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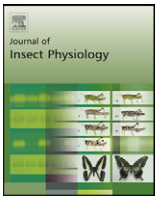

Hyperthermic aphids: Insights into behaviour and mortality

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ABSTRACT

Although the impact of warming on winter limitation of aphid populations is reasonably well understood, the impacts of hot summers and heat wave events are less clear. In this study, we address this question through a detailed analysis of the thermal ecology of three closely related aphid species: *Myzus persicae*, a widespread, polyphagous temperate zone pest, *Myzus polaris*, an arctic aphid potentially threatened by climate warming, and, *Myzus ornatus*, a glasshouse pest that may benefit from warming. The upper lethal limits (ULT_{50}) and heat coma temperatures of the aphid species reared at both 15 and 20 °C did not differ significantly, suggesting that heat coma is a reliable indicator of fatal heat stress. Heat coma and CT_{max} were also measured after aphids were reared at 10 and 25 °C for one and three generations. The extent of the acclimation response was not influenced by the number of generations. Acclimation increased CT_{max} with rearing temperature for all species. The acclimation temperature also influenced heat coma; this relationship was linear for *M. ornatus* and *M. polaris* but non-linear for *M. persicae* (increased tolerance at 10 and 25 °C). Bacteria known generically as secondary symbionts can promote thermal tolerance of aphids, but they were not detected in the aphids studied here. Assays of optimum development temperature were also performed for each species. All data indicate that *M. persicae* has the greatest tolerance of high temperatures.

1. Introduction

Climate warming is predicted to benefit many agricultural pest insects. The relaxation of low temperature limitation of overwintering survival, the beneficial effects of warmer springs in terms of rapid individual and population development, and the potential for additional generations per year, are all considered likely to lead to increases in pest population sizes (Cannon, 1998; Bale et al., 2002; Diffenbaugh et al., 2008, but see Adler et al., 2007). Furthermore, the poleward march of many species and the survival of invasive species at previously inhospitable latitudes provide clear evidence that many pest (and non-pest) species are expanding their ranges in response to climate warming (Cannon, 1998; Parmesan, 1996; Thomas and Lennon, 1999; Walther et al., 2002; Ward and Masters, 2007; Diffenbaugh et al., 2008). These observations are consistent with the dominant paradigm that lower temperature limits have a greater influence on species' distributions than upper limits (Addo-Bediako et al., 2000; Chown, 2001).

However, few studies have considered the potential effects of extreme summer temperatures. Models of future change suggest that extreme heat events including '*hot days, hot nights, and heat waves*' will increase in frequency (IPCC, 2007). Meta-analyses of insect thermal tolerance show that lower lethal temperatures (LLT) and latitude show a significant negative relationship (Addo-Bediako et al., 2000). Thus, warming may result in a steady poleward shift of species' climate envelopes (albeit not necessarily of suitable habitats) (Parmesan, 1996; Thomas and Lennon, 1999; Walther et al., 2002). By contrast, no relationship is observed between upper lethal temperatures and latitude (Addo-Bediako et al., 2000), which suggests that the likely effects of the increasing incidence of heat waves on geographic distribution and mortality will be less predictable. Nevertheless, many aphid species (particularly those which feed on herbaceous plants) are vulnerable to exposure to potentially lethal high temperatures at the soil surface (Hodjat and Bishop, 1978; Roitberg and Myers, 1979; Hodgson, 1991) and to a lesser extent, whilst feeding on leaves (Salvucci et al., 2000; Böcher and Nachman, 2001). The risk of exposure is exacerbated by rapid population growth, which imposes a two-fold burden of enhanced competition and predation (Dixon, 1998). Both problems initiate local migration by individual aphids as they walk or drop from the host plant in response to

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natural enemies (Roitberg and Myers, 1979; Dill et al., 1990; Nelson et al., 2004), or cues associated with crowding and poor quality hosts (Hodjat and Bishop, 1978; Hodgson, 1991). Therefore, research to elucidate the dynamic between high temperatures and the activity and survival of aphids is of critical importance when considering the impacts of global warming.

In this study, we analyze the relationships between thermal tolerance traits, habitat, and rearing temperature for three species of aphid from the genus *Myzus* in order to develop predictions as to the effects of high temperature events on aphid populations. *Myzus persicae* (Sulzer) is an agricultural pest throughout the temperate zone and may therefore be most likely to experience extreme high temperatures. *Myzus polaris* (Hille Ris Lamber) is restricted to dry, sparsely vegetated sites in Greenland and northern Canada. The microclimate at such sites is highly variable (Böcher and Nachman, 2001); however, predator pressure, and the probability of populations growing large enough to compete may be lower at high latitude. Thus, *M. polaris* may embark on fewer small scale migrations and in consequence, will experience fewer high temperature exposures than *M. persicae*. Little is known of the origins of *Myzus ornatus* (Laing); this species is rarely common on field crops and has a broadly sub-tropical distribution, over wintering in the temperate zone in glasshouses and on pot plants (Blackman and Eastop, 1984).

Upper thermal tolerance tends to be measured either as the upper lethal temperature (ULT_{50}), or the critical thermal maximum ($\mathrm{CT}_{\max}$) (Lutterschmidt and Hutchison, 1997; Beitinger et al., 2000). A wide variety of methods are used to measure ULT ranging from direct plunge methods to dynamic methods employing both stepped and humped thermal profiles (Lutterschmidt and Hutchison, 1997; Beitinger et al., 2000). Regardless of the exposure method, ULT is always estimated by analysis of mortality recorded hours, or more often days, post-exposure. Techniques for measuring $\mathrm{CT}_{\max}$ always involve gradual heating (Lutterschmidt and Hutchison, 1997; Beitinger et al., 2000). Observations are then made constantly or at regular time/temperature intervals and the point at which each individual reaches $\mathrm{CT}_{\max}$ is inferred with reference to their behaviour. The actual recorded behaviours frequently vary between studies but most conform to the original definition of $\mathrm{CT}_{\max}$ as 'the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death' (Cowles and Bogert, 1944). Estimates of both ULT (when dynamic methods are utilised) and $\mathrm{CT}_{\max}$ depend on the heating rate (Jumbam et al., 2008), while ULT is also strongly influenced by the length of the hold period (Beckett et al., 1998).

The aim of this study is to investigate the response of aphid species to high temperature, and to elucidate the relationship between $\mathrm{CT}_{\max}$ and ULT_{50} (defined here as temperature and not a time x temperature interaction) in aphids by measuring three separate traits, $\mathrm{CT}_{\max}$, ULT_{50} (using a short hold time to minimise time × temperature interactions), and heat coma (defined as the high temperature at which all movement ceases). If mortality is associated with synaptic dysfunction (Klose and Robertson, 2004), then the temperatures at which neuromuscular transmission fails (heat coma) and the temperatures at which the insects die will be closely related. We also consider the relationship between rearing temperature, heat coma, and $\mathrm{CT}_{\max}$. It has been suggested that a trade-off between maximum levels of thermal tolerance and the extent of plasticity of tolerance traits may influence the limits of species distributions (Stillman, 2003; Pörtner et al., 2006). Thus, knowledge of the extent of plasticity in these traits can form the basis of hypotheses concerning the likely future distribution of each species. For example, the distributions of species with limited ability to respond to increased temperatures are likely to be reduced under climate change (Stillman, 2003; Calosi et al., 2008).

Recent work has implied a possible role of bacteria known generically as primary symbionts and secondary symbionts in determining survival of pea aphids, *Acrythosiphon pisum* exposed to high temperature (Russell and Moran, 2006; Dunbar et al., 2007). The thermal tolerance of the primary symbiont common to all aphid species, *Buchnera aphidicola* (Munson et al., 1991a,b) is determined in part by a point mutation affecting expression of a small heat shock protein (Dunbar et al., 2007). This mutation twice became fixed in laboratory reared pea aphid populations within 5 years and is present in field populations of pea aphid (Dunbar et al., 2007). The secondary symbionts *Serratia symbiotica* (a.k.a. PASS, R-type) and *Hamiltonella defensa* (a.k.a. PABS, T-type) have also been shown to increase tolerance of high temperatures (Chen et al., 2000; Montllor et al., 2002; Russell and Moran, 2006) while *Regiella insecticola* (a.k.a. PAUS, U-type) reduces tolerance (Russell and Moran, 2006). The extent to which such interactions may determine thermal tolerance in other aphid species is unknown and requires further investigation.

Here we report the results of assays to identify the secondary symbiont faunas, the optimum temperature for development, the ULT_{50}s of aphids reared at constant 15 and 20 °C, the $\mathrm{CT}_{\max}$ and heat coma temperatures and the responses of these traits to both short-term single generation and longer-term three generation acclimation at high (25 °C) and low (10 °C) rearing temperatures for three species of aphid from the genus *Myzus*. We address the following questions: (1) Do secondary symbionts differ between species, and if so, could this variation affect thermal tolerance? (2) How are ULT, heat coma, and $\mathrm{CT}_{\max}$ related to one another, and is the pattern similar across species? (3) Does intergenerational acclimation allow thermal tolerance to increase greatly beyond the response available through single generation acclimation, and if so, how far? (4) Does rearing temperature affect $\mathrm{CT}_{\max}$ or heat coma temperature, and if so, do species show similar rearing temperature x threshold response curves?

2. Materials and methods

2.1. Insect material

Myzus polaris were collected from *Cerastium alpinum* in the area around Kangerlussusuaq, Greenland (67°01′N) during June 2007. Fundatrices and their offspring were returned to the U.K. and established on stems of *C. alpinum* (4–8 cm in length) secured in Blackman boxes (Blackman, 1971) and maintained at 15 °C 24 h light.

Four clones of *M. persicae* were used in most experiments. One clone (HRI) was collected from the field from *Brassica oleracea* (see Hazell et al., 2008 for details), while the remainder (clones T1V, 4255A, and 4106A (a.k.a. Braveheart, type J)) were obtained from (Rothamsted Research (Hertfordshire, U.K.)), and were originally collected from *Beta vulgaris*, *Brassica napus*, and *Solanum tuberosum*, respectively. Clones HRI, T1V, and 4255A were collected from central England and clone 4106A from southern Scotland. For simplicity, in this study clone 4106A is referred to as *M. persicae* 1, HRI as *M. persicae* 2, 4255A as *M. persicae* 3, and T1V as *M. persicae* 4. Two additional *M. persicae* clones (LA (Almeria, S. Spain) and 2922B1 (S.F., Rothamstead)) were included in the secondary symbiont assay. All *M. persicae* were maintained on excised Chinese cabbage (*Brassica rapa* var. Wingbok) leaves in Blackman boxes at a 15 °C 16:8 L:D regime.

Myzus ornatus were collected from *Fuchsia* sp. at the University of Melbourne greenhouses, Melbourne, Australia (37°47′S) during June 2007 (University of Melbourne). They were sent to the U.K. where a single monoclonal lineage was established. This lineage, from here on in referred to simply as *M. ornatus* was maintained on