (Jonsson *et al*., 2009; Sand & Brittain, 2009). Additional generations would lead to greater population growth and accelerate potential pest outbreaks (Altermatt, 2009). It is therefore understandable that knowledge of aphid thermal biology is vital to the understanding of how aphids may react to potential climate change with implications for the prediction of pest outbreaks.

1.6.2 Species distribution

In the face of global change, species are expected to track changing climate, either through adapting to new conditions or by shifting distribution (Walther *et al*., 2002), since failure to do so could result in population extinction. Bio-indicator species, such as Lepidoptera, provide information on alterations to species distribution. In a study of 35 non-migratory butterfly species, 63% were found to have shifted their range northwards by between 35-240km over the previous century, which is considered a direct impact of regional warming (Parmesan *et al*., 1999). Additionally, the southern green stinkbug *Nezara viridula* has extended its range 70km further north in Japan since the 1960s, displacing the closely related *Nezara antennata* (Musolin, 2007). Similar range shifts have been reported for bird species, for example, with the northern margins of British breeding birds having extended northwards by an average of 18.9km over a 20 year period (Thomas & Lennon, 1999).

Climate tracking is not always achievable and species re-distribution can be prevented when geographical formations, habitat loss and fragmentation impede movement or when movement is limited by additional factors, for example, light limitation preventing the re-distribution of reef building corals (Hoegh-Guldberg, 1999). Failure to reach climatically favourable habitats is apparent in the forests of Monteverde, Costa Rica, where 20 of 50 species of toads and frogs have become locally extinct and one species, the golden toad *Bufo periglenes*, has become globally extinct, as a consequence of cloud bank migration to higher altitudes (Pounds *et al*., 1999). In addition to species distribution, climate change is expected to impact the phenology and physiology of organisms, community interactions, and ecosystem dynamics (Walther *et al*., 2002). Information on variation in thermal tolerance of *M. persicae* along a latitudinal gradient and the potential differences in acclimation ability will provide an indication of how the distribution of the species could change in a changing global climate, with implications for pest management.

1.6.3 Alien species

Species are moved beyond their natural ranges, both deliberately and accidentally, by human interference and this too is true of aphids. For example, the spotted alfalfa aphid *Therioaphis trifolii* was introduced into the USA in the 1950s, the Russian wheat aphid *Diuraphis noxia*

into South Africa in the 1970s (Blackman & Eastop, 2000), and the rose-grain aphid *Metopolophium dirhodum* into New Zealand as recently as the 1980s (Nicol *et al*., 1997). Fortunately, most invasions fail with successful establishment of alien species being prevented when new habitats are not climatically matched to the native range. The 'tens rule' provides a general rule of thumb to the success of invasions, with invasions progressing through a series of stages: import to the country, escape into the wild, establishment of a population and finally development into a pest species. The rule theorizes that only 10% of invasive species successfully progress to the next stage (Williamson & Brown, 1986; Williamson, 1996, 2006). Nonetheless, it is estimated that naturalized alien species constitute approximately 2-33% of the flora of continental areas and 80% of islands (Vitousek *et al*., 1997).

Alien species can have detrimental ecological impacts on native species via direct predation, competition, spreading diseases, hybridization and altering the habitat. Economic impacts can further be imposed. Introduction of the zebra mussel *Dreissena polymorpha* into North America resulted in costs of up to $2 billion annually for the removal of the species from fouled water pipes and inlets (Lodge *et al*., 1993; MacIsaac, 1996). Insects, in particular, can cause great economic losses due to many species being pests of important agricultural crops. The introduction of the Mexican rice borer *Eoreuma loftini* into Louisiana, Southern America, is predicted to cost the rice industry $45 million in lost revenue once all of Louisiana is infested (Reay-Jones *et al*., 2008). Global change and shifting temperatures could alter current patterns of climatic matching, potentially facilitating the successful establishment of alien species. This highlights once again the importance of understanding the thermal biology of such a prolific and major agricultural pest as *M. persicae*, should global climate change result in expansion outside of its current range.

1.7 Study hypotheses and experimental considerations

Previous research on aphid thermal biology has focused on the variation of cold hardiness between different life stages and the effects of rapid cold hardening. To date, no study has

focused on variation in thermal biology within a single aphid species along a latitudinal gradient. Such knowledge is important in the forecasting of how the distribution of pest species such as *M. persicae* will be impacted by global climate change and will further provide information regarding the level of gene flow between populations and the potential for future speciation.

It can be hypothesised that populations of *M. persicae* will display regional adaptations. At the northern end of the range, it would be predicted that populations would have greater cold tolerance (indicated by lower lethal and coma temperatures) and lower developmental thresholds. At high latitude, populations would further be expected to display a greater ability to acclimate at lower temperatures, although a lesser ability to acclimate to higher temperatures, than populations at the range centre and southerly range margin. The reverse would be predicted for populations from lower latitudes. It would therefore be expected that a gradient of increasing cold tolerance but decreasing heat tolerance would become apparent as *M. persicae* populations progress from Mediterranean, to temperate and then sub-polar climates. It could also be predicted that a shift from anholocycly to holocycly would occur from southerly latitudes to more northerly latitudes due to extreme low temperatures selecting against the anholocyclic lifecycle in sub-polar regions and favourable temperatures preventing natural climatic control of anholocyclic clones in the Mediterranean.

On careful consideration of the predictions stated above, the reality is perhaps less straightforward. Mediterranean countries, such as Spain, would be expected to have an abundance of anholocyclic clones due to a lack of winter stress. However, Spain also has a plentiful supply of the primary host, the peach tree, with Spain being the fourth largest producer of peaches in the world after China, Italy and the USA (Llacer *et al.,* 2009). Availability of the primary host will favour existence of the holocyclic lifecycle, and thus, anholocyclic and holocyclic lifecycles could both be common in Spain. As a result, extensive migrations are not required and regionally adapted populations displaying greater heat tolerance could be expected. At the northerly range margin of *M. persicae* in Scandinavia, holocycly would be favoured due to extreme winter temperatures which would select against the anholocyclic lifecycle. However, peach trees are required to support the holocyclic lifecycle, and, these are uncommon in Scandinavia. If holocyclic aphids cannot persist due to

a lack of the primary host and likewise anholocyclic aphids due to extreme sub-zero winter temperatures, it is likely that *M. persicae* populations cannot reside all year round in Scandinavia, unless in protected areas such as glasshouses, and an annual influx of aphids must therefore occur in order to build up the populations that are found outdoors in summer. If annual immigrations into Scandinavia occur from other parts of Europe, aphids collected in Scandinavia would not be expected to display greater levels of cold tolerance or reduced heat tolerance.

The current study is designed to determine inter-clonal variation in the thermal biology of *M. persicae* in relation to geographical variation. However, aphid clones display variation in biological characteristics that, although are not the target of the investigation, have the potential to impact on thermal tolerance. As a consequence, aphid characteristics including lifecycle type, clonal type, insecticide resistance status, colour morphology and the presence of symbionts, need to be considered prior to the investigation to ensure that results observed are a consequence of geographical variation associated with the sites of collection.

1.7.1 Aphid lifecycle

M. persicae exists as two distinct lifecycle types, anholocycly and holocycly, with a further two intermediate lifecycle types, androcycly and intermediate. It is well recognized that anholocyclic clones are less cold tolerant than overwintering holocyclic eggs, at least in their respective overwintering stages (Hutchinson & Bale, 1994). The lifecycle of all clones used in the current study must be determined because failure to determine the lifecycle of study clones will prevent any interpopulational variation in temperature tolerance being attributed to geographical distribution, since disparity in lifecycle type could also account, at least in part, for observed differences.

1.7.2 Aphid clonal type

The ability of *M. persicae* to reproduce clonally, both within the anholocyclic and holocyclic lifecycles, results in the production of genetically distinct aphid clones within a population (Fenton *et al*., 1998; Fenton *et al*., 2005; Kasprowicz *et al*., 2008). All aphid clones collected for study will be subjected to microsatellite analysis to determine variation at the chosen loci and to assign a 'type'. Aphids with identical microsatellite patterns at the loci examined are classified as the same type and, although this does not indicate that the clones are genetically identical, it does determine that clones originated from the same stem mother through asexual propagation (Kasprowicz *et al*., 2008). Such information will further allow investigation into whether aphids of the same type are more alike in relation to thermal tolerance than clones of different types.

1.7.3 Insecticide resistance

Aphids are a major pest worldwide owing to the ease in which they transmit plant viral diseases and due to resultant structural damage caused to the plant when feeding. The control of aphid pests using insecticides has led to an increase in insecticide resistant aphid clones.

Recent studies on aphid insecticide resistance suggest a trade off between resistance and thermal tolerance. In *M. persicae*, winter survival is inversely related to insecticide resistance, leading to the conclusion that selection for insecticide resistance is counteracted by the selection pressures of British winters (Foster *et al*., 1996). In addition, the mobility of *M. persicae* at low temperatures is inversely related to levels of insecticide resistance (Foster *et al*., 1997). All study clones will therefore be resistance typed prior to use in experiments.

1.7.4 Colour morphology

Many aphid species exist in distinct and stable colour morphs. Examples include the pea aphid *Acyrthosiphon pisum* which has green and red forms (Dixon, 1998), the walnut aphid *Chromaphis juglandicola* with yellow and white forms (Hougardy & Mills, 2008) and the

study species *M. persicae* which also has green and red forms (Blackman, 1987). The colour of the study clones will be noted in case colour morphology influences thermal tolerance.

1.7.5 Bacterial symbionts

All phloem feeding Homoptera possess symbiotic microorganisms (Douglas, 1998). In aphids, the primary symbiont is the bacterium *Buchnera aphidicola*. Transmitted maternally via a process known as transovarial transmission, the bacteria are housed in specialised cells known as bacteriocytes or mycetocytes and provide the aphids with nutrients and essential amino acids lacking from the phloem sap diet (Douglas, 1998). This symbiotic relationship is essential to aphid development, without which growth and reproductive rates are low (Moran & Dunbar, 2006). *Buchnera* are sensitive to high temperatures, with relatively short exposures of a few hours at high temperatures (37°C) being sufficient enough to kill *Buchnera* and have detrimental affects on the aphids (Ohtaka & Ishikawa, 1991).

In addition to the *Buchnera* primary symbiont, aphids are host to a variety of secondary symbionts. Such secondary symbionts have been found to confer resistance against parasitic wasps (Oliver *et al*., 2005) and fungal infections (Scarborough *et al*., 2005), expand the number of host plants available for utilization (Tsuchida *et al*., 2004), and even impact life history traits such as dispersal and mating habits which would reduce gene flow and ultimately aid speciation (Leonardo & Mondor, 2006). Additionally, specific symbionts have been demonstrated to increase heat tolerance (Chen *et al*., 2000; Montllor *et al*., 2002; Russell & Moran, 2006).

1.8 Aims, objectives and hypotheses tested

The main aim of the study is to investigate aspects of the thermal biology of the aphid *Myzus persicae* to ascertain how tolerance to extreme temperatures varies between clones collected from different locations along the aphid's latitudinal distribution in Europe.

Specific objectives include:

- To establish in culture nine clones of *M. persicae*, three collected from each of three regions of the aphid's European distribution: Southern Spain, Britain and Scandinavia (Sweden).
- To estimate the lower and upper lethal temperatures (LT_{50} min and LT_{50} max) of these clones.
- To determine the movement thresholds and chill and heat coma temperature of the clones.
- To determine the mobility of the clones at varying temperatures (0°C to 25°C).
- To study the effects of acclimation at 10°C and 25°C for one and three generations on the above thresholds of all clones.

In meeting these objectives, four main hypotheses will be tested:

- Clones collected from Sweden will be more cold tolerant than those collected from Britain or Spain.
- Clones collected from Southern Spain will be more heat tolerant than clones collected from Britain or Sweden.
- No latitudinal trend in thermal tolerance will be evident. Severe winter temperatures and a lack of the primary host plant in Scandinavia will prevent permanent *M. persicae* populations existing and therefore populations will rely on annual migrations from other parts of Europe to persist. Extensive annual seasonal

migrations results in an annual redistribution of aphid clones that will override any regional adaptation.

- Aphid clones of the same type, as determined by microsatellite analysis, will be more similar with respect to thermal tolerance than clones of differing types.