

Warming decreases thermal heterogeneity of leaf surfaces: implications for behavioural thermoregulation by arthropods

Robin Caillon^{*1}, Christelle Suppo¹, Jérôme Casas¹, H. Arthur Woods² and Sylvain Pincebourde¹

¹Institut de Recherche sur la Biologie de l'Insecte (IRBI CNRS UMR 7261), Université François Rabelais, Faculté des Sciences et Techniques, 37200 Tours, France; and ²Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA

Summary

1. Ectotherms rely heavily on the spatial variance of environmental conditions to thermoregulate. Theoretically, their fitness is maximized when they can find suitable microhabitats by moving over short distances – this condition is met when spatial variance is high at fine spatial scales. Strikingly, despite the diversity of organisms living in leaf microhabitats, little is known about the impact of warming on the spatial variance of climatic conditions at the scale of individual leaf surfaces.

2. Here, we used experimental manipulation of ambient conditions to quantify the effects of environmental change on the thermal heterogeneity within individual leaf surfaces. We also explored the implications for behavioural thermoregulation by arthropods at a single leaf surface.

3. Using thermography, we characterized the apple leaf microclimate in terms of span and spatial aggregation of surface temperatures across a range of air temperatures and relative humidities. Then, we assessed how thermal heterogeneity within individual leaves affected behavioural thermoregulation by the two-spotted spider mite (*Tetranychus urticae* Koch) under both near-optimal and sublethal conditions in this microhabitat. We measured the upper lethal temperature threshold of the mite to define sublethal exposure.

4. Thermal heterogeneity of individual leaves was driven mainly by ambient air temperature. Higher air temperatures gave both smaller ranges and higher spatial aggregation of temperatures at the leaf surface, such that the leaf microclimate was homogenized.

5. *Tetranychus urticae* used behavioural thermoregulation at moderate air temperature, when thermal heterogeneity was high at the leaf surface. At higher air temperature, however, heterogeneity declined and spider mites did not perform behavioural thermoregulation.

6. Warming decreases thermal heterogeneity of leaf surfaces with critical implications for arthropods – behavioural thermoregulation alone is not sufficient to escape the heat in the leaf microhabitat. Information on spatial variance of microclimatic conditions is critical for estimating how readily organisms can buffer global warming by moving.

Key-words: aggregation index, climate change, ectotherms, lethal temperature, microclimate, microhabitat, spatial variance, temperature, thermal stress, thermography

Introduction

A common approach in global change biology is to relate the average change of a climate signal to biological processes (Whittaker 2001). However, focusing on mean effects can be unrealistic because the variance, in time or space,

can also be ecologically meaningful. The role of temporal variance has mostly been assessed by studying the ecological impacts of the occurrence of extreme events (Easterling *et al.* 2000; Denny *et al.* 2009; Pincebourde *et al.* 2012). For example, a change in temporal variance was shown to reverse the effect of the mean change in some cases (Benedetti-Cecchi *et al.* 2006). By contrast to the temporal variance and despite its impact on the thermoregulation

*Correspondence author. E-mail: robincaillon@gmail.com

opportunities of ectotherms (Kearney, Shine & Porter 2009), the role of spatial variance (i.e. spatial heterogeneity) has been largely disregarded in global change biology until recently (Scherrer & Korner 2010; Sears, Raskin & Angilletta 2011; Buckley, Miller & Kingsolver 2013; Scheffers *et al.* 2013). Moreover, the link between microclimate heterogeneity and its impacts on ectotherms has been traditionally studied at scales above metres (see Angilletta 2009). Climate change is expected to modify the spatial heterogeneity of microhabitats – that is, the spatial arrangement of temperature patches – because of nonlinear relationships between climate variables and performance of organisms (Kearney 2013). Strikingly, little is known on the impact of environmental changes on the spatial heterogeneity of temperatures at very fine scales such as individual leaf surfaces despite the great diversity of organisms living in the leaf microhabitat (Pincebourde & Woods 2012).

Ectotherms directly depend on spatial heterogeneity of temperatures because they need to perform well by choosing temperatures that give high rates of development and to escape lethal temperatures (Kearney, Shine & Porter 2009). They have evolved several thermoregulatory mechanisms to cope with temperature heterogeneity (Chown & Nicolson 2004; Angilletta 2009). Although endothermic strategies exist (Krogh & Zeuthen 1941; Lahondère & Lazzari 2012), most arthropods rely on an external heat source to control their body temperature (May 1979). This control often involves behaviour – moving rapidly over short distances or among different microhabitats to approach optimal temperatures for performance. To this end, arthropods must be able to sense and respond to local variation in temperatures – such responses can be quicker when the variation occurs at finer scales. In addition, the level of heterogeneity must be sufficient to enable arthropods to find suitable temperatures within local scales. For instance, flat-rock spiders can select precisely suitable microhabitats for egg development that are only a few centimetres apart from unsuitable locations underneath hot rocks in Australian temperate habitats during summer (Pike, Webb & Shine 2012). A particular case of behavioural thermoregulation is thermal avoidance where organisms stay away from extreme temperatures in their environment (Robertson, Kuhnert & Dawson 1996; Li & Wang 2005). Overall, behavioural thermoregulation must be considered to assess the impact of macroclimate and climate change on organisms (Kearney, Shine & Porter 2009).

Here, we focused on the ability of spider mites to thermoregulate within the leaf microclimate. Leaves are particularly suitable habitats for studying behavioural thermoregulation, because they impose finite extents over which arthropods movements can easily be studied and because single leaf surfaces can show substantial thermal heterogeneity (Jones 1999). This spatial heterogeneity is generated through two biophysical processes underlying heat exchanges between the plant and the environment (Jones 1992; Campbell & Norman 1998; Pincebourde &

Woods 2012): (i) the boundary layer, which is the air layer at the interface between the leaf and nearby free-stream air and (ii) the stomatal patchiness, which is the spatial and temporal heterogeneity of the stomatal conductance over a single leaf surface (Jones 1999; Mott & Buckley 2000). The thickness of the boundary layer can reach 10 mm depending on wind speed, on the size and shape of the leaf and on the density of trichomes at its surface (Burrage 1971; Campbell & Norman 1998). Stomatal patchiness modifies local conditions within the boundary layer on the lower side of a leaf, where stomata are located in most dicotyledonous plants. Small arthropods such as spider mites or insect eggs (<1 mm) are directly influenced by the temperature deviations between leaf surface temperature and air temperature (Potter, Davidowitz & Woods 2009). However, it remains unclear whether small arthropods can exploit the thermal heterogeneity of the leaf microclimate to perform behavioural thermoregulation within a single leaf surface (Woods 2013).

Leaf microclimates have mostly been characterized in terms of mean temperatures without accounting for thermal heterogeneity (Pincebourde & Woods 2012). For instance, the mean leaf temperature excess (i.e. temperature deviation between leaf surface and ambient air) can go up to 10 °C (Cook, Dixon & Leopold 1964; Pincebourde *et al.* 2007) or even 20 °C in some alpine plants in full sunlight (Linacre 1967). Thermal heterogeneity of intra-leaf surfaces has been quantified in fewer studies. Tomato leaves had a 1 °C range of temperature over a single leaf surface (Cook, Dixon & Leopold 1964), while French bean and soybean leaves had ranges up to 4 °C (Jones 1999; Aldea *et al.* 2005). Nevertheless, these studies report levels of thermal heterogeneity only under partially controlled environmental conditions (i.e. in greenhouses). Fully controlled experimental conditions would allow a more precise assessment of the thermal heterogeneity of leaf surfaces. In particular, its response to an increase in ambient temperature is not known. Consequently, the potential effects of global warming on the behavioural thermoregulation abilities of leaf-dwelling arthropods remain largely unexplored (Pincebourde & Woods 2012).

To understand whether and how spider mites thermoregulate, we must first characterize the spatial heterogeneity of temperatures at the leaf surface. Therefore, we used experimental manipulation of ambient conditions to compare the thermal heterogeneity of individual leaves under moderate and harsh ambient temperature conditions. We studied the leaf surface at a high spatial resolution using thermography to characterize temperature heterogeneity in terms of composition (i.e. range of temperature values) and configuration (i.e. spatial pattern of the temperature values). Then, we explored the potential effects of intra-leaf heterogeneity on the behavioural thermoregulation abilities of spider mites under moderate and harsh ambient temperature conditions. We measured *T. urticae*'s lethal temperature threshold to ensure that sublethal thermal conditions were applied during this second experiment.

Materials and methods

BIOLOGICAL MODEL

Leaf surface temperature measurements were made on the Golden cultivar of the apple tree *Malus domestica* (Rosaceae). Apple seedlings (<3 years old) were grown in pots (15 cm in diameter) in a greenhouse with variable meteorological conditions, with air temperature ranging from 14.5 to 42.5 °C and air relative humidity from 29.5% to 95% during the study period (minima and maxima). Seedlings were watered generously about every 2–3 days according to conditions, and each pot received nitrogen-enriched fertilizer (N-P-K: 5-3-7). Local populations of the two-spotted spider mite (Fig. 1) *Tetranychus urticae* (Acarid: Tetranychidae) were sampled around Tours (France) in early spring of 2011. Spider mites were then reared directly on seedlings in the greenhouse. Both last-instar larvae and adults were used for the experiments, which were carried out in the morning when stomata open rapidly in response to blue-enriched light in nature (Zeiger *et al.* 2002; Shimazaki *et al.* 2007; Wang, Noguchi & Terashima 2011).

EXPERIMENT 1 – LEAF MICROCLIMATE CHARACTERIZATION

The aim was to quantify the spatial heterogeneity of leaf surface temperatures under different environmental conditions. We determined the impact of shifting air temperature and relative humidity on the leaf surface temperatures. The temperature heterogeneity of the leaf surface was measured focusing on two components: the

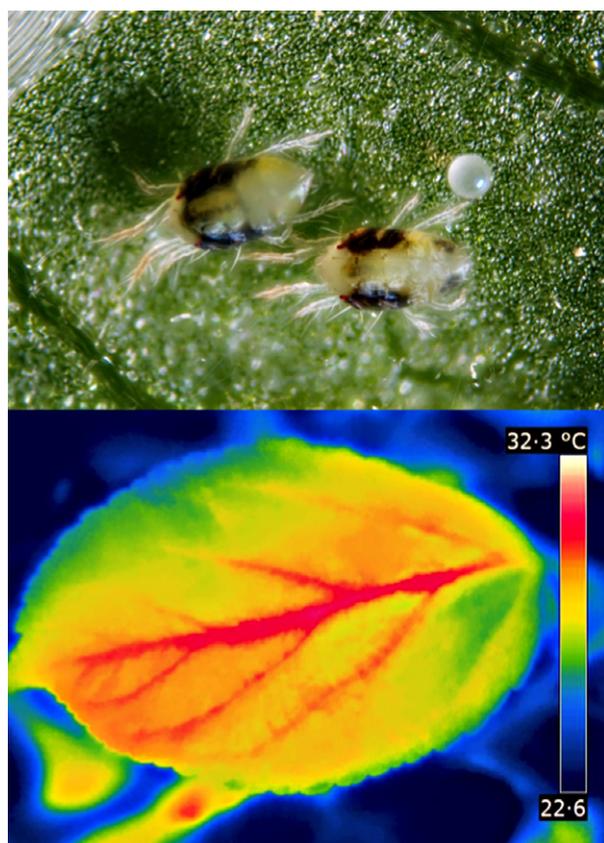


Fig. 1. Primary organism, *T. urticae* (top, photo credit: Gilles San Martin) and infrared image of an apple leaf surface taken in the field under full sunlight (bottom, photo credit: Sylvain Pincebourde).

composition, which refers to the range of temperature values, and the configuration, which refers to their spatial pattern. Apple trees were placed in a climatic chamber 24 h before the experiment, with a photoperiod of 14:10 and temperatures of 25:18 °C (day: night). This acclimation period was set to homogenize physiological states of apple seedlings. The climatic chamber (VB 1014-A; Vötsch, Balingen Frommern, Germany) allowed for precise control of temperature and relative humidity with limited, constant and spatially homogeneous air flows (0.3 m·s⁻¹). Three air temperature treatments (25, 30 and 39 °C) were crossed with three air relative humidity treatments (30%, 60% and 90%), resulting in nine different treatments with three replicates each. This spans a wide range of conditions that spider mites may encounter in their natural environments. For each replicate, the leaf surface was positioned flat, right below the lamp (hydrargyrum medium-arc iodide lamp, HSI-T SX 400W, Sylvania Britelux). Incident radiation received by all replicates was in the range of 220–240 Wm⁻².

The measurement of leaf surface temperature started 15 min after the target air temperature was reached in the climatic chamber. Leaf surface temperatures were measured with a thermal imaging device (FLIR Systems, B335, Wilsonville, OR, USA) equipped with a macro lens (IR LENS 10 mm; FLIR Systems) 1 h after the experiment started, giving a thermal image with a temperature value for each pixel decomposing the leaf surface (Fig. 1). The camera's lens was positioned 20 cm from the leaf surface, resulting in a spatial resolution of 0.49 ± 0.11 mm and a thermal resolution of 0.05 °C. This resolution approximates the body sizes of adult spider mites (0.3–0.5 mm; Kreiter 2003). The emissivity was set at 0.99 as determined previously by comparing the temperature reading on the camera with the actual leaf surface temperature measured using a type-T thermocouple (copper-constantan) at the very same location.

EXPERIMENT 2 – LETHAL TEMPERATURE THRESHOLD OF *T. URTICAE*

The lethal temperature threshold of *T. urticae* was measured for comparison with leaf surface temperature values. For each temperature treatment, a group of 10 spider mites was exposed to a single air temperature treatment during 1 h in the climatic chamber. We tested exposure to temperature from 40 °C (for group one) to 47 °C (for group nine). The experimental duration (1 h) was in the range of exposure to daily maximal temperature for small arthropods on leaf surfaces in the field (Pincebourde *et al.* 2007).

Prior to the experiment, spider mites were in the greenhouse, where they were submitted to daily natural variations of environmental conditions. Single spider mites were put in an Eppendorf tube with wet cotton to ensure high and constant relative humidity and placed in the climatic chamber once the target air temperature was reached. A fine thermocouple was inserted in one Eppendorf tube to measure actual temperatures. Temperature was assumed to be the same in each Eppendorf tube. After the treatment, spider mites were removed from the chamber and mortality was measured 24 h later. The lethal temperature threshold is the temperature at which 50% mortality is observed (lethal dose 50%; LD₅₀).

EXPERIMENT 3 – DISTRIBUTION OF *T. URTICAE* ON THE LEAF SURFACE

The characterization of the leaf microclimate (Experiment 1) identified the air temperature needed to reach sublethal temperatures for spider mites at the leaf surface, based on the lethal temperature threshold measurement (Experiment 2). The aim of Experiment 3 was to measure the impact of different climatic conditions on the distribution of spider mites at the leaf surface according to the thermal heterogeneity of the leaf surface. Like in Experiment 1, apple

seedlings were conditioned 24 h before the experiment. Two air temperature treatments of 25 °C (moderate condition) and 37 °C (harsh condition) were applied, with six replicates for each (six leaves from six different plants). Relative humidity was fixed at 60% at both temperatures. For each replicate, the leaf surface was placed in a horizontal position right below the lamp. Incident radiation received by all replicates was in the range of 220–240 W·m⁻².

For each replicate, an apple seedling was exposed alone to the experimental temperature in the climatic chamber for 15 min. Then, 20 spider mites were put on the lower surface of a leaf and given 15 min to acclimate. After that, the experiment started and the distributions of both spider mites and leaf surface temperatures were recorded simultaneously for 1 h (total time was 90 min). A video camera (HDC-SD200; Panasonic Kadoma, Osaka, Japan) was used to follow spider mites at the lower leaf surface. The same thermal imaging device described above for Experiment 1 (FLIR Systems, B335) was used to measure upper leaf surface temperatures at the beginning and end of experiment to compare thermoregulation opportunities. The thermal conductivity between the upper and lower leaf surfaces, and the thinness of the integument, are such that surface temperature is considered equal on both sides (Jones 1999). The body temperature of a spider mite was assumed to be equal to the leaf surface temperature since they were indistinguishable on infrared images and since they are deeply immersed within the leaf boundary layer due to their small size (Ferro & Southwick 1984). We acknowledge, however, that the humidity within the leaf boundary layer may differ between the two leaf sides since the apple leaf is hypostomatous – stomata are present only on the lower side.

STATISTICAL ANALYSIS

Data on leaf surface temperatures were extracted from raw images with ThermoCam Researcher software (FLIR Systems). In terms of temperature range, descriptive statistics such as mean, maximal and minimal temperatures at the leaf surface were used. These descriptive statistics were calculated on the temperature excess between leaf surface pixels and the ambient air. To assess a per area range of temperature, the Temperature Range Index (TRI) was calculated on the pixel temperature values of the leaf surface with Fragstats software (University of Massachusetts, Landscape Ecology Lab). This index is based on the patch richness density that is widely used in landscape ecology and which allows the comparison among landscapes (i.e. leaves) of different sizes (McGarigal & Marks 1994). It is close to 0 when the range of temperature is very low and increases with it. In terms of spatial pattern, the Aggregation Index (AI), also used in landscape ecology, was computed to assess the extent to which elements of the same class (i.e. temperature pixels of the same value) are aggregated or clumped in a landscape (i.e. at the leaf surface) (He, DeZonia & Mladenoff 2000). Expressed as a percentage of maximal aggregation, AI goes from 0 when the landscape is minimally aggregated to 100 when it is maximally aggregated (i.e. a single compact patch for each temperature value). A multifactorial analysis of variance (two-way ANOVA) and *post hoc* Tukey tests were performed with the R package 'stats' (R Development Core Team 2013) to determine which descriptors (mean, minimum, maximum, TRI, AI) were influenced by environmental conditions.

The upper lethal temperature threshold of *T. urticae* was estimated by nonlinear regression of survival rate against air temperature (Fig. S1, Supporting information). A logistic dose-response regression was performed in Table Curve (Systat Software Inc., Chicago, Illinois, USA) to estimate the lethal temperature threshold leading to 50% of individuals to die (LD₅₀).

In Experiment 3, we studied the behavioural thermoregulation abilities of spider mites. Leaf surface temperature distribution involved up to 9000 observations because of the spatial resolution of the infrared camera. In contrast, the body temperature distribu-

tion of spider mites corresponded to no more than 80 observations for each temperature treatment (25 and 37 °C). Thus, a direct comparison of the two distributions is not reasonable. The null hypothesis is that spider mites do not thermoregulate. Furthermore, by randomly distributing themselves at the leaf surface, they experience the leaf temperature distribution. Therefore, the most probable body temperature value corresponds to the most represented temperature value at the leaf surface: the mode of the frequency distribution of leaf surface temperatures. In contrast, we would observe a deviation between body temperature of spider mites and the mode of the leaf surface temperature when they actively thermoregulate. To determine whether spider mites were randomly located on the leaf surface, we expressed their body temperature in relation to the mode of the frequency distribution of leaf surface temperatures. Body temperature was extracted from the thermal images according to mite position at the leaf surface for both air temperature treatments (25 and 37 °C) (Fig. S2, Supporting information). The temperature deviation between surface temperature at the spider mite location and the mode of the leaf surface temperature was calculated. A deviation of their body temperature from the mode of the leaf surface temperature is indicative of preferred temperature at the leaf surface and thus of behavioural thermoregulation. A multiple regression analysis was performed to assess the relationship between body temperature deviation and the mode of the leaf surface temperature at the first and last time of the experiment for each air temperature treatment. Then, each model was improved keeping only those variables showing a significant effect. Leverage was checked to identify over-influential observations ($h > 3h$; with h the leverage of a given observation, and h the mean leverage). The regression analysis was performed again after removing over-influential data points to check for robustness of the statistical model.

Results

EXPERIMENT 1 – LEAF MICROCLIMATE CHARACTERIZATION

In terms of composition, air temperature and air relative humidity had opposing effects on the leaf temperature excess. The mean leaf temperature excess – the mean leaf surface temperature minus the ambient air temperature – varied inversely with air temperature (ANOVA, temperature: $F_{2,18} = 27.11$, $P < 0.001$) (Fig. 2). When the air relative humidity was 30%, for example, the mean leaf temperature excess decreased from 6.9 to 5.2 °C as the ambient temperature rose from 25 to 39 °C. The effect of air relative humidity on the mean leaf temperature excess was significant (ANOVA, relative humidity: $F_{2,18} = 4.48$, $P = 0.027$) and was most pronounced at an intermediate temperature of 30 °C, as indicated by a significant interaction between air relative humidity and temperature (ANOVA, temperature*relative humidity: $F_{4,18} = 3.39$, $P = 0.031$). Minimal and maximal leaf temperature excesses – the minimal and maximal leaf surface temperature minus the ambient air temperature – were calculated for a more accurate understanding of the microclimatic response. The minimal leaf temperature excess was significantly affected by air relative humidity (ANOVA, relative humidity: $F_{2,18} = 16.40$, $P < 0.001$) and temperature (ANOVA, temperature: $F_{2,18} = 3.67$, $P = 0.046$) (Fig. 3). The minimal leaf temperature excess was the lowest at an air relative humidity of

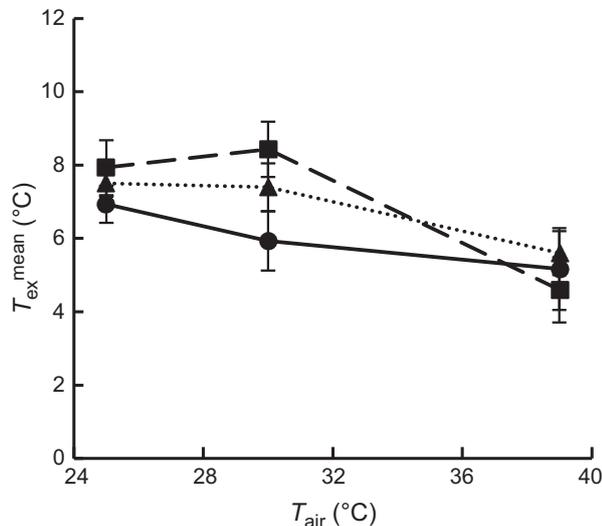


Fig. 2. Temperature excess between mean leaf surface temperature and ambient air ($T_{\text{ex}}^{\text{mean}}$) according to air temperature and air relative humidity conditions (line and circles: 30%, dashed line and squares: 60% and dotted line and triangles: 90%).

30% and decreased with increasing air temperature (ANOVA, temperature: $F_{2,18} = 3.67$, $P = 0.046$) (Fig. 3). The interaction between air relative humidity and temperature was not significant for the minimal leaf temperature excess (ANOVA, temperature*relative humidity: $F_{4,18} = 2.18$, $P = 0.112$). The maximal leaf temperature excess showed no significant relationship with air relative humidity (ANOVA, relative humidity: $F_{2,18} = 3.08$, $P = 0.071$), but varied inversely with air temperature (ANOVA, temperature: $F_{2,18} = 36.81$, $P < 0.001$). The interaction between air rela-

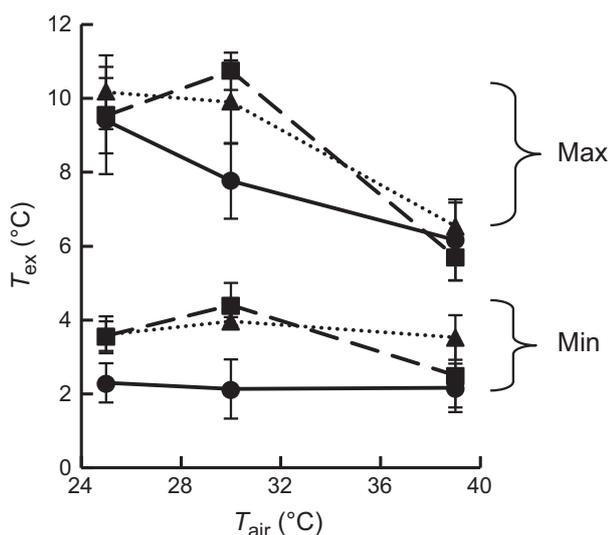


Fig. 3. Temperature excess (T_{ex}) between maximal (top) and minimal (bottom) leaf surface temperature and ambient air according to air temperature and air relative humidity conditions (line and circles: 30%, dashed line and squares: 60% and dotted line and triangles: 90%).

tive humidity and temperature was not significant for the maximal leaf temperature excess (ANOVA, temperature*relative humidity: $F_{4,18} = 2.59$, $P = 0.071$). In addition, TRI showed no significant relationship with air relative humidity (ANOVA, relative humidity: $F_{2,18} = 2.77$, $P = 0.089$) but was inversely related to air temperature (ANOVA, temperature: $F_{2,18} = 14.35$, $P < 0.001$) (Fig. 4). The interaction between air relative humidity and temperature was not significant for TRI (ANOVA, temperature*relative humidity: $F_{4,18} = 1.35$, $P = 0.291$). Therefore, the range of temperatures available at the leaf surface responds mostly to air temperature and warming led to a decrease of this range.

In terms of configuration, a similar pattern to the one of TRI was found for the spatial distribution of leaf surface temperatures (Fig. 5). AI increased with air temperature (ANOVA, temperature: $F_{2,18} = 20.80$, $P < 0.001$). It ranged from 61.8% to 72.6% at an air temperature of 25 and 39 °C, respectively. Air relative humidity and the interaction between air temperature and relative humidity had no detectable effect (ANOVA, relative humidity: $F_{2,18} = 0.43$, $P = 0.656$; ANOVA, temperature*relative humidity: $F_{4,18} = 1.05$, $P = 0.410$). Therefore, the spatial pattern of temperatures at the leaf surface responds mostly to air temperature and warming led to more aggregated leaf surface temperatures.

EXPERIMENT 2 – UPPER LETHAL TEMPERATURE THRESHOLD FOR *T. URTICAE*

The logistic dose-response regression showed a drop in the survival rate of spider mites between 45.5 and 46 °C, from 70% to 0% (logistic regression: $r^2 = 0.93$; $F_{2,6} = 62.5$; $P < 0.001$) (Fig. S1). The nonlinear regression estimated the LD₅₀ at 45.9 °C.

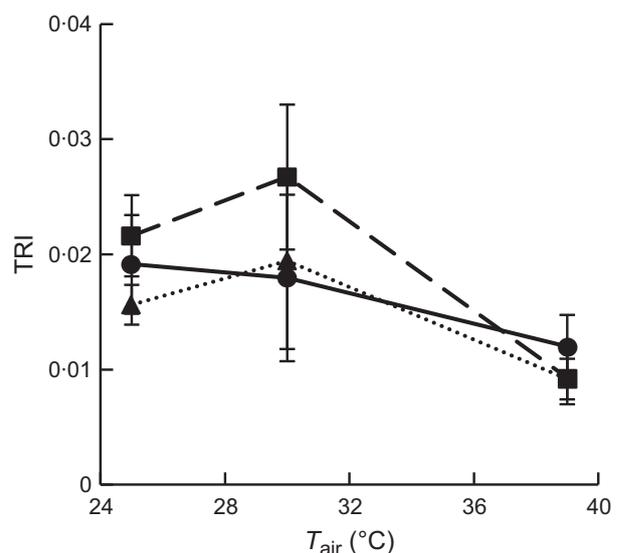


Fig. 4. Temperature Range Index (TRI) according to air temperature and air relative humidity conditions (line and circles: 30%, dashed line and squares: 60% and dotted line and triangles: 90%).

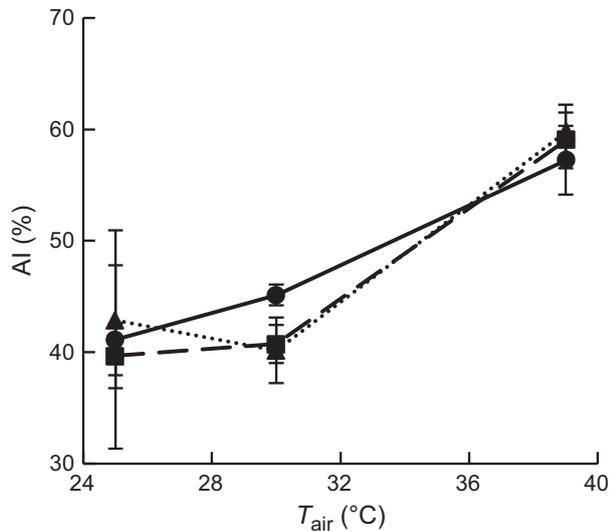


Fig. 5. Aggregation Index (AI) according to air temperature and air relative humidity conditions (line and circles: 30%, dashed line and squares: 60% and dotted line and triangles: 90%).

EXPERIMENT 3 – THERMOREGULATION BY *T. URTICAE*

On average, at the end of the 1 h experiment, 5.7 and 5.8 spider mites were found at the leaf surface at 25 and 37 °C air temperature, respectively. The rest of the 20 spider mites put on the leaf may have spun a web to drop off the leaf or may have moved to another part of the plant via the petiole. Overall, the body temperature of spider mites increased with the distribution mode of the leaf surface temperatures in the two treatments (Fig. 6). At 25 °C air temperature, the body temperature deviation varied inversely to the mode of the leaf surface temperatures (linear regression, mode: t -value = -2.85 , $P = 0.006$) and time had no significant effect (linear regression, time: t -value = -0.0281 , $P = 0.978$) (Fig. 7a). Keeping the significant variables, the improved statistical model showed that body temperature deviated from the mode of the leaf surface temperatures with an estimated parameter of -0.26 (linear regression, mode: t -value = -3 , $P = 0.004$). Leverage statistics revealed two points with high leverage (grey symbols in Fig. 7). Once those points were removed, body temperature deviated from the mode of the leaf surface temperatures with an estimated parameter of -0.29 (linear regression, mode: t -value = -2.64 , $P = 0.010$). For the experiment at 37 °C, neither the mode of the leaf surface temperatures (linear regression, mode: t -value = -0.99 , $P = 0.325$) nor time (linear regression, time: t -value = 1.49 , $P = 0.141$) showed significant effect. In the improved model, only the intercept was fitted (linear regression, intercept: t -value = -2.86 , $P = 0.006$) with a significant value of -0.25 (Fig. 7b). Leverage statistics did not reveal any over-influential data point in the treatment at 37 °C air temperature.

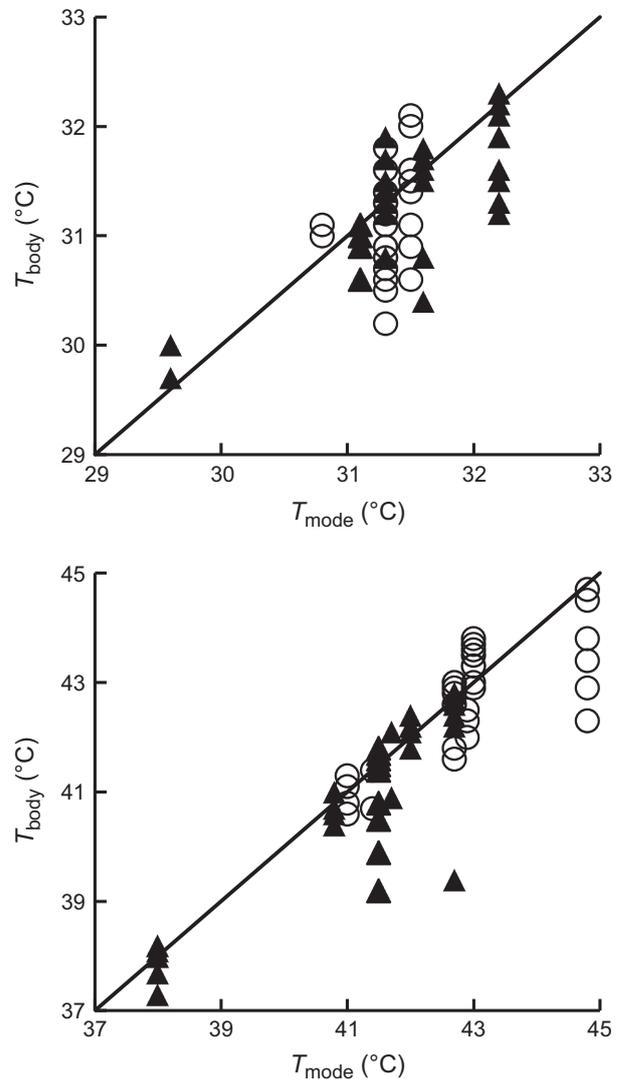


Fig. 6. Body temperature of spider mites (T_{body}) as a function of the mode of the leaf surface temperatures (T_{mode}) at 25 °C (top) and 37 °C (bottom) air temperature. Graphs show data for the first (black triangles) and last (empty circles) time of the experiment, after 1 h.

Discussion

Leaf surface temperature can be highly heterogeneous, especially in full sunlight (Jones 1999). Here, we show how environmental conditions modulate the thermal heterogeneity of individual leaves in terms of composition (temperature range) and configuration (spatial distribution of temperatures). Our study is the first to detail this relationship between environmental conditions and the level of thermal heterogeneity at a single leaf surface. Despite an impact on the leaf temperature excess, relative humidity did not affect the thermal heterogeneity of the leaf surface itself. By contrast, air temperature had large effects on leaf temperature heterogeneity. Warming both decreased the range of temperatures available at the leaf surface and increased the aggregation of similar temperature values.

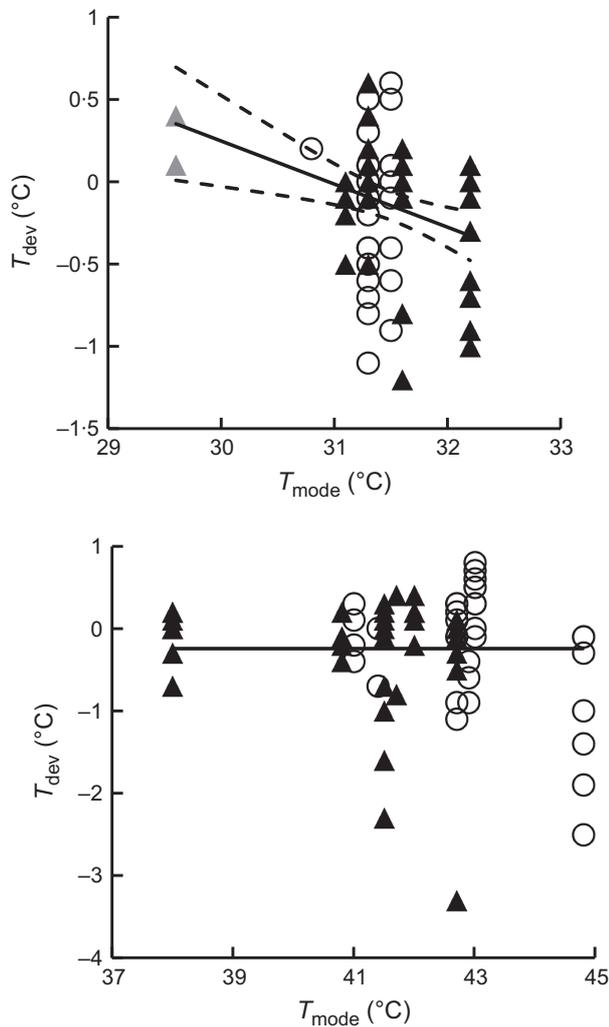


Fig. 7. Deviation of spider mite body temperature from the mode of leaf surface temperatures (T_{dev}) as a function of this mode at 25 °C (top; confidence interval in dashed lines) and 37 °C (bottom) air temperature. Graphs show data for the first (black triangles) and last (empty circles) time of the experiment, after 1 h. Observations with high leverage are identified in grey.

Together, these two effects homogenized the leaf microclimate. In addition, the leaf partially buffered the amplitude of the warming, as the maximal temperature excess was lower at higher ambient temperatures.

Leaves provide microclimates for leaf-dwelling arthropods. We explored the potential effects of the heterogeneity of leaf surface temperatures on the behaviour of spider mites. Behavioural thermoregulation should be more difficult when the leaf surface is homogeneous at high ambient temperature. Indeed, we found that spider mites do not thermoregulate under sublethal conditions, although a narrow range of colder temperatures could have been selected. The ability of organisms to avoid extreme heat would therefore depend much more on the ability of the leaf to buffer unusual climatic conditions than on their own success in avoiding sublethal temperatures, as long as they remain at the leaf surface. *Tetranychus urticae*, however, is

a web spinner, especially at high population density (Le Goff *et al.* 2010). This skill allows it to escape harsh conditions at the leaf surface by moving out onto the web. In addition, daily migration of spider mites towards upper plant parts, which is mainly driven by light stimulus, is much reduced under warm conditions (Mori 1962; Pralavorio, Fournier & Millot 1989). This suggests that spider mites would avoid fully illuminated leaves when air temperatures are high. Finally, *T. urticae* is quite resistant to high temperatures, with a lethal temperature threshold of 45.9 °C. This threshold may explain the passive behaviour of spider mites in the homogeneous matrix of leaf surface temperatures, even though this matrix contains temperatures close to the lethal threshold.

In contrast to extreme conditions, moderate temperatures gave greater scope for behavioural thermoregulation. At 25 °C air temperature, the range of temperatures at the leaf surface encompassed the optimal developmental temperature of *T. urticae*, which is around 30 °C (Bounfour & Tanigoshi 2001). On average, the body temperature of spider mites increased by 0.71 °C for each 1 °C increment in the mode of the leaf surface temperatures above this optimal temperature (Fig. 6). Therefore, spider mites buffer about 29% of the leaf surface warming by thermoregulating in moderate environmental conditions. Thus, spider mites select leaf areas with surface temperatures close to their optimum for developmental rate or other temperature-dependent life-history traits, leading potentially to high rates of population growth. We suggest that behavioural thermoregulation within single leaf surfaces could play a role in the population dynamics of small arthropods, but this link remains to be explored. Unfortunately, few studies have considered the leaf temperature as a driver of leaf-dwelling arthropod population growth (Kingsolver 1979; Pincebourde & Woods 2012; see also Bernard *et al.* 2013; for a parallel with a plant–phytopathogen interaction).

In a single canopy into the field, between-leaf heterogeneity adds up to intra-leaf heterogeneity. In fact, the range of mean apple leaf temperatures within a single plant canopy (sunny and shaded leaves differ by about 5 °C; Pincebourde *et al.* 2007) compares well with the range of temperatures over single apple leaf surfaces under moderate conditions (about 6 °C). In addition, the range of within-canopy variation in leaf temperatures varies from about 6 °C up to more than 10 °C for eight deciduous tree species in a temperate forest (Leuzinger & Körner 2007). Arthropods have evolved particular behaviours in response to such within-canopy heterogeneities, for example by moving up and down in a canopy (Willmer 1982). We show that behavioural responses also occur at the within-leaf level, although the time scale of the behaviours is likely to differ between the leaf and the canopy scale.

The leaf temperature excess clearly depends on meteorological conditions. In particular, incoming radiation raises leaf temperature above ambient temperature (Field, Chiariello & Williams 1982). However, our experimental

measurements are likely to underestimate the leaf temperature excess in natural conditions, because natural level of radiation can reach much higher values than the moderate ones we used (220–240 Wm^{-2}) (Gates 1980). With all others parameters fixed (radiative heat flux, but also relative humidity, convection and water status of the plant), we demonstrate a homogenization of temperatures at the leaf surface with warming (at 39 °C air temperature). In our study region, maximal air temperature reached 37.8 °C in 2012 and 34.9 °C in 2013 (meteorological data collected at about 30 km from our greenhouse). While the air temperature applied in our laboratory experiment is close to the extremes that mites experience in our region, radiative heat flux in nature could be more than four times higher than the one we applied. With similar air temperature conditions and such high radiative heat fluxes, heat and water stresses are likely to be important, leading to similar or even stronger reductions in both leaf mean temperature excess and temperature heterogeneity as transpiration rate is increasing and stomata are closing, respectively. The leaf temperature excess also depends on the properties of the leaf. Because of the structure of the boundary layer at the leaf surface, leaf edges are colder than central parts. The boundary layer tends to be thinner at the leaf edges, especially at the leading edge, and thicker in the middle. Hence, the resistance to heat loss is higher in central portions of a leaf, leading to higher temperatures (Oke 1978). Also, the wind regime was turbulent, causing the boundary layer to develop from all edges of the leaf. This turbulent regime explains the overall circular distribution of leaf surface temperatures from warm leaf centre to cool leaf edges (see Fig. 1).

Warming decreased the mean leaf temperature excess. The interesting feature is that the minimal temperature excess varied only slightly, while the maximal one decreased markedly with ambient warming. In other words, at high ambient temperatures, the maximal leaf surface temperatures came to resemble minimal temperature excess, which perhaps reflects actions taken by the plant to avoid or minimize thermal stress to its tissues. The different behaviour of the minimal and maximal temperature excesses in response to warming explains the decrease of the temperature range index at high air temperature. Therefore, warming leads to both a shift and a decrease of the range of temperature available at the leaf surface, thereby depressing the temperature heterogeneity. Furthermore, the aggregation index increases with ambient temperature. Warming tends to gather together temperature pixels of the same value into bigger and better-defined patches at the leaf surface. A more aggregated spatial pattern of temperatures decreases temperature heterogeneity in terms of configuration. Overall, warming constrains considerably the thermal environment within the leaf microclimate in a predictable way.

The homogenization of the leaf microclimate with warming can be caused by the response of the plant to environmental stress. Apple leaves were under near-

optimal conditions in the moderate treatment. With the radiation level we imposed, the stomatal conductance was at about 70% of its maximal value (as inferred from the light response curves of apple leaves; Massonnet 2004; Pincebourde *et al.* 2006). Under such favourable conditions, the latent heat losses from a leaf are at least as important as convective heat losses. Hence, variation in stomatal conductance over a single leaf surface should lead to significant deviations in transpiration rate and ultimately generate heterogeneity in surface temperatures, as emphasized in our results. By contrast, high air temperature exposes leaves not only to heat stress but also to water stress because of the increase of the leaf vapour pressure deficit (VPD) with air temperature (Gates 1980). Nevertheless, the relationship between leaf VPD, transpiration rate and stomatal conductance is nonlinear: At high air temperature and thus high VPD, the stomatal conductance decreases to control transpiration rate (Monteith 1995; Bunce 1996; Massonnet 2004; Pincebourde & Casas 2006) and thus limit water stress. The exponential decrease in stomatal conductance with increasing leaf VPD can only limit the increase in transpiration rate at a plateau at which leaf surface temperature becomes independent of stomatal activity (Oren *et al.* 1999). Therefore, at high leaf VPD, transpiration remains significant despite stomatal closure, leading to a decrease in leaf temperature excess. Hence, under extreme air temperature, slight spatial variations in the level of stomatal closure are expected to have little effects on the transpiration rate and therefore on the leaf surface temperatures, leading to homogenization of the leaf surface temperatures. As a result, extreme air temperatures both depress the leaf temperature excess and homogenize leaf surface temperatures.

Global warming is now recognized as a major threat to organisms and to the ecosystems they occupy. A key problem for the scientific community is to predict the consequences of global warming at the small scales at which most organisms live (Potter, Woods & Pincebourde 2013). Significant advances have been made in understanding the role of temporal variance in the response of organisms to global warming (Benedetti-Cecchi *et al.* 2006; Pincebourde *et al.* 2012). Here, for the first time, we report the impacts of environmental changes on the spatial heterogeneity of temperatures at the scale of individual leaf surfaces. We highlight the importance of analysing spatial variance in microhabitat temperatures. Spatial variance is critical for understanding the opportunities for organisms to buffer daily temperature extremes or, in a broader context, global warming. The grid used to decompose the environment must be fine enough to capture the actual spatial heterogeneity available to organisms during behavioural thermoregulation (Kearney, Shine & Porter 2009) and dispersal (Buckley, Miller & Kingsolver 2013). For leaf-dwelling arthropods, the leaf surface shows different patterns of spatial heterogeneity according to the proximate ambient environment, which itself varies from leaf to leaf within plant canopies (Sinoquet *et al.* 2001; Pincebourde *et al.*

2007). Therefore, two leaves close to each other in a canopy can present different opportunities for behavioural thermoregulation by tiny organisms. This emphasizes the difficulty of generalizing climate downscaling models because of the complexity of microhabitats. It remains to determine whether movement by organisms within such small spatial scales will significantly help them to buffer some part of the climatic changes as measured at global or regional scales.

Acknowledgements

We are grateful to Marie-Cécile Dalstein, from the experimental research station of La Morinière (Saint Epain, 37800 Tours, France), for her help in collecting spider mites in orchards and Serge Kreiter, from the centre of biology for population management, for the identification of spider mites (34988 Montferrier-sur-Lez, France). We also acknowledge Bertanne Visser and the ESMB group at IRBI for useful comments on earlier draft and Gilles San Martin for his photo of *T. urticae* (see www.flickr.com/photos/sanmartin/ for more pictures). Finally, we are grateful to Joel Kingsolver and an anonymous reviewer for constructive comments on an earlier version of the manuscript. This work was supported by ANR Blanc MicroClimMite (ANR-2010 BLAN-1706-02) and by INEE (PEPS-2010 THERMAL-GAMES).

References

- Aldea, M., Hamilton, J.G., Resti, J.P., Zangerl, A.R., Berenbaum, M.R. & DeLucia, E.H. (2005) Indirect effects of insect herbivory on leaf gas exchange in soybean. *Plant, Cell and Environment*, **28**, 402–411.
- Angilletta, M.J. (2009) *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, Oxford.
- Benedetti-Cecchi, L., Bertocci, I., Vaselli, S. & Maggi, E. (2006) Temporal variance reverses the impact of high mean intensity of stress in climate change experiments. *Ecology*, **87**, 2489–2499.
- Bernard, F., Sache, I., Suffert, F. & Chelle, M. (2013) The development of a foliar pathogen does react to leaf temperature. *New Phytologist*, **198**, 232–240.
- Bounfour, M. & Tanigoshi, L.K. (2001) Effect of temperature on development and demographic parameters of *Tetranychus urticae* and *Eotetranychus carpini borealis* (Acari: Tetranychidae). *Annals of the Entomological Society of America*, **94**, 400–404.
- Buckley, L.B., Miller, E.F. & Kingsolver, J.G. (2013) Ectotherm thermal stress and specialization across altitude and latitude. *Integrative and Comparative Biology*, **53**, 571–581.
- Bunce, J.A. (1996) Does transpiration control stomatal responses to water vapor pressure deficit? *Plant, Cell and Environment*, **19**, 131–135.
- Burrage, S.W. (1971) The microclimate at the leaf surface. *Ecology of the Leaf Surface Micro-organisms* (eds T.F. Preece & C.H. Dickinson), pp. 91–101. Academic Press, London.
- Campbell, G.S. & Norman, J.M. (1998) *An introduction to Environmental Biophysics*. Springer Verlag, New-York.
- Chown, S. & Nicolson, S. (2004) *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, Oxford.
- Cook, G.D., Dixon, J.R. & Leopold, A.C. (1964) Transpiration: its effects on plant leaf temperature. *Science*, **144**, 546–547.
- Denny, M.W., Hunt, L.J.H., Miller, L.P. & Harley, C.D.G. (2009) On the prediction of extreme ecological events. *Ecological Monographs*, **79**, 397–421.
- Easterling, D.R., Evans, J.L., Groisman, P.Ya., Karl, T.R., Kunkel, K.E. & Ambenje, P. (2000) Observed variability and trends in extreme climate events: a brief review. *Bulletin of the American Meteorological Society*, **81**, 417–425.
- Ferro, D.N. & Southwick, E.E. (1984) Microclimates of small arthropods: estimating humidity within the leaf boundary layer. *Environmental Entomology*, **13**, 926–929.
- Field, C., Chiariello, N. & Williams, W.E. (1982) Determinants of leaf temperature in *California mimulus* species at different altitudes. *Oecologia*, **55**, 414–420.
- Gates, D.M. (1980) *Biophysical Ecology*. Springer-Verlag, New-York.
- He, H.S., DeZonia, B.E. & Mladenoff, D.J. (2000) An aggregation index (AI) to quantify spatial patterns of landscapes. *Landscape Ecology*, **15**, 591–601.
- Jones, H.G. (1992) *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*. Cambridge university press, Cambridge.
- Jones, H.G. (1999) Use of Thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. *Plant, Cell and Environment*, **22**, 1043–1055.
- Kearney, M. (2013) Activity restriction and the mechanistic basis for extinctions under climate warming. *Ecology Letters*, **16**, 1470–1479.
- Kearney, M., Shine, R. & Porter, W.P. (2009) The potential for behavioural thermoregulation to buffer “cold-blooded” animals against climate warming. *Proceedings of the National Academy of Sciences USA*, **106**, 3835–3840.
- Kingsolver, J.G. (1979) Thermal and hydric aspects of environmental heterogeneity in the pitcher plant mosquito. *Ecological Monographs*, **49**, 357–376.
- Kreiter, S. (2003) *Les Acariens Phytophages des Plantes*, pp. 19–60. INRA, ENSA, Montpellier.
- Krogh, A. & Zeuthen, E. (1941) The mechanism of flight preparation in some insects. *Journal of Experimental Biology*, **18**, 1–10.
- Lahondère, C. & Lazzari, C. (2012) Mosquitoes cool down during blood feeding to avoid overheating. *Current biology*, **22**, 40–45.
- Leuzinger, S. & Körner, C. (2007) Tree species diversity affects canopy leaf temperatures in a mature temperate forest. *Agricultural and Forest Meteorology*, **146**, 29–37.
- Le Goff, G.J., Mailleux, A.-C., Detrain, C., Deneubourg, J.-L., Clotuche, G. & Hance, T. (2010) Group effect on fertility, survival and silk production in the web spinner *Tetranychus urticae* (Acari: Tetranychidae) during colony foundation. *Behaviour*, **147**, 1169–1184.
- Li, X. & Wang, L. (2005) Effect of thermal acclimation on preferred temperature, avoidance temperature and lethal thermal maximum of *Macrobatus harmsworthi* Murray (Tardigrada, Macrobiotidae). *Journal of Thermal Biology*, **30**, 443–448.
- Linacre, E.T. (1967) Further notes on a feature of leaf and air temperatures. *Theoretical and Applied Climatology*, **15**, 422–436.
- Massonnet, C. (2004) Variabilité architecturale et fonctionnelle du système aérien chez le pommier (MALUS domestica Borkh.): Comparaison de quatre cultivars par une approche de modélisation structure-fonction. PhD Thesis, Ecole Nationale Supérieure Agronomique de Montpellier.
- May, M.L. (1979) Insect thermoregulation. *Annual Review of Entomology*, **24**, 313–349.
- McGarigal, K. & Marks, B.J. (1994) *Fragstats: Spatial Pattern Analysis Program for Quantifying Landscape Structure*. Oregon state university, Forest science department, Corvallis.
- Monteith, J.L. (1995) A reinterpretation of stomatal responses to humidity. *Plant, Cell and Environment*, **18**, 357–364.
- Mori, H. (1962) The effects of photo-stimulus on the thermal reaction in four species of spider mites. *Journal of the Faculty Agriculture*, **52**, 10–19.
- Mott, K.A. & Buckley, T.N. (2000) Patchy stomatal conductance: emergent collective behaviour of stomata. *Trends in Plant Science*, **5**, 258–262.
- Oke, T.R. (1978) *Boundary Layer Climates*. Routledge, New-York.
- Oren, R., Sperry, J.S., Katul, G.G., Pataki, D.E., Ewers, B.E., Phillips, N. et al. (1999) Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant, Cell and Environment*, **22**, 1515–1526.
- Pike, D.A., Webb, J.K. & Shine, R. (2012) Hot mothers, cool eggs: nest-site selection by egg-guarding spiders accommodates conflicting thermal optima. *Functional Ecology*, **26**, 469–475.
- Pincebourde, S. & Casas, J. (2006) Multitrophic biophysical budgets: thermal ecology of an intimate herbivore insect-plant interaction. *Ecological Monographs*, **76**, 175–194.
- Pincebourde, S. & Woods, H.A. (2012) Climate uncertainty on leaf surfaces: the biophysics of leaf microclimate and their consequences for leaf-dwelling organisms. *Functional Ecology*, **26**, 844–853.
- Pincebourde, S., Frak, E., Sinoquet, H., Regnard, J.L. & Casas, J. (2006) Herbivory mitigation through increased water-use efficiency in a leaf-mining moth-apple tree relationship. *Plant, Cell and Environment*, **29**, 2238–2247.
- Pincebourde, S., Sinoquet, H., Combes, D. & Casas, J. (2007) Regional climate modulates the canopy mosaic of favourable and risky microclimates for insects. *Journal of Animal Ecology*, **76**, 424–438.
- Pincebourde, S., Sanford, E., Casas, J. & Helmuth, B. (2012) Temporal coincidence of environmental stress events modulates predation rates. *Ecology Letters*, **15**, 680–688.

- Potter, K.A., Davidowitz, G. & Woods, H.A. (2009) Insect eggs protected from high temperatures by limited homeothermy of plant leaves. *The Journal of Experimental Biology*, **212**, 3448–3454.
- Potter, K.A., Woods, H.A. & Pincebourde, S. (2013) Microclimatic challenges in global change biology. *Global Change Biology*, **19**, 2932–2939.
- Pralavorio, M., Fournier, D. & Millot, P. (1989) Activité migratoire des Tétranyques: mise en évidence d'un rythme. *Entomophaga*, **34**, 129–134.
- R Development Core Team (2013). *R: A Language and Environment for Statistical Computing*. R foundation for statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, Available online from <http://www.R-project.org>.
- Robertson, R.M., Kuhnert, C.T. & Dawson, J.W. (1996) Thermal avoidance during flight in the locust *locusta migratoria*. *The Journal of Experimental Biology*, **199**, 1383–1393.
- Scheffers, B.R., Edwards, D.P., Diesmos, A., Williams, S.E. & Evans, T.A. (2013) Microhabitats reduce animal's exposure to climate extremes. *Global Change Biology*, **20**, 495–503.
- Scherrer, D. & Korner, C. (2010) Infra-red thermometry of alpine landscapes challenges climatic warming projections. *Global Change Biology*, **16**, 2602–2613.
- Sears, M.W., Raskin, E. & Angilletta, M.J. (2011) The world is not flat: defining relevant thermal landscapes in the context of climate change. *Integrative and Comparative Biology*, **51**, 666–675.
- Shimazaki, K.-I., Doi, M., Assmann, S.M. & Kinoshita, T. (2007) Light regulation of stomatal movement. *Annual Review of Plant Biology*, **58**, 219–247.
- Sinoquet, H., Le Roux, X., Adam, B., Amaglio, T. & Daudet, F.A. (2001) RATP: a model for simulating the spatial distribution of radiation absorption, transpiration and photosynthesis within canopies: application to an isolated tree crown. *Plant, Cell and Environment*, **24**, 395–406.
- Wang, Y., Noguchi, K. & Terashima, I. (2011) Photosynthesis-dependent and -independent responses of stomata to blue, red and green monochromatic light: differences between the normally oriented and inverted leaves of sunflower. *Plant and Cell Physiology*, **52**, 479–489.
- Whittaker, J.B. (2001) Insects and plants in a changing atmosphere. *Journal of Ecology*, **89**, 507–518.
- Willmer, P.G. (1982) Microclimate and the environmental physiology of insects. *Advances in Insect Physiology Volume 16* (eds M.J. Berridge, J.E. Treherne & V.B. Wigglesworth Sr), pp. 1–58. Academic Press, London.
- Woods, H.A. (2013) Ontogenetic changes in the body temperature of an insect herbivore. *Functional Ecology*, **27**, 1322–1331.
- Zeiger, E., Talbott, L.D., Frechilla, S., Srivastava, A. & Zhu, J. (2002) The guard cell chloroplast: a perspective for the twenty-first century. *New Phytologist*, **153**, 415–424.

Received 20 December 2013; accepted 2 May 2014

Handling Editor: Kwang Lee Pum

Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Survival rate of *T. urticae* (%) according to temperature (°C). The line represents the non-linear regression (see text).

Fig. S2. Distribution frequency of (top) leaf surface temperatures at the start (line) and end (dashed line) of the experiment, and (bottom) body temperature of spider mites at the start (triangles) and end (circles) of the experiment.