

Insect overwintering in a changing climate

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Summary

Insects are highly successful animals inhabiting marine, freshwater and terrestrial habitats from the equator to the poles. As a group, insects have limited ability to regulate their body temperature and have thus required a range of strategies to support life in thermally stressful environments, including behavioural avoidance through migration and seasonal changes in cold tolerance. With respect to overwintering strategies, insects have traditionally been divided into two main groups: freeze tolerant and freeze avoiding, although this simple classification is underpinned by a complex of interacting processes, i.e. synthesis of ice nucleating agents, cryoprotectants, antifreeze proteins and changes in membrane lipid composition. Also, in temperate and colder climates, the overwintering ability of many species is closely linked to the diapause state, which often increases cold tolerance ahead of temperature-induced seasonal acclimatisation. Importantly, even though most species can invoke one or both of these responses, the majority of insects die from the effects of cold rather than freezing. Most studies on the effects of a changing climate on insects have focused on processes that occur predominantly in summer (development, reproduction) and on changes in distributions rather than winter survival *per se*. For species that routinely experience cold stress, a general hypothesis would be that predicted temperature increases of 1°C to 5°C over the next 50–100 years would increase winter survival in some climatic zones. However, this is unlikely to be a universal effect. Negative impacts may occur if climate warming leads to a reduction or loss of winter snow cover in polar and sub-polar areas, resulting in exposure to more severe air temperatures, increasing frequency of freeze–thaw cycles and risks of ice encasement. Likewise, whilst the dominant diapause-inducing cue (photoperiod) will be unaffected by global climate change, higher temperatures may modify normal rates of development, leading to a decoupling of synchrony between diapause-sensitive life-cycle stages and critical photoperiods for diapause induction. In terms of climate warming and potential heat stress, the most recent predictions of summer temperatures in Europe of 40°C or higher in 50–75 years, are close to the current upper lethal limit of some insects. Long-term data sets on insect distributions and the timing of annual migrations provide strong evidence for ‘positive’ responses to higher winter temperatures over timescales of the past 20–50 years in North America, Europe and Asia.

Key words: insect, cold hardiness, diapause, climate change, distributions, invasive species.

Introduction

As poikilothermic animals, insects have limited ability to regulate their body temperature and whilst this lack of internal thermal homeostasis might be viewed as disadvantageous, Meglitsch nevertheless described insects as among the most successful of animals (Meglitsch, 1972). A review of the likely impacts of a changing climate on the low temperature biology of insects is clearly a complex subject but there are three factors that are particularly important to consider: the cold tolerance of species under prevailing climatic conditions, the occurrence of diapause, and the direct and indirect effects of higher temperatures on one or both of these overwintering processes.

There has been longstanding interest in the mechanisms by which insects are able to survive in the most severe of winter climates, in which the main principles were established by the pioneering studies of Salt (Salt, 1961). What has sometimes been overlooked is firstly, that whilst polar regions may appear inhospitable to life on earth, permanent winter snow cover usually provides a buffered and thermally stable microclimate for overwintering invertebrates, and secondly, the great majority of studies on insect cold hardiness have focused on ‘cold hardy insects’, i.e. those that regularly experience some level of winter cold stress. The first part of this review will therefore provide a summary of the main features of the freeze-tolerant and freeze-avoiding strategies, consider the range of thermal

tolerances exhibited by species inhabiting different climatic zones, and then highlight some of the possible direct and indirect effects of higher temperatures on insect survival in winter.

Diapause is an essential requirement for the overwintering success of many temperate and colder climate species, and the diapause state is known to confer increased cold hardiness in the absence of low temperature acclimatisation (this usually follows naturally in the transition from summer through to autumn and to winter) (Pullin and Bale, 1989); and yet the research disciplines of ‘insect cold hardiness’ and ‘diapause’ have often appeared ‘separate’ (Denlinger, 1991; Pullin, 1996). In reviewing the literature on how a changing climate might affect insect overwintering and diapause, there are few studies that have addressed this question directly by experimentation. Against this background, the second part of this review will place the diapause response in the context of insect overwintering, explain the role of ‘sensitive’ and ‘diapausing’ life-cycle stages within the framework of diapause induction, maintenance and termination, and then use this knowledge to discuss how higher temperatures may disrupt successful progression through the sequential phases of the diapause syndrome.

Whilst experiments targeted at elucidating the effects of higher temperatures on both insect overwintering and diapause are limited, long-term data collected originally for other purposes provide some of the most compelling evidence for the ability of insects to exploit

the more favourable conditions experienced during this era of climate warming. For this reason, the third part of the review will describe a series of investigations on insect distributions, pest outbreaks and invasive species to exemplify how information gained from physiological studies is not only important in explaining patterns and changes observed under recent and current climatic conditions but also provides a basis for predicting future responses and, particularly, the species and regions of the world where the effects of climate change will range from beneficial or tolerable to stressful or life-threatening.

Global climate change – the thermal context

From the detailed review of global climate change by MacDonald, there are three facets that are highly relevant to our understanding of the likely impacts of higher temperatures on the physiology and ecology of terrestrial invertebrates, particularly insects and mites: (1) since around 1860 Earth has been undergoing a period of climate warming, which is predicted to continue throughout this century. (2) This warming is not consistent across the globe – increases in temperature are likely to be greatest at higher latitudes, such as the high Arctic. (3) Whilst current temperatures, and those predicted to occur over the next 50–100 years are within the range that organisms have experienced in previous climate cycles, both the rate of temperature change and the predicted future levels of CO₂ are unprecedented (MacDonald, 2010).

Strategies of insect cold hardiness

By way of a summary, it is often stated that there are two main strategies of insect cold hardiness – freeze tolerance and freeze avoidance by supercooling. The key difference between these strategies is the synthesis of ice nucleating agents (proteins) in freeze-tolerant species and the removal of all potential nucleators, such as gut content, from freeze-avoiding species. Ice nucleating agents are usually synthesised in autumn and initiate freezing at temperatures in the region of -4°C to -10°C , in so-called ‘safe extracellular areas’ such as the haemocoel; as a result of ice freezing out of solution, water then moves progressively from cells to these areas to re-establish the osmotic equilibrium (Fig. 1). The net effect is that when the insect experiences the lowest temperatures of mid-winter, the cell fluids have become sufficiently ‘cryo-concentrated’ to avoid intracellular freezing, which is regarded as deleterious or lethal in most insects (see also Lee et al., 1993). Both freeze-tolerant and freeze-avoiding species may also contain polyols and sugars (e.g. glycerol and trehalose, respectively) and antifreeze (thermal hysteresis) proteins, although the function of these agents differs between the two strategies (Fig. 2). As the number of studies on freeze-tolerant species has increased, the temperatures at which the nucleators initiate freezing at the ‘supercooling point’ (SCP) have been found to be generally consistent between species (mean usually above -10°C). However, the lethal temperature has been shown to be much more variable, ranging from -10°C in the sub-Antarctic beetle *Hydromedion sparsutum* (Müller) (Bale et al., 2000) to -25°C in the temperate hoverfly *Syrphus ribesii* L. (Hart and Bale, 1997) and -40°C or lower in the North American gallfly *Eurosta solidaginis* Fitch (Bale et al., 1989a; Bale et al., 1989b). The survival of species such as *H. sparsutum* with a ‘high’ lethal temperature in a sub-Antarctic climate would seem to depend on the thermal protection provided by the decaying leaves of its host plant *Parodiochloa flabellate* (Lam.) and the cover of winter snow. As well-studied species, *E. solidaginis* and the autumnal moth *Epirrita autumnata* Bkh. exemplify the features of the freeze-tolerant and freeze-avoiding strategies – in the latter case, the overwintering eggs

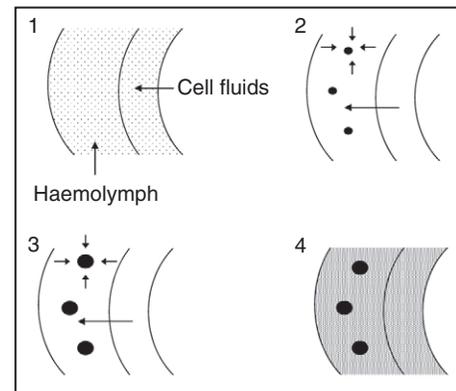


Fig. 1. Schematic representation of the function of extracellular ice nucleating agents in a freeze-tolerant insect [● indicate sites of nucleation in extracellular areas and increasing ice masses at progressively lower sub-zero temperatures from summer (1) to mid-winter (4)] (see Bale, 2002).

of *E. autumnata* survive through the harsh Scandinavian winters above snow level as long as the air temperature does not fall below the SCP of -36°C (Tenow and Nilssen, 1990).

One of the problems of trying to place the cold hardiness of insects in general within the alternative strategies of freeze tolerance and avoidance is that these strategies were derived from studies on cold-hardy insects living in cold and very cold climates, whereas the majority of species live in much less severe climates, and biodiversity is greatest in the tropics. A range of studies have shown that whilst most insects have considerable potential to ‘supercool’ (in the absence of gut content), a low SCP is not necessarily indicative of cold hardiness. For example, there are many species with winter SCPs of -25°C but when held at higher sub-zero temperatures, mortality increases after exposures of 1–3 months [e.g. the beech weevil *Rhynchaenus fagi* L. (Bale, 1991)] or only a few minutes [e.g. the aphid *Myzus persicae* (Sulz.) (Bale et al., 1988; Clough et al., 1990)]. With species of tropical and Mediterranean origin, high mortality can occur whenever the insects experience temperatures below the developmental threshold, i.e. between 10°C and 0°C (Hatherly et al., 2005); hence, overwintering of such species in cool temperate climates is dependent on a high level of thermal protection. With respect to the effects of cold exposure, Bale described species such as *R. fagi*, *M. persicae* and tropical insects as ‘chill tolerant’, ‘chill susceptible’ and ‘opportunistic survivors’, respectively. In reality, the terminology is not important – the key point is that most insects are neither freeze tolerant nor freeze avoiding (because they are killed by the effects of cold, not freezing), and this diversity of thermal tolerance needs to be recognised in any generalised consideration of the impacts of climate warming on insect winter survival (Bale, 1993; Bale, 1996).

Impacts of climate warming on insect overwintering

There have been few studies that have investigated the direct effects of higher winter temperatures on insects, although there have been many experiments on the effects of higher summer temperatures on rates of development and reproduction, which have used various climate manipulation systems such as cloches (Coulson et al., 1993; Strathdee and Bale, 1993), solardomes (Brooks and Whittaker, 2001), free air carbon enrichment (FACE) (Hillstrom and Lindroth, 2008) and controlled environments, e.g. the ecotron (Lawton et al., 1993) (for a review, see Bale et al., 2002). Predictions of the likely effects of climate warming on overwintering insects have therefore

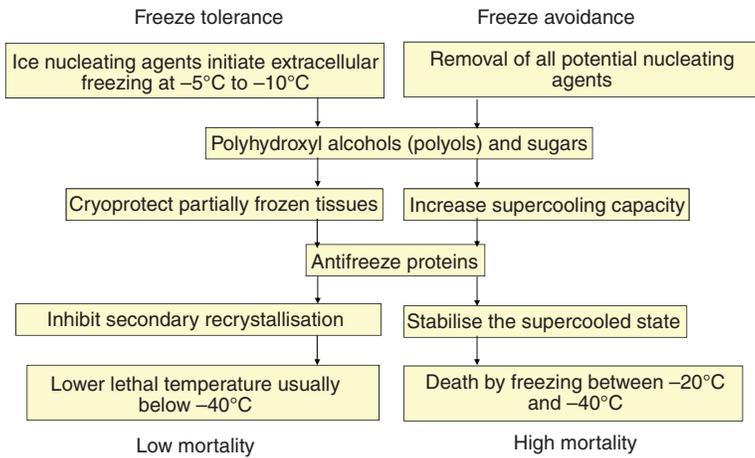


Fig. 2. Schematic representation of the main biochemical components involved in the freeze-tolerance and freeze-avoidance strategies of an overwintering insect (see Bale, 2002).

been based largely on knowledge of thermal tolerance under laboratory and natural exposures and in a limited number of manipulation experiments (Coulson et al., 2000; Konestabo et al., 2007). Under experimental conditions, insect survival usually declines with decreasing temperature or with increasing period of exposure. As a general hypothesis it would therefore be expected that for insects that currently experience winter cryo-stress, increases in temperatures in the range of 1°C to 5°C would increase survival. However, a number of situations can be envisaged in which higher winter temperatures may lead directly or indirectly to higher mortality in winter, such as the reduction of winter snow cover and the resultant exposure of organisms to the more severe air temperatures or to a greater frequency of freeze-thaw cycles. By contrast, for those species with very cold-hardy overwintering stages, reduced winter snow cover or early snow clearance will have the advantage of creating a longer ‘summer’ season. The following section describes a range of species that might become ‘winners’ and ‘losers’ in a scenario of climate warming in polar areas.

The Arctic and Antarctic may appear inhospitable for life on Earth, especially for organisms such as insects that have limited ability to regulate their body temperature, but some species such as the Antarctic collembolan *Cryptopygus antarcticus* Willem and the mite *Alaskozetes antarcticus* (Michael) can be found in very high numbers. For such soil- and moss-inhabiting species, winter snow cover provides an effective thermal buffer against very low air temperatures. As exemplified in Fig. 3 (M. R. Worland, personal

communication), at the beginning of the Antarctic winter (March–April), soil surface and 5 cm depth temperatures track air temperature but later in the winter after some snowfall, as the air temperature drops to -20°C to -25°C, soil surface temperature also declines, but only to around -10°C, and also without the sharp fluctuations in temperature occurring above the snow.

It seems that the designation of a species as freeze tolerant has usually been based on a single laboratory freeze-thaw event, and under certain circumstances such as freezing under thermally buffering snow cover, it is the case that an individual may freeze and remain in the frozen state for much of the winter. However, when snow cover does not occur at all in winter, or arrives ‘late’ and is lost ‘early’, freeze-tolerant insects may undergo a number of ‘internal’ freeze-thaw cycles, and simulations of such events have produced some interesting, and perhaps, unexpected results. In a study on the freeze-tolerant larvae of *H. sparsutum*, when individuals were frozen and thawed at daily intervals for 10 days, all larvae froze at a consistent temperature (from -2°C to -4°C) during the first freezing event. Thereafter, the majority of larvae showed a decrease in their SCP in the second or later freezing event, and all of these larvae died during the consecutive freezing exposures. A small number of larvae froze at the same temperature on every occasion, and these were the only individuals alive after the 10 freeze-thaw events (Bale et al., 2001). In a similar study with the freeze-tolerant larvae of *S. ribesii* frozen on five occasions at daily or weekly intervals, the effect of ‘re-freezing’ was to both

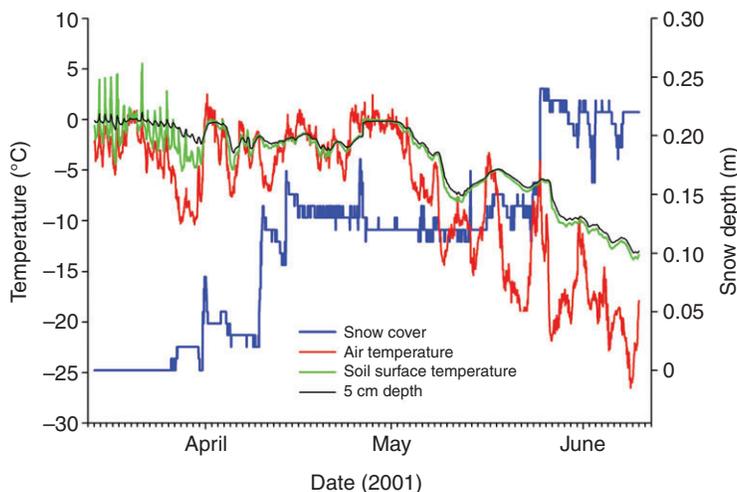


Fig. 3. Effect of snow cover on soil surface and 5 cm deep temperatures in relation to air temperature on Anchorage Island, Rothera (Antarctic) (data from British Antarctic Survey).

lower and increase the range of SCPs, with some larvae freezing as low as -28°C (Brown et al., 2004). Freezing, thawing and re-freezing of some freeze-tolerant insects seems to decrease the efficiency of the nucleator such that a proportion of the population lose their freeze-tolerant status and then rely on supercooling for survival – which will depend on their SCP relative to the minimum temperature experienced. Here, it is also important to note that in the absence of snow cover, the mid-winter SCPs of ‘freeze-avoiding’ species such as *C. antarcticus* and *A. antarcticus* overlap Antarctic winter air temperatures (Worland and Convey, 2001), which further illustrates the crucial importance of winter snow in protecting terrestrial invertebrates (Fig. 3).

An associated feature of snow loss and intermittent freeze–thaw cycles will be an increased availability of ‘temporary’ water, increased ice cover on the soil surface and penetration to deeper levels. A range of factors are at play during freeze–thaw cycles, which will affect the outcome in terms of life or death, e.g. whether the organism is in contact with water that then freezes to ice, becomes encased in ice or is in an ‘icy’ microhabitat but not in direct contact with the ice – and all of these might be affected by the cuticular properties of the organism. In a manipulation of the winter ice surface layer in a High Arctic soil community, Coulson et al. simulated changes in ice thickness under predicted climate change to assess if this might represent an environmental catastrophe (Coulson et al., 1993). An ice layer of 25 cm reduced the total number of soil microarthropods by 50%; however, mortality was greatest in Collembola, with mites being relatively unaffected. In fact, even thin, naturally occurring ice layers significantly reduced Collembola abundance, suggesting a possible shift in soil arthropod community structure under climate change. Differential susceptibility among microarthropod taxa could be the result of multiple factors, including ‘ice encasement’ or to the effects of ‘external water’ freezing on the cuticle of the organism. As a further interesting observation, when the Arctic collembolan *Megaphorura arctica* (Tullberg) was held over ice for 16 days with a gradual decrease in temperature from 5°C to -5°C , the body water content declined from 2.43 g g^{-1} dry mass to 0.68 g g^{-1} dry mass and over the same time period the SCP decreased from -7°C to -17°C (Worland et al., 1998); this ‘cryoprotective dehydration’ thus increases cold hardiness, illustrating that even within a single taxa, the difference between ‘water’ and ‘ice’ and the proximity of the organism to the substrate can result in a lethal or beneficial outcome. There is also a major difference in the effects of the freezing of cuticular water on survival between the two main overwintering strategies; if water freezes on the cuticle and penetrates through to internal tissues and fluids, all insects other than freeze-tolerant species would be killed by the freezing (Duman, 2001). By contrast, external nucleation seems beneficial to some freeze-tolerant insects, as it induces internal freezing at a higher temperature than would occur under normal ice nucleating agent activity (Lee et al., 1996).

The end of winter ice and snow thaw creates fast-flowing meltwater streams that are an important dispersal mechanism for Collembola, and would allow an annual redistribution and recolonisation process where populations had been locally depleted by events linked to the loss of snow cover and freeze–thaw cycles. Over a two-day period, 1000 *C. antarcticus* were caught in a net placed in a meltwater stream at Rothera in the Antarctic (S.A.L.H., M. R. Worland, P. Convey and J.S.B., unpublished), and Hawes et al. found that 50% of a sample of this collembolan could survive without food for around eight months on the surface of freshwater and for two months on seawater (Hawes et al., 2008).

It is probably an understatement to say that the interactions between snow, water, ice and the survival of polar invertebrates are complicated but it is certainly the case, provided the organism can survive through winter, an increase in the length of the summer ‘favourable’ season is likely to be beneficial, and some species have life cycles that enable them to exploit such changing conditions. The Arctic aphid *Acyrtosiphon svalbardicum* Heikinheimo overwinters as eggs on its host plant *Dryas octopetala* L.; the eggs of *A. svalbardicum* have a mean SCP of $-36.2\pm 0.5^{\circ}\text{C}$, which was lowered to $-38.0\pm 0.6^{\circ}\text{C}$ after a month of acclimation at -10°C , with some eggs freezing as low as -44°C . These low SCP values were linked to long-term cold hardiness in which 80% of eggs survived for a month at -30°C (Strathdee et al., 1995). The life cycle of *A. svalbardicum* is genetically programmed (unlike temperate aphids) with a minimum of two generations (fundatrix and the sexual morphs, ovipara and male) and a maximum of three generations, in which the fundatrix produces some viviparae (in addition to the males and oviparae) and these give rise to a third generation consisting exclusively of the two sexual morphs (Strathdee et al., 1993b). Developmental analyses found that 470 day-degrees above a threshold of 0°C were required to complete the two generation egg → fundatrix → sexual morphs → egg life cycle whereas 710 day-degrees were required for the egg → fundatrix → vivipara → sexual morphs → egg route (Strathdee et al., 1993a). When natural populations of *A. svalbardicum* eggs were enclosed within cloches that raised the daily temperature by $1\text{--}2^{\circ}\text{C}$ over the entire summer season at Ny Alesund (78.9°N) on Svalbard, there was a 10-fold increase in the end of summer egg density, from around 500 eggs m^{-2} in areas without any warming to over 5700 eggs m^{-2} inside the cloches (Strathdee et al., 1993a). Retrospective climatic analyses for the Ny Alesund area indicated that prior to 1990, only once in every 25 years would there be a summer ‘thermal budget’ of >710 day-degrees that would allow completion of the three generations and the 10-fold ‘bonus’ of ‘extra’ eggs. However, the combination of a cold-hardy overwintering egg and a ‘risk-free’ summer life cycle that includes some flexibility suggests that *A. svalbardicum* will be one of the ‘winners’ in a scenario of continued climate warming.

There are two further important observations to make in concluding this section on the impacts of climate warming on terrestrial arthropods with different overwintering strategies, different levels of cold hardiness and living in different climatic zones. Firstly, the great majority of studies are carried out on a ‘species’ but there are now several examples of species with sub-species or other forms of sub-specific variation that are genetically distinct and differ in their thermal tolerance (Hoffmann et al., 2005; Hoffmann and Weeks, 2007). For example, in a comparison of the cold hardiness of four clones of the aphid *Myzus persicae* (distinguished by microsatellite analysis), the lower lethal temperature (LLT_{50}) differed by 1.2°C (from -12.7°C to -13.9°C) but the upper lethal temperature (ULT_{50}) differed by only 0.2°C (from 41.7°C to 41.9°C) (Hazell et al., 2010a; Hazell et al., 2010b). Care is required in attributing thermal tolerance properties to species that have wide latitudinal distributions and genetically distinct ‘strains’. Secondly, there is a growing body of evidence to suggest that ‘cold region’ and temperate species have sufficient phenotypic plasticity to survive and succeed in a warmer climate but species currently enduring heat stress have little ‘room for manoeuvre’; whilst the LLT can be depressed, the ULT is already ‘close to the ceiling’ and in the absence of a genetic response, even modest warming in tropical areas will impose a severe physiological stress.

Rapid responses to changing temperatures

Whilst the main focus of this review with regard to a 'changing world' concerns gradual increases in temperature through global warming and its seasonal impact on overwintering, many insects experience changes in temperature on timescales of a few hours, and are able to invoke physiological responses to aid survival or maintain other activities, such as movement or flight. The phenomenon of rapid cold hardening (RCH) was first described by Lee et al. with the flesh fly *Sarcophaga crassipalpis* Macquart and has since been described in a wide range of species (Lee et al., 1987a; Overgaard et al., 2007). RCH was initially regarded as physiologically interesting but ecologically irrelevant because in natural environments insects do not experience the sudden changes in temperature that cause 'cold shock' mortality, e.g. a rapid transfer from 20°C to -8°C, nor a period at 0°C (1–3 h) that is commonly used in laboratory experiments to induce the RCH response. However, rapid cold hardening is now recognised as a common occurrence, manifest across a range of responses, and of undoubted ecological importance. For example, a series of studies have shown that in many species, an acclimatory period at any temperature between 10°C and 0°C can produce a 'rapid' increase in survival in a subsequent sub-zero exposure but, more importantly, cooling at physiological rates experienced in nature (e.g. from 20°C to 0°C at 0.1°C min⁻¹) also increases survival at sub-zero temperatures (Kelty and Lee, 1999; Powell and Bale, 2004); and 'seasonally-acclimated' insects maintain the ability to rapidly cold harden as demonstrated by the aphid *Sitobion avenae* (Fab.) (Powell and Bale, 2005). Two other observations exemplify the scope of rapid cold hardening. Firstly, Worland and Convey observed a diurnal change in the SCP of the Antarctic collembolan *C. antarcticus* with the majority of the population freezing around -10°C in the daytime and at -25°C in the night; these are the mean freezing temperatures that usually characterise the summer-feeding and winter-starved states of *C. antarcticus* but, remarkably, the organisms could achieve this major change in their SCP profile in a matter of hours (Worland and Convey, 2001). Secondly, when samples of *Drosophila melanogaster* Meigen were collected in Ohio (OH, USA) during a natural thermoperiod with a daytime high of 23°C and a night time low of 9°C and then exposed to -7°C for 2 h, survival increased from 5% in the daytime population to 63% in flies collected at night; also, the cold torpor temperature decreased from 7.9°C to 6.0°C between day and night (Kelty and Lee, 2001). It is clear that *D. melanogaster* would not experience a temperature of -7°C in northern USA in summertime but, collectively, the studies on *S. avenae*, *C. antarcticus* and *D. melanogaster*, although carried out on different species inhabiting different climatic zones and with changes in different thermal thresholds (lethal and non-lethal), are all indicative of an important and common ability, i.e. insects are able to sense and track patterns of changing environmental temperature, which in the case of a decreasing profile, is not initially harmful or restrictive (in terms of flight or walking ability) but rather, acts as a 'token stimulus' – an indicator that much lower temperatures may follow that would prevent movement, cause cry-injuries or may be lethal. As a result, across different species, insects can lower their cold torpor (chill coma) temperature to maintain movement, decrease their non-freezing lethal temperature or rapidly modify their SCP to prevent freezing – all within minutes or a few hours. Also, in a study with *S. avenae*, it has been shown that RCH is achieved without any cost in terms of rate of development or fecundity (Powell and Bale, 2004; Powell and Bale, 2005).

It seems unlikely that higher temperatures in the region of 1°C to 5°C would have a negative impact on these various rapid

responses, although for cold climate and temperate species, they may be induced less often.

Diapause and insect cold hardiness

What is diapause?

Diapause is a genetically programmed pre-emptive developmental response to changing seasons and environmental conditions. It is an environmentally adaptive dormancy that can occur during any stage of development (egg, larva, pupa or adult) but the diapausing stage is consistent and specific within a species. Diapause is distinct from quiescence, which can occur at any stage in the life cycle and represents an immediate response to stress. Most commonly, insects have a facultative diapause, in which there is a developmental stage that is responsive to specific environmental 'cues' (the sensitive stage) that programme the diapause. Thus, the sensitive stage makes the 'physiological decision' to either diapause or delay entry into diapause for another generation (Tauber et al., 1986; Denlinger, 2002). Species having an obligate diapause arrest development at the same developmental stage every generation regardless of prevailing environmental conditions. However, the exact seasonal timing of diapause initiation can vary from year to year, and between locations, because of differences in thermal developmental budgets, e.g. in the spruce budworm *Choristoneura fumiferana* (Clem.) (Han and Bauce, 1998). In both the facultative and obligatory response, diapause represents an alternative developmental pathway, initiated by unique patterns of gene expression, which allows insects to survive seasonally recurring chronic forms of environmental stress, and to coordinate growth, development and reproduction with annual cycles of favourable environmental conditions (Denlinger, 2002). Both obligate and facultative diapause are likely to be affected by climate change but this review will primarily focus on facultative diapause.

In temperate and polar zones, i.e. above 30°N latitude, day length is the primary cue programming diapause. This represents a strong, noise-free indicator of the changing seasons, and has remained highly reliable over evolutionary time. Patterns of day length change will of course remain unaffected by climate change but their effectiveness as an indicator of seasonal changes in temperature may diminish. For each species of insect there is a critical day length (CDL), which will programme the diapause response. This is designated as the photoperiod that induces a 50% incidence of diapause, and changes with latitude, e.g. the CDL of the blow fly *Calliphora vicina* (R-D) at 65°N is 16.5 h whereas at 51°N it is 14.5 h (McWatters and Saunders, 1996). This represents an adaptation to the fact that winter occurs earlier, and more rapidly, at higher latitudes, and small changes in photoperiod either side of the CDL produce a dramatic shift in diapause incidence (Fig. 4). Low temperature can enhance the diapause response (greater incidence and longer duration) but temperature by itself does not typically induce diapause.

The adaptive significance of this seasonal response to changing day length, termed photoperiodism, lies in the anticipatory nature of switching off development and reproduction in preparation for winter, even when the immediate conditions may be favourable (Bradshaw and Holzapfel, 2010). Thus, diapause is a pivotal point in the life cycle of insects and crucial to the survival of seasonal adversity. Furthermore, given the diverse roles insects play in ecosystem function, many other processes are synchronous with the diapause programme (plant consumption/pollination, predator-prey interactions, etc.). Consequently, any disruption of diapause as a result of climate change could have profound effects on ecosystem stability.

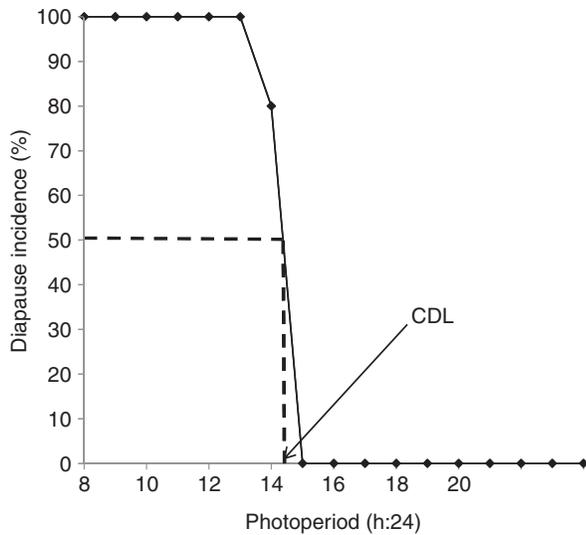


Fig. 4. Photoperiodic response curve for the production of diapausing larvae by adults of the blow fly *Calliphora vicina* at 20°C (flies collected from latitude of 51°N). Larvae from eggs deposited on day 12 post-eclosion and reared in constant darkness at 11°C. The critical day length (CDL) is 14.5h:24 h (see Saunders, 1987).

Diapause and cold tolerance

In most insects, diapause enhances cold stress tolerance (Denlinger, 2002). Indeed, if cold tolerance is broken down into its component mechanisms, a close relationship between the cold stress response and diapause is supported by an increasing number of studies. Thus, within the diapause programme, a suite of stress response mechanisms is initiated to facilitate overwintering survival (Fig. 5). These include the synthesis of cold-protective metabolites, e.g. glycerol, termed cryoprotectants (Lee et al., 1987b; Han and Bauce, 1998; Košťál and Šimek, 2000), changes in membrane lipid composition (Hodková et al., 1999; Michaud and Denlinger, 2006; Tomčala et al., 2006), a general cessation of the cell cycle (Nakagaki et al., 1991; Tamariello et al., 1999), and the synthesis of molecular chaperones, such as heat shock proteins (Hsps) (Rinehart et al., 2007). Here it is important to recognise that these mechanisms are initiated solely as a result of entering diapause; they are not a response to low temperature, although they may be enhanced by subsequent cold exposure.

Membrane adaptation in particular is recognised as the front line of protection against cold stress, as it represents the interface between the insects' cells and their environment. This process also provides a comparison of the similarities between diapause and the cold stress response. Acute cold exposure causes either injury or disruption to membrane function but prior conditioning (acclimation to less severe temperatures) alters the physical properties of the membrane to offer protection. Increasing the proportion of unsaturated fatty acids (UFAs) in membrane phospholipids, at the expense of saturated fatty acids (SFAs), is one strategy to compensate for the rigidifying effects of cooling (Cossins, 1994). Other cold-induced changes in membrane composition include a shift in the proportion of phosphatidylethanolamine (PE) molecular species relative to phosphatidylcholines (PC), the conical shape of PEs being regarded as favourable in terms of cold adaptation (Hazel, 1995). Interestingly, changes in membrane composition within diapause often mirror that seen during cold acclimation (Michaud and Denlinger, 2006; Hodkova et al., 1999). However, this process is known to be under

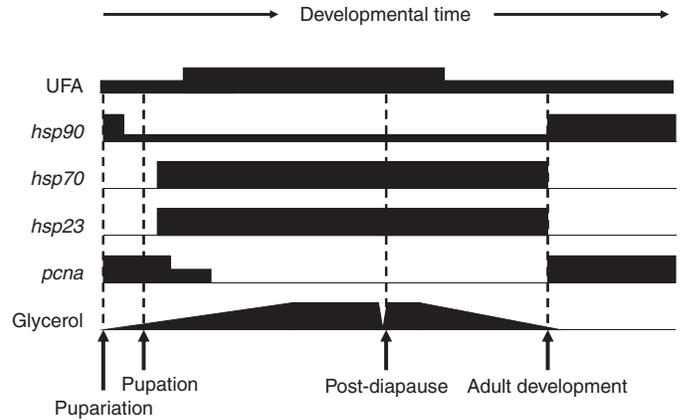


Fig. 5. Schematic representation of temporal changes in membrane unsaturated fatty acid content (UFA) [data from Michaud and Denlinger (Michaud and Denlinger, 2006)], expression patterns of key diapause-associated genes [heat shock proteins (Hsp), proliferating cell nuclear antigen (PCNA)] and glycerol content during pupal diapause in the flesh fly *S. crassipalpis* from the time of pupariation through to pharate adult development (Hayward et al., 2005).

photoperiodic control and is not a direct response to low temperature (Hodkova et al., 2002). Thus, membrane adaptation represents an early preparation for the diapause state, before the onset of winter conditions, and could critically influence both the progression of diapause and its stress-tolerance phenotype. The impact of climate change on diapause membrane adaptation, or indeed any diapause stress response mechanism, remains unknown but higher temperatures could certainly disrupt these processes.

Tropical insects

Global warming is occurring faster in winter than in summer, and is greatest at high latitudes (IPCC, 2007). Thus, insects with a hibernal diapause are likely to experience the most significant changes in their thermal environment. However, tropical insects may already be closer to their physiological limits of high temperature and drought tolerance. This means that even small changes at these latitudes could have dramatic consequences on survival. Tropical diapause is again utilised to span periods of seasonal adversity, and there is some evidence that changing day length can play a role in programming diapause induction (Denlinger, 1986). Changes in temperature, moisture, population density or food composition, however, tend to be the dominant-inducing stimuli of tropical diapause (Tauber et al., 1986). Unlike the temperate zone, there is considerable variation in the incidence of diapause in the tropics, mainly because seasonal cycles at low latitudes do not always produce such clear-cut changes in environmental conditions that entirely restrict continued development. This is not true of all tropical habitats, however, and there are numerous examples of prolonged and intense periods of diapause (Denlinger, 1986) to survive either severe drought in the dry season, e.g. the African grasshopper *Zonocercus variegatus* (L.) (Page, 1980), or avoid being active during the rainy season, e.g. the anthophorid bee *Epicharis zonata* (F. Smith) (Roubik and Michener, 1980). A slight warming or drying of the climate in the tropics could have dramatic effects on the incidence, duration or successful completion of diapause in many species.

Diapause in a changing climate

Current greenhouse gas levels suggest that all ecosystems are locked into a trajectory of continued warming for several decades, regardless of any mitigating actions. It is crucial, therefore, to assess the impact of climate warming on the seasonal physiology of insects. Typically, at temperate latitudes, the diapause programme spans three seasons: induction and initiation take place in late summer and autumn; the diapausing stage occurs in the first part of winter; and diapause termination and post-diapause quiescence follow during the latter part of winter and early spring. When addressing the effect of climate change, therefore, it is important to consider the influence of higher temperatures across these phases of diapause.

Diapause induction: decoupling photoperiod and temperature cues

In the temperate zone, virtually all insects rely on diapause to overwinter (Denlinger, 2002). The decision to enter diapause is initiated when the sensitive stage experiences the shortening days of late summer and autumn, while developmental arrest (the diapausing stage) is usually initiated later in the life cycle, and can even occur in the subsequent generation. For example, in *C. vicina* the sensitive stage is the adult fly, while diapause occurs in the following third instar larva (L3) (Vingradova and Zinovjeva, 1972; Saunders et al., 1986). For the silk moth *Bombyx mori* (L.), the sensitive stage is the egg, with diapause occurring in the next generation of eggs (Yamashita and Hasegawa, 1985). Thus, each individual has its own genetically determined response to day length and, based on that response, either enters diapause or continues development (Bradshaw and Holzapfel, 2010).

For many species, warmer conditions experienced during the photoperiodic induction of diapause, i.e. autumn, are likely to reduce diapause incidence and duration. For example, in *C. vicina*, adults reared at 20°C produce fewer diapausing offspring, and diapause is shorter, than adults reared at 15°C (McWatters and Saunders, 1998). This is thought to be because more rapid development means that fewer diapause-inducing photoperiods are experienced during the sensitive stage (Saunders, 1987). As well as reduced diapause incidence, the 'decision' to enter diapause can also be aborted if a threshold temperature is exceeded, e.g. UK populations of *C. vicina* enter diapause under short day conditions at temperatures below 15°C but will abort diapause above this temperature (Vaz Nunes and Saunders, 1989; Saunders and Hayward, 1998). Given that the CDL of *C. vicina* populations at 51°N (14.5 h) occurs at the end of August, it seems likely that diapause is already currently being averted in the progeny of adults first experiencing the CDL, as prolonged periods above a soil temperature of 15°C are regularly being recorded at this time of year [Rothamstead Research and The UK Environmental Change Network (http://www.rothamsted.bbsrc.ac.uk/aen/ecn/Soil_T10_YR.htm)]. Several other species also avert diapause under high temperatures, e.g. *Sesamia nonagrioides* (Lefebvre) (Fatinou and Kagkou, 2000), and for species that can complete another generation prior to winter or can survive winter outside of diapause, this capacity to avert diapause may provide a selective advantage (Tougou et al., 2009). For many species, however, the decoupling of temperature and photoperiodic cues brought about by climate warming, presents a number of significant problems. If diapause is crucial for overwintering survival then, once averted, higher temperatures will be required to continue to allow development to the next diapausing generation before the onset of winter conditions. Thus, for many temperate and sub-polar insects, late entry into diapause introduces the risk of encountering cold stress outside of diapause – before cold tolerance mechanisms have

become established (Bradshaw et al., 2004; Rinehart et al., 2007; Bradshaw and Holzapfel, 2010). This is believed to determine the current northern margin of the green stinkbug *Nezara viridula* (L.) in Japan. Only diapausing adults of *N. viridula* are able to overwinter but the timing of diapause induction at its northern margin, 34.1°N, means that individuals only reach nymphal stages prior to winter, and so are destined to die (Musolin, 2007). Further south, there is a sufficiently long growing season to allow the pre-winter generation to reach adulthood, and at these locations *N. viridula* has been displacing *N. antennata* (Scott) (Tougou et al., 2009).

Not all species will have their diapause adversely affected by climate change however. For example, the flesh fly, *S. crassipalpis* enters diapause even at high temperatures (25°C) that are favourable for continued development (Denlinger, 1972). Thus, a warming climate will not necessarily reduce the incidence of diapause in this species, although it could impact on other diapause characteristics, such as duration, which is shorter at higher temperatures (Denlinger, 1972). Recent evidence also suggests that certain insects may be capable of adapting their photoperiodic response to rapid climate change (Bradshaw and Holzapfel, 2010), which could overcome some of the problems of seasonal synchrony previously described. Thus, the prolonged growing season has imposed selection on altering the timing of seasonal activities such as diapause, and can result in a change in the CDL required to induce diapause. A clear example of this is the genetic shift towards shorter (more southerly) CDLs in northern populations of the pitcher plant mosquito *Wyeomyia smithii* (Coq.) (Bradshaw and Holzapfel, 2001a; Bradshaw and Holzapfel, 2001b).

Diapausing stage: reduced stress-tolerance phenotype

Climate warming is unlikely to occur consistently throughout winter. The impact of intermittent warmer periods on the development of diapause stress tolerance is therefore an important consideration. For many insects, overwintering stress-tolerance mechanisms only reach their full potential upon exposure to cold conditions, e.g. *Ostrinia nubilalis* (Hubner) (Grubor-Lajsic et al., 1992) and *Pyrrhocoris apterus* (L.) (Tomčala et al., 2006). Consequently, higher autumn and early winter temperatures may inhibit this acclimation response and increase mortality if more severe conditions occur later in winter. Even if chilling is not specifically required to induce stress-tolerance mechanisms, a change in the seasonal timing of diapause initiation under warmer conditions could also influence winter mortality. For example, in the obligate diapause of the spruce budworm *C. fumiferana*, warmer conditions induce an early entry into diapause and a subsequently modified metabolic rate within diapause. This causes an early synthesis and utilisation of glycerol before the coldest part of winter, resulting in high winter mortality (Han and Bauce, 1998). Similar, temperature-dependent metabolic shifts during diapause appear to be a common characteristic (Adedokun and Denlinger, 1985). Evidence from laboratory studies shows that diapausing individuals from a broad range of species are more cold tolerant following cold acclimation (Denlinger, 1991). To date, however, there are few detailed studies that have investigated how climate change may impact on the molecular mechanisms underpinning the diapause stress-tolerance phenotype (see also Hayward et al., 2005).

Diapause termination and spring emergence: a complex situation

Diapause in many temperate insects is known to terminate in mid-winter, long before the return of favourable conditions in spring (Denlinger, 1991; Hodek, 1996). For example, the diapause of the

flesh fly *Sarcophaga bullata* (Parker) typically ends in early January in the USA (Denlinger, 1972). The stage beyond diapause has been termed post-diapause quiescence (Hodek, 1996), and often retains many of the stress-tolerance mechanisms of the diapausing stage (Hayward et al., 2005). However, individuals within post-diapause are primed and ready to initiate development as soon as favourable conditions are experienced. Upon the resumption of development, many of the stress-tolerance mechanisms are 'switched off', and the stress-tolerance phenotype is significantly reduced (Hayward et al., 2005). Thus, brief warm spells after the termination of diapause could initiate a premature resumption of development, leaving animals vulnerable to later cold spells. This sequence of events has been hypothesised as an explanation for 1990 population collapse of both larch bud moth *Zeiraphera diniana* (Guénée) (Baltensweiler, 1993) and a contributing factor to the reduced spring emergence of *C. fumiferana* (Han and Bauce, 1998). Sustained periods below the threshold for development during post-diapause quiescence are also thought to be an important synchronising mechanism, because they allow individuals that may have had a staggered entry into diapause, and different rates of diapause progression, to all complete diapause before the environment allows development to resume (Tauber et al., 1986). Recent earlier and warmer springs, therefore, have resulted in an earlier and more staggered emergence in several species (Roy and Sparks, 2000). However, a direct consideration of how climate change influences diapause, which underpins this phenomenon, has rarely been undertaken (but see Tobin et al., 2008).

Early emergence

The duration of diapause can be influenced by many factors: accumulated chilling, moisture, food and day length (Tauber et al., 1986). For many species, however, a general principle is that diapause duration is shorter at higher temperatures. For example, *S. crassipalpis* remains in diapause for 118 days at 17°C, 70 days at 25°C and 57 days at 28°C (Denlinger, 1972). The reasons for this are not fully understood but one hypothesis is that a higher metabolic rate during diapause at elevated temperatures depletes stored nutrient resources more quickly (Hahn and Denlinger, 2007). Many species accumulate greater energy reserves in preparation for diapause (Lees, 1955; Tauber et al., 1986), and there appears to be a greater minimum energy requirement for entry into diapause vs continued non-diapause development (Saunders, 1997), although little is known about the mechanisms underpinning this resource allocation (see also Robich and Denlinger, 2005). Furthermore, it is not known whether these strategies are adaptable to different temperature conditions. Low temperatures are a powerful force reducing the energetic costs of diapause and contribute to the retention of nutrient reserves required for post-diapause processes (Hahn and Denlinger, 2007). For example, after a larval diapause, energy reserves must fuel pupation, metamorphosis, construction of adult tissues, as well as post-diapause feeding and (possibly) reproduction (Leather et al., 1993). Thus, if climate warming disrupts the metabolic balance of diapause, there may be a significant impact on survival and fecundity as well as diapause incidence or duration. Early spring emergence has been recorded for many species over recent decades (Roy and Sparks, 2000; Gordo and Sanz, 2006; Hassal et al., 2007; van Asch et al., 2007) but the comparative fitness of early and late emerging individuals has received little attention.

Late emergence

Another outcome of warmer autumns and winters could actually be delayed emergence or possibly even sustained diapause and death. Exposure to high temperatures during early diapause can deepen

the period of dormancy in some instances, with insects requiring longer periods and/or different temperature conditions to complete diapause development (Danks, 1978; Han and Bauce, 1998). As well as enhancing cold tolerance within diapause, periods of chilling can also be essential for diapause termination in some species (Tauber et al., 1986). For insects requiring a chilling stimulus to terminate dormancy, diapause duration is again temperature dependent, being shortest at the optimal temperature and longer under either higher or lower temperatures. Thus, in the northern grasshopper *Melanoplus borealis* (Fieber), if a chilling cue is not received individuals may emerge late or not at all (Fielding, 2008). There are also examples of butterflies where the vernal development has been delayed despite warmer springs (Roy and Sparks, 2000). Clearly, any change in spring emergence could result in a loss of synchrony with the environment. Prolonged diapause has also been associated with increased mortality, a lower incidence of mating and lower fecundity (Denlinger, 1981).

Loss of synchrony with environment

Synchrony in phenology between an insect and its environment is crucial to maximising an individual's fitness. Loss of synchrony could also have a significant impact, at the ecosystem level, on community dynamics. A key issue with respect to climate change is whether the phenologies of interacting species respond in parallel to a warming environment. There is evidence that some plant–pollinator interactions have remained cohesive (Hegland et al., 2009) whereas others have become temporally mismatched under recent climate change (Memmott et al., 2007). Many plant–insect herbivore interactions have also been altered by climate warming (Parmesan, 2007). The demographic consequences of these decoupled interactions, however, remains largely unknown. One of the best studied examples is the winter moth *Operophtera brumata* (L.), where egg hatch has advanced significantly, relative to budburst in its host plant the pendunculate oak *Quercus robur* (L.) (Visser and Holleman, 2001; van Asch and Visser, 2007). When considering the impact of diminished synchrony between insects and their host it is also important not to assume the 'starting point' prior to climate change represented 'perfect' synchrony. Thus, Feeny observed that before recent global warming, *O. brumata* routinely suffered 90% mortality due to mis-matches between egg hatch and budburst, suggesting the system may be highly vulnerable to small changes in synchrony (Feeny, 1970). Recent evidence, however, predicts a rapid response to selection pressure on the timing of egg hatch in *O. brumata*, leading to a restoration of synchrony with *Q. robur* bud burst (van Asch et al., 2007). Thus, as with adaptations in the photoperiodic induction of diapause in *W. smithii* (Bradshaw and Holzapfel, 2001a; Bradshaw and Holzapfel, 2001b), the genetic mechanisms underpinning adaptability to climate change and the selection pressures acting upon them, need to be considered before species responses can be predicted with any accuracy.

Applications of insect ecophysiology

Over the last 25 years there have been a number of national and international research programmes that have investigated the effects of higher concentrations of CO₂ and higher temperatures on plants and their associated insect herbivores [e.g. International Tundra Experiment (ITEX) and the UK's 'Terrestrial Initiative in Global Environmental Research' (TIGER)]. In addition, several species of insect have been routinely monitored for longer periods of time, and although these schemes were initiated with specific objectives and prior to any concerns about 'climate warming', the patterns in the data reveal some of the most compelling evidence for the impact

of higher temperatures on insect overwintering and the environmental consequences. The areas that will be covered in this review are changes in distributions, forecasting the risk of pest outbreaks, and predicting the likelihood of establishment of non-native and invasive species. In each case, the underlying explanations for the observations are physiological, but the outcomes are ecological, and often of economic importance.

Changes in distributions

Insects are generally more mobile than plants, leading to the view that through their powers of flight, insects regularly ‘disperse’ outside of existing ranges; however, permanent establishment in these new areas is prevented by the much slower distributional changes of their host plants (Lawton, 1995). Whilst this view is undoubtedly correct, there are also many examples where the historical distribution of plants, particularly crop species and forests, has been wider than that of some of their associated insect herbivores but the latter species have shown distinct shifts in their distribution over the most recent 30–50 years, and increases in the minimum and mean winter temperature are often cited as the most likely explanation for such changes. This section provides three examples of species inhabiting different climates and with different overwintering biologies (for a review, see Bale, 2010).

The northern limit in Japan in the 1960s of the southern green stinkbug *Nezara viridula* (L.) was 34.1°N, delineated by the 5°C mean temperature isothermal line for coldest winter month (January). By 2000, *N. viridula* was found 70 km further north and had displaced the closely related *Nezara antennata* Scott; between 1960 and 2000 the minimum and mean winter temperatures increased by 1–2°C (Musolin 2007). In Scandinavia, 100 year records of the distributions of two geometrid moths *E. autumnata* (autumnal moth) and *Operophtera brumata* L. (winter moth) and occurrence of damage to birch trees suggest that changes in the ranges of the two species are linked to interactions between the cold hardiness of the overwintering stages and higher winter temperatures (Jepsen et al., 2008). Historical differences in the distribution of the two moth species have been attributed to the lower SCP of the overwintering eggs of *E. autumnata* but over the past 15–20 years there has been an increase in the minimum and mean winter temperatures. During this period, *O. brumata* has shifted its distribution north-eastwards, becoming dominant in areas previously occupied by *E. autumnata*, and at the same time, *E. autumnata* has expanded its range into even colder continental areas where, in some winters, the minimum temperature is consistently above the SCP of overwintering eggs (35°C) (Tenow and Nilssen, 1990).

The winter pine processionary moth *Thaumetopoea pityocampa* Den. and Schiff. provides an interesting example of a species that has shown a gradual altitudinal range expansion of 230 m in the Italian Alps over the past 30 years (Battisti et al., 2005). The larvae of *T. pityocampa* overwinter in nests spun from silk and, depending on temperature, leave the nest at night to feed on pine needles; winter survival is determined by the number of feeding hours per day during the coldest part of winter from December to February (Battisti et al., 2005; Buffo et al., 2007). One interesting feature of *T. pityocampa* is that larvae feed in winter, which would normally be considered incompatible with a freeze-avoidance strategy, because food particles in the digestive system are regarded as potent ice nucleators. Recent research suggests that a proportion of larval population is freeze tolerant to 10°C below the SCP and the remainder rely on supercooling to survive (Battisti et al., 2005; Battisti et al., 2006; Buffo et al., 2007). One of the predicted features of global climate change, alongside progressively higher

temperatures, is an increase in so-called ‘extreme events’ – such as abnormally hot or cold days or longer periods, high precipitation and flooding. It has been suggested that such events pose a particular risk for new colonies at range margins and may lead to a reversal of the previous range expansion (Battisti et al., 2006). *Thaumetopoea pityocampa* provides an example of the effect on an extreme event that has led to a ‘one off’ rapid range expansion that seems to have become permanent; during a period of very warm nights in 2003 with temperatures regularly above the flight threshold, moths colonised high altitude sites considerably above the existing range margin and the resultant larval populations overwintered successfully (Battisti et al., 2006).

The examples of the changes in distribution of *N. viridula*, *E. autumnata*, *O. brumata* and *T. pityocampa* are essentially correlative – the species have shifted their range into previously unoccupied areas that would seemingly have posed a lethal winter physiological stress without the 30–50 years of recent climate warming. In a further example that has included a detailed analysis of cold hardiness, it appears that the winters have not yet ‘warmed’ sufficiently to allow a permanent range expansion. The large yellow underwing moth *Noctua pronuba* (L.) is a resident and common noctuid species in the UK overwintering as larvae that feed on a wide range of host plants; adults of the related dark sword grass moth *Agrotis ipsilon* Hufnagel are often found in large numbers in late summer but the species has so far seemed unable to establish permanently in the UK. A likely explanation for this failure is the difference in the larval cold hardiness of the two species. Although the larvae have a similar SCP (around –12°C), whereas acclimation depressed the freezing temperature of *N. pronuba* to –15.4°C, there was no response in larvae of *A. ipsilon*. Similarly, the LT₅₀ (lethal temperature) was lower in *N. pronuba* (–15.8°C compared with –8.9°C), LTime₅₀ (lethal time) at –5°C was longer (9 and 5.8 days, respectively), and in a field exposure, larvae of *N. pronuba* survived throughout the winter whereas there was 100% mortality of *A. ipsilon* after six weeks (Bale and Walters, 2001). The study on *N. pronuba* and *A. ipsilon* therefore provides direct ‘physiological’ evidence of a lack of overwintering ability as an explanation for the observed distributions, compared with the previously cited ‘correlative analyses’ of changes in distribution in relation to known increases in environmental temperature. However, it nevertheless lacks sufficient quantitative climatological insight to address the most intriguing question for *A. ipsilon* and other species with ‘climate-limited’ distributions: how much warmer does it need to be and are there other critical requirements to allow permanent establishment in areas commonly visited by summer migrants?

Forecasting the risk of pest outbreaks

Aphids are among the most effective insect vectors of plant virus diseases, such as the transmission of potato leaf roll virus (PLRV) and potato virus Y (PVY) by the peach-potato aphid *M. persicae* and barley yellow dwarf virus (BYDV) by the grain aphid *S. avenae*. Most aphids of economic importance have two genetically distinct types of life cycle within the one species: holocyclic and anholocyclic. In the holocyclic life cycle, multiple generations reproduce asexually from spring through to summer and to early autumn and then, in response to decreasing photoperiod, sexual morphs (males and egg-laying females – the oviparae) are produced, which mate and lay the overwintering eggs. Aphid eggs are very cold hardy (Strathdee et al., 1995) and within northern Europe it seems that winter minimum temperatures are not a threat to survival. Egg hatch occurs at much the same time each spring, after which

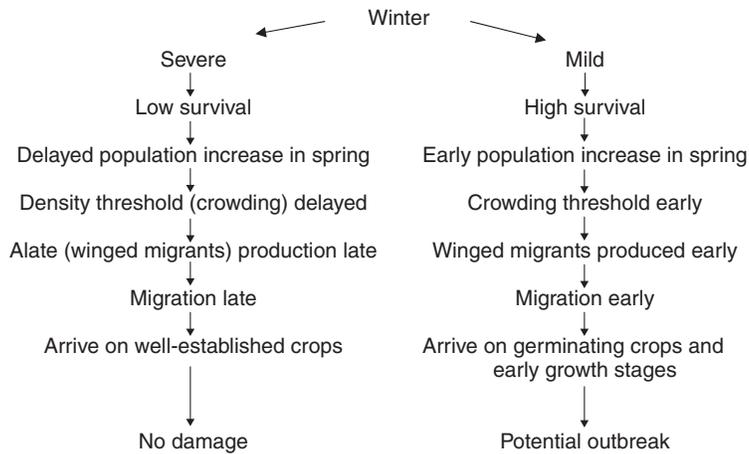


Fig. 6. Schematic representation of the effects of mild and severe winters on spring and summer populations and related damage potential of aphids overwintering as anholocyclic clones.

subsequent generations reproduce asexually, eventually reaching a critical density that triggers the production of winged aphids that fly to crops such as potato and cereals. The key point is that this migration usually occurs in summer at a time when spring-sown crops are well established and therefore less affected by aphid feeding and virus infection.

Anholocyclic clones arise from a genetically stable mutation that renders the aphids unresponsive to the decreasing photoperiodic cue that triggers the sexual morphs and egg production in the autumn. Anholocyclic clones therefore reproduce asexually the whole year round, with increases and decreases in the rates of development and reproduction governed by seasonal changes in temperature. However, in contrast to their eggs, the overwintering 'active stages' of anholocyclic clones are much less cold hardy – mortality increases from 0% to >90% as the temperature decreases from around -5°C to $<-15^{\circ}\text{C}$ (Bale et al., 1988; Clough et al., 1990). Thus, depending on the temperatures experienced in winter and duration of exposure, the relative size of the post-winter population ranges from 'very high' (mild winter), 'average' (normal winter) or 'very low' (cold winter), and it is the size of these populations, acting through a series of ecophysiological processes (Fig. 6) that determine the timing of the 'spring' migration each year, the numbers of migrating aphids and, in turn, the risk of a virus epidemic on crops (Harrington et al., 1991; Harrington et al., 2007). A 50 year record of the time when the first migrating *M. persicae* is caught each year in a suction trap (at Rothamsted Research, Harpenden, Herts, UK) has identified a strong correlation with winter temperature (January–February mean) as shown in Fig. 7A. The timing of the migration can differ by over three months between years, and this difference represents a potential epidemic (April migration) or essentially 'no damage' (July migration). Also, the number of migrating aphids is higher after mild winters (Fig. 7B). The interesting feature highlighted in Fig. 7A is the high proportion of mild winters and relatively early migrations over the past 20 years compared with 40–50 years ago. Furthermore, in absence of any major change in the dynamics of aphid parasitism or predation by natural enemies, it seems likely that continued climate warming will exacerbate current aphid problems.

Establishment of invasive species

Species with the potential to be invasive in previously uncolonised countries and wider areas are transported around the world, accidentally or intentionally, by various mechanisms and whilst climate does not usually impact on the transport process, the

temperatures experienced in the 'receiving environment' can have a direct bearing on the likelihood of establishment. The use of non-native insects and mites for the biological control of pest species is an example of intentional introduction and release of organisms that under certain circumstances may become 'invasive'. For example, the Harlequin ladybird *Harmonia axyridis* (Pallas) originates in Asia and was first released in Europe around 1990 in field trials in France and then commercially released in 1995 as a control agent against aphids. From 2000 onwards, the beetle spread through central Europe, across the English Channel to the UK and northwards into Scandinavia (for a review, see Brown et al., 2008). Later studies have shown that the ladybird overwinters as an adult, is cold hardy, can diapause, is polyphagous (predating many species of non-target prey), is highly dispersive and likely to predate and displace native Coccinellids in some European countries (Berkvens et al., 2008; Pell et al., 2008; Adriaens et al., 2008). This raises the interesting question of how this species came to be imported into Europe and released. The USA, Canada, Australia and New Zealand have stringent regulations for the import and release of non-native species as biological control agents. In Europe, there is no EU-wide regulation; rather, some countries have regulation (e.g. UK, The Netherlands) and others (e.g. France, Italy) do not (for a review, see Hunt et al., 2008). This means that a species can be released in a country without regulation and spread to neighbouring countries in which its release would not have been allowed – which is the situation with *H. axyridis*. But even in some countries with regulation, the method used to assess the risk of establishment has been simplistic (based on 'climate matching' rather than a direct assessment of cold tolerance), the assumption being that species of tropical origin would not be able to survive in a colder temperate climate (Bale, 2005; Bale et al., 2009). The problems with this approach came to light when the predatory mite *Neoseiulus californicus* (McGregor), released in to UK glasshouses as a control of spider mite *Tetranychus urticae* Koch, was found to have established outdoors in orchards (Jolly, 2000). A series of studies found that a proportion of the commercially supplied populations were able to diapause (trait was probably introduced when stock cultures were refreshed with field collected material), and when these mites escaped from glasshouses, they responded to diapause-inducing cues and were able to overwinter; additionally, non-diapausing mites were also capable of long-term survival through UK winters (Hart et al., 2002).

Over the last 10 years there has been a focus in Europe on devising an environmental risk assessment (ERA) process for non-native

biocontrol agents, with the dual aims of ensuring environmental safety of the released organisms without imposing prohibitive costs on the biocontrol industry (van Lenteren et al., 2006; Bale et al., 2008). This initiative has resulted in a generic template for risk assessment as summarised in Fig. 8 (van Lenteren et al., 2006). Whilst the sequential ‘tests’ of this ERA are flexible, for species that will be released in temperate or colder climates there is strong argument to make ‘establishment’ the first assessment, i.e. where a species is to be released exclusively into glasshouses (‘augmentative and inudative control’), if it can be shown that there is no risk of winter survival, then any escapees are of no concern, and there is no need to assess host range and non-target effects. By the same token, if it is a ‘classical control’ scheme where permanent establishment is a pre-requisite for success, then it would be essential to know that the species could survive through a seasonal climate and winter cold stress. An analysis of the cold hardiness of eight non-native control agents that have been released in the UK and (i) established outdoors, (ii) failed to establish, or (iii) are candidates for release has identified a strong correlation between the LT_{50} at 5°C and duration of survival in the field in winter (Fig. 9) (Bale et al., 2009). There is a distinct cluster of species that cannot survive for more than 3–4 weeks in the field, and these could be regarded as ‘safe’ for release into glasshouses. Interestingly, the analysis identified retrospectively *N. californicus* as a species with adequate cold hardiness to survive through UK winters, and this has proved to be the case. As with the changes in distribution and forecasting of pest outbreaks, this is a further example of how physiological data can be applied to a problem of both economic and conservation importance.

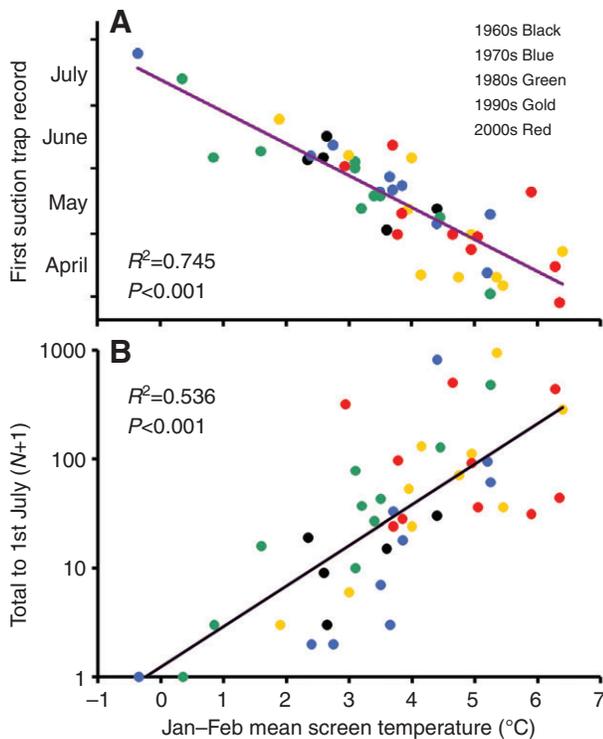


Fig. 7 (A) Date of first record of the aphid *Myzus persicae* in the Rothamsted Research suction trap from 1965 to 2009 (courtesy of Rothamsted Research). (B) Total migrant population (to 1 July) of the aphid *Myzus persicae* in the Rothamsted Research suction trap from 1965 to 2009 (courtesy of Rothamsted Research).

Temperature and activity thresholds

The concept of ‘survival in a changing world’ could be viewed in terms of the ways in which climate, and particularly temperature, can impact directly on organisms with potentially lethal outcomes – but climate warming may affect ‘survival’ in other ways. As an example, all insects and their natural enemies have species-specific thermal characteristics and thresholds, such as rates of development, activity and critical temperatures for mobility and flight. In a predator–prey interaction, if the predator can develop at a lower temperature than its prey or remain active when the prey have become mobile, it is more likely to be successful than if it is thermally less competitive. In the natural world, such thresholds will clearly impact on the balance between different trophic levels but where organisms are being selected for a specific purpose, for example, biological control, then knowledge of any differences between the control agent and the intended target would be valuable information in the selection process. Various methods have been used to assess activity thresholds of insects such as chill coma but all of these involve direct observation by eye, and some require regular disturbance of the organism to test for the ‘righting response’ – the ability of an individual when placed on a surface on its dorsum to ‘turn over’ to its normal walking position (Klok and Chown, 1997). A new system has recently been developed in which multiple specimens are placed in a small temperature-controlled arena (constructed in aluminium) and their behaviour continuously monitored by video capture recording. This system allows assessment of walking speed at different temperatures, variation in chill coma temperature across a population and even the critical temperature at which there is the last movement in an appendage

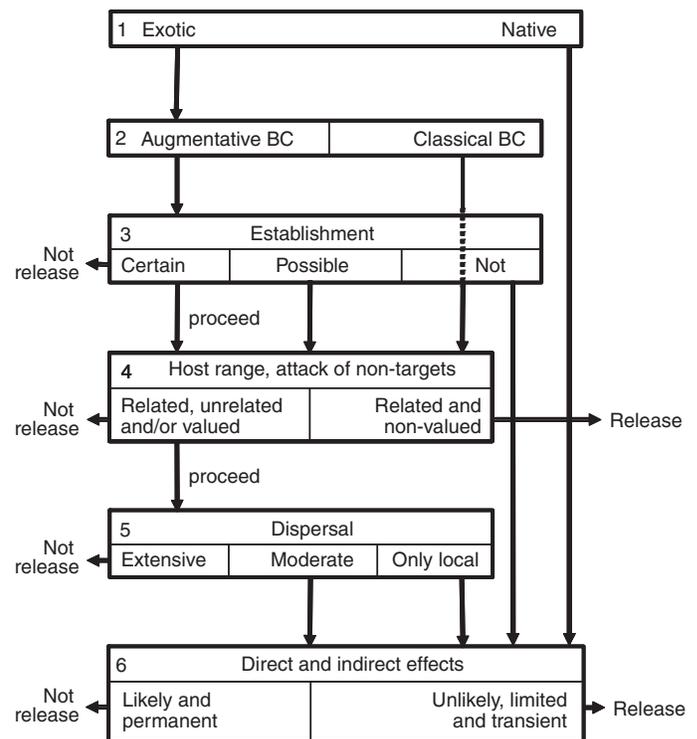


Fig. 8. Flow chart summarising a hierarchical environmental risk assessment scheme for arthropod biocontrol agents (modified from van Lenteren et al., 2006).

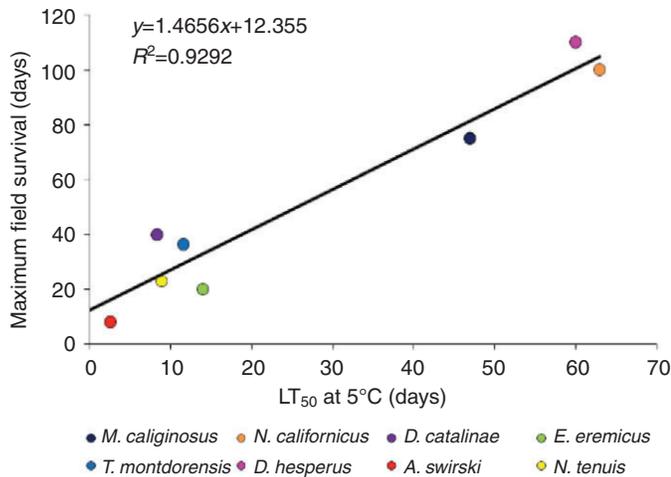


Fig. 9. Relationship between LT_{50} (lethal time) at 5°C (laboratory) and winter field survival of eight non-native glasshouse biocontrol agents (*Macrolophus caliginosus*, *Neoseiulus californicus*, *Delphastus catalinae*, *Eretmocerus eremicus*, *Typhlodromips montdorensis*, *Dicyphus hesperus*, *Amblyseius swirskii* and *Nesidiocoris tenuis* (see Bale et al., 2009).

(leg or antenna), and there is a permanent record of each experiment (for details, see Hazell et al., 2008). This type of analysis has recently been applied to two predatory biological control agents [the mite *Nesidiocoris tenuis* (Reuter) and the mite *Amblyseius swirskii* Athias-Henriot] and has identified clear differences in their thermal activity profiles (Bale et al., 2009). With respect to climate warming, it will be important to assess how these ‘non-lethal’ thresholds change with natural and experimental acclimation, and whether the differentials between species under prevailing regimes are maintained under different climatic conditions.

Conclusions

Global climate change has been described as the greatest natural experiment in the history of science. Across a range of species from different climatic zones there is evidence that over the past 30–50 years there have been changes in distributions – with northward advances into ‘colder areas’, in phenologies (with earlier migrations, and in the establishment of invasive species in new locations) (Bale, 2010). In addition, in the majority of examples of these events cited in this review these ‘changes’ have been attributed to a progressive alignment between the winter thermal tolerance of the insect and a gradual amelioration of the climate. For the most part, therefore, the central hypothesis set out in the Introduction to this review – that a climate warming of 1°C to 5°C would be beneficial for insects currently experiencing some level of cryo-stress in parts of their distribution – has been supported by the evidence; and furthermore, it seems likely that the physiological plasticity of organisms inhabiting these regions will allow the observed trends to continue over the next 30–50 years. However, this generalised conclusion comes with some major caveats. It is clear that winter snow cover is an effective thermal buffer against severe air temperatures in polar areas, and as this protection is reduced, becomes intermittent or is completely lost, the effects on some species may be profound; some freeze-tolerant species cannot survive through repeated freeze–thaw cycles, the winter-enhanced supercooling ability of some freeze-avoiding insects may be inadequate in the face of very low environmental temperatures, encasement in ice may be differentially lethal for some taxa and the more frequent occurrence of cuticular

surface moisture in areas experiencing irregular sub-zero temperatures may be highly detrimental for some species.

In a similar way, whereas diapause has been regarded as both an essential component of the overwintering strategy of many insects, and a phenological synchronising mechanism between insects, food resources and favourable abiotic conditions, there would seem to be a series of clearly identifiable sequential events in the diapause syndrome where higher temperatures could disrupt the normal process – averting diapause completely, delaying entry into diapause, decoupling development from induction cues, removing required chilling stimuli and promoting de-synchronised termination and post-diapause emergence. Thus, the net impact of climate warming on diapausing insects is challenging to predict (Huey, 2010). At present, these possibilities have in the main been identified from a theoretical ‘overlaying’ of future climate scenarios on to the thermal profile and requirements for the induction, maintenance and termination phases of diapause, in combination with experimental exposure to higher temperatures envisaged to represent different ‘end points’ of current climate change. It may well be that it will require the ongoing ‘natural experiment’ to reveal the extent to which the potential disruptions play out, are found to fall within the limits of plasticity of the diapause process, or require an evolutionary adaptation over time.

Although climate warming may occur to a lesser extent in tropical areas, insects living in ‘hot climates’ close to their upper physiological limit may be least able to cope with the higher temperatures associated with a changing climate. In one of the few studies that have investigated the upper and lower thermal thresholds and acclimatory ability of a group of closely related species of polar [*Myzus polaris* (Hille Ris Lambers)], temperate [*Myzus persicae*] and tropical [*Myzus ornatus* (Laing)] origin, Hazell et al. (Hazell et al., 2010a; Hazell et al., 2010b) found that across all species, there was more variation in the lower than the upper thresholds, and low temperature acclimation depressed the lower limits by more than high temperature raised the upper thresholds. These responses are broadly consistent with the large scale patterns described by Addo Bediako et al. and Chown (Addo Bediako et al., 2000; Chown, 2001). Compared with temperate zones, not only do tropical areas lack reliable seasonal cues that would allow phenotypic plasticity to evolve (Kingsolver and Huey, 1998; Ghalambor et al., 2006), for those species living close to their upper thermal limits under the prevailing climate, survival at higher temperatures may be beyond that which can be achieved by plasticity alone (Stillman, 2003; Pörtner et al., 2006).

In overall conclusion, there is no reason to doubt the view of Meglitsch that insects are very successful animals and, as a taxon, will survive through the physiological challenges of a warming climate; and although their diversity is their strength, this review has highlighted many situations and species for which a warming climate may not be beneficial (Meglitsch, 1972). Interestingly, whilst the extent of warming may be greatest at high latitudes and least in tropical areas, it is within these two zones that the impacts of higher temperatures may be most profound – the loss of snow cover exposing insects to a range of potentially lethal effects, and the high temperatures of the tropics increasing beyond the limits that can be overcome by phenotypic plasticity, and perhaps for some species, through natural selection and evolution.

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