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Effects of simulated heat waves on an experimental plant–herbivore–predator food chain

ARNAUD SENTIS*†, JEAN-LOUIS HEMPTINNE† and JACQUES BRODEUR*

Département de sciences biologiques, Institut de recherche en biologie végétale, Université de Montréal, Montréal, QC, Canada, H1X 2B2, †Université de Toulouse – École nationale de formation agronomique, UMR 5174 CNRS/Université Toulouse III/ENFA 'Evolution et Diversité Biologique', BP 22687, F-31326, Castanet-Tolosan Cedex, France

Abstract

Greater climatic variability and extreme climatic events are currently emerging as two of the most important facets of climate change. Predicting the effects of extreme climatic events, such as heat waves, is a major challenge because they may affect both organisms and trophic interactions, leading to complex responses at the community level. In this study, we set up a simple three-level food chain composed of a sweet pepper plant, *Capsicum annuum*; an aphid, *Myzus persicae*; and a ladybeetle, *Coleomegilla maculata,* to explore the consequences of simulated heat waves on organism performance, trophic interactions, and population dynamics. We found that (1) heat waves do not affect plant biomass, significantly reduce the abundance and fecundity of aphids, and slightly affect ladybeetle developmental time and biomass, (2) heat waves decrease the impact of ladybeetles on aphid populations but do not modify the effect of aphids on plant biomass, and (3) food chains including predatory ladybeetles are more resistant to heat waves than a simple plant–aphid association, with aphid abundance being less influenced by heat waves in the presence of *C. maculata*. Our results suggest that more biodiverse ecosystems with predators exerting a strong biotic control are likely to be less influenced by abiotic factors and then more resistant to extreme climatic events than impoverished ecosystems lacking predators. Our study emphasizes the importance of assessing the effects of climatic change on each trophic level as well as on trophic interactions to further our understanding of the stability, resilience, and resistance of ecological communities under climatic forcing.

Keywords: climatic change, extreme climatic event, heat stress, insects, trophic interactions

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Introduction

Climate shapes the abundance and distribution of species as well as their interactions at the population and community levels. However, distribution ranges and communities are changing in response to climatic change; this has become a major concern in ecology. Several pieces of evidence indicate that not only higher mean temperatures but also increased climatic variability can have significant effects on organisms and ecosystems. Extremes, such as heat waves, will become more frequent and display larger amplitudes (IPCC, 2007). Although their impacts are thought to be more important to many organisms than shifts in average temperatures (Easterling *et al*., 2000), most studies have only focussed on the effects of increasing mean temperature on organism performance, thereby neglecting extreme temperature events (see Bale *et al*., 2002; Brown *et al*., 2004; Harrington *et al*., 1999; Parmesan & Yohe, 2003 for example of studies ignoring extreme temperature events).

Contrary to the progressive increase in average temperature, which is a long-term process, extreme climatic events are sporadic and can induce rapid consequences for organisms, populations, and communities that can be measured using short-term experiments (Ciais *et al*., 2005; Jentsch *et al*., 2007; De Boeck *et al*., 2010; Bannerman *et al*., 2011; Smith, 2011; Gillespie *et al*., 2012). Heat waves could be particularly severe for poikilothermic animals because they cannot regulate their internal temperature. Prior studies on arthropods reported that, when the constant temperature exceeds the upper temperature threshold for growth and reproduction, organism development rate is reduced, reproduction fails, and populations crash (Lamb & Gerber, 1985; Asin & Pons, 2001; Petavy *et al*., 2001; Gillespie *et al*., 2012). However, these negative effects may not only depend on the temperature *per se* but also on how long organisms are exposed to temperatures exceeding their upper temperature threshold. As exposure time increases, negative effects become more important and, ultimately, provoke death. On the other hand, insects can cope with high

Correspondence: Arnaud Sentis, Département de sciences biologiques, Institut de recherche en biologie végétale, Université de Montréal, Montréal, QC, Canada H1X 2B2, tel. +514 343 6111 #82548, fax +514 343 2288, e-mail: arnaud.sentis@umontreal.ca

temperatures when they have sufficient time to recover between successive episodes of thermal stress (Davis *et al.*, 2006; Mironidis & Savopoulou-Soultani, 2008). Together, these results suggest that organism performance decreases with the frequency and amplitude of heat waves.

The outcome of climate change for a species also depends on consequential changes in the nature and strength of interactions with other organisms within the community (Davis *et al.*, 1998; Petchey *et al.*, 1999; Post *et al.*, 1999; Suttle *et al.*, 2007). In some cases, direct effects of temperature on organisms can be weaker than indirect effects through trophic and guild interactions (Barton *et al.*, 2009; Barton, 2011). Understanding the effects of climatic variability on community dynamics therefore requires knowledge of how species interact and how these interactions are affected by temperature variation (Stenseth *et al.*, 2002; Tylianakis *et al.*, 2008; Van Der Putten *et al.*, 2010). However, studies on extreme climatic events have generally focussed on a single trophic level, mainly plants (Ciais *et al.*, 2005; Davison *et al.*, 2010; De Boeck *et al.*, 2010), and few have examined trophic interactions (but see Bannerman *et al.*, 2011; Gillespie *et al.*, 2012; Harmon *et al.*, 2009; Thibault & Brown, 2008). In an original study, Thibault & Brown (2008) analyzed the consequences of an exceptional flood on a desert community dominated by rodents. This catastrophic event provoked species-specific mortalities and altered interspecific interactions and community species composition, resulting in a rapid and extensive reorganization of this community. The Thibault & Brown (2008) study showed that (1) the consequences of extreme climatic events on ecosystems are difficult to predict because of species-specific responses and (2) it is crucial to investigate how the effects of extreme events cascade through trophic chains to understand and predict changes in population dynamics and community structure; minor changes in trophic interactions can greatly modify community structure (Thibault & Brown, 2008; Tylianakis *et al.*, 2008).

Climatic disturbance can also have important consequences for the stability of communities by modifying the strength of trophic interactions (Petchey *et al.*, 1999; Walther *et al.*, 2002; Smith, 2011). According to theory, complex ecological systems are generally more stable and resistant to biotic invasion or climatic change than simpler ecosystems because strong, potentially destabilizing consumer–resource interactions are dampened by numerous weak interactions in complex food webs (Petchey *et al.*, 1999; Mccann, 2000). For instance, Wilmers & Post (2006) showed that the presence of wolves in Yellowstone National Park alleviates the effects of warming on carrion abundance during an El Niño episode. Carrion availability for the scavenger community was a function of climatic variation before wolf reintroduction but became determined primarily by wolves following their reintroduction because wolves became the major factor driving winter carrion availability. They concluded that ecosystems with top predators are likely to exhibit stronger biotic regulation and should be more resistant to climate change than ecosystems without top predators. Apart from this example, there is poor understanding of the extent to which higher trophic-level interactions may increase or decrease the resistance of food webs to extreme climatic events.

Under laboratory conditions, we investigated the effects of temperature regimes, characterized by differences in the frequency and amplitude of temperature peaks, on organisms and their interactions using a plant–aphid–ladybeetle mesocosm system. Experiments were also performed to determine the effects of peak amplitudes on aphid fecundity and mortality. We finally examined the role of ladybeetles on the resistance of the trophic chain to simulated heat waves.

Materials and methods

Biological system

Ubiquitous and abundant in terrestrial ecosystems, aphids are contagiously distributed in time and space (Van Emden & Harrington, 2007). Their colonies grow rapidly and are exploited by a large number of predator, parasitoid, and pathogen species (Dixon, 1998; Van Emden & Harrington, 2007). Aphids and their natural enemies are sensitive to temperature changes and constitute excellent models to explore temperature effects on food-web interactions. We studied a system composed of the sweet pepper plant *Capsicum annuum* L. cv. Bell Boy, the green peach aphid *Myzus persicae* Sulzer (Homoptera: Aphididae), and the predatory ladybeetle *Coleomegilla maculata* lengi Timberlake (Coleoptera: Coccinellidae). These species have overlapping niches and may coexist in nature or in greenhouses (Boiteau, 1983).

Sweet pepper plants were grown from seed and fertilized twice a week with Nitrophoska (12-4-14) with a nitrogen concentration of 100 ppm (Plant-Prod ©, Montréal, Canada). Plants were used for maintenance of a colony of *M. persicae*, established from individuals collected in greenhouses of Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu (45°19′N, 73°16′W, Québec, Canada). About 4000 adult *C. maculata* were collected in October 2009 in a field at Saint-Mathieu de Beloeil (45°35′N, 74°45′W, Québec, Canada), brought back to the laboratory, reared in mesh cages (60 × 40 × 40 cm), and fed pollen and *M. persicae*. All insects and plants were held in growth chambers (Conviron® E15; Controlled Environments, Inc., Winnipeg, Manitoba, Canada) at 24 ± 1 °C, 50–60% relative humidity, and under a 16L : 8D photoperiod at a light intensity of 150 μmol m^{-2} s^{-1}.

Experimental design

We conducted two experiments under a relative humidity of 70 ± 9% and a photoperiod of 16L : 8D. Four-week-old pepper plants having four unfurled leaves were individually placed in plastic cylinders (ø: 20 cm; h: 45 cm) glued to a plastic disk platform. The top of the cylinder and the two lateral openings were covered with mesh muslin for ventilation. Adult *M. persicae* used in all experiments were obtained from synchronous cohorts. During the experiments, temperature and humidity were recorded continuously using Hobo U12 (Hobo®; Onset Computer Corporation, Inc., Bourne, MA, USA) units.

Experiment 1. Effects of heat waves on organisms and trophic interactions

A factorial experiment was set up to measure the effect of extreme fluctuating temperature regimes (i.e., heat waves) on plants, aphids, and coccinellids. The temperature regimes were (1) a constant temperature of 23 °C, (2) one peak of 30 °C twice a week, (3) one peak of 30 °C per day, (4) one peak of 40 °C twice a week, and (5) one peak of 40 °C per day (Fig. 1). Temperature was kept constant at 23 °C on days without a peak. On days including a peak, temperature started to increase at 10:00 hours, reached the maximum (i.e., 30 or 40 °C) from 12:00 to 13:00 hours, and then decreased until 15:00 hours. Temperature was kept then constant for the rest of the day at 22 and 20 °C for peaks of 30 and 40 °C, respectively. Each temperature regime was designed so that the daily average temperature was 23 °C.

There were three experimental food chain lengths called treatments in this study: (1) Plant treatment (*C. annuum* only), (2) Aphid treatment (*C. annuum* + *M. persicae*), and (3) Predator treatment (*C. annuum* + *M. persicae* + *C. maculata*). During the experiment, pepper plants were fertilized every 3 days as described above. At the onset of the experiment, four parthenogenetic adult female *M. persicae* were transferred to the upper leaves of each plant in the Aphid and Predator treatments using a fine camel hair brush. In the Predator treatment, aphids were left 24 h to acclimatize and reproduce, and then a newly hatched first instar *C. maculata* larva was introduced near the aphid colony. After 10 days, the number of aphids per plant was recorded for the Predator and Aphid treatments, and the aerial plant fresh mass was measured for the three food chain length treatments. *C. maculata* larvae from the Predator treatment were isolated in Petri dishes (ø 90 mm) at 23 °C, starved for 24 h, and then weighed to the nearest microgram using a microbalance (Mettler Toledo MT5; Mettler-Toledo, Inc., Mississauga, Ontario, Canada). They were next fed *ad libitum* with *M. persicae* and their developmental time from eclosion to pupation was recorded.

Because of logistical constraints, only two temperature-controlled growth chambers were available to run the experiment. We therefore set up an experimental design that contributes to reduce potential pseudoreplication issues. At the beginning of the experiment, two temperature regimes were randomly selected and each assigned to a growth chamber. Because each temperature regime was repeated twice over the experiment, we avoided running the two repetitions at the same time to ensure that differences between temperature regimes would not be an effect of experimental date. Furthermore, the two repetitions were not performed in the same growth chamber to have a balanced design and avoid a potential growth chamber effect. For each temperature regime, a repetition or experimental date thus corresponds to one growth chamber. Each growth chamber contained 18 cylinders that were placed on the floor and arranged in three blocks of six cylinders to control for potential environmental heterogeneity within the chamber. Within each block, the cylinders were placed at random to avoid experimental bias or pseudoreplication issues. For the first repetition of each temperature regime, two blocks had one Plant treatment, three Aphid treatments, and two Predator treatments. The third block consisted of one Plant treatment, two Aphid treatments, and three Predator treatments. For the second repetition, two blocks had one Plant treatment, two Aphid treatments, and three Predator treatments. The remaining block consisted of one Plant treatment, three Aphid treatments, and two Predator treatments. As a

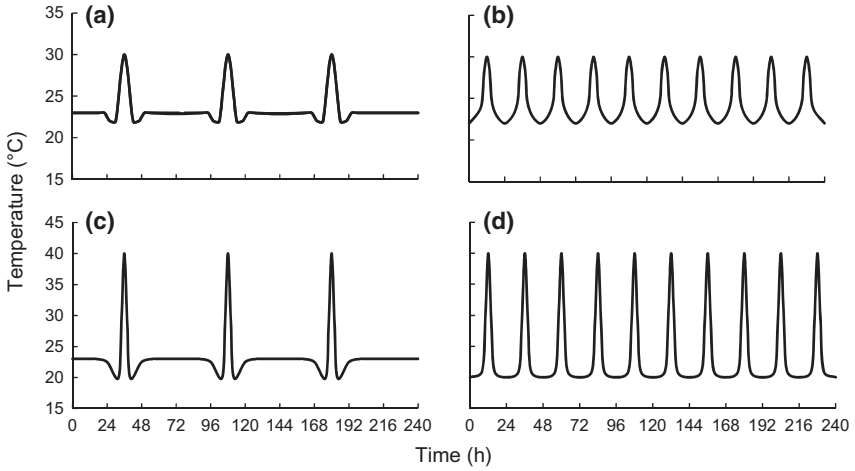

Fig. 1 The four regimes in which temperature varied: (a) one peak of 30 °C twice a week, (b) one peak of 30 °C per day, (c) one peak of 40 °C twice a week, and (d) one peak of 40 °C per day.

result, for each temperature regime, the experiment was repeated 15 times for the Predator treatment and Aphid treatment and six times for the Plant treatment. Furthermore, two or three Hobo U12 (Hobo®) units were placed in each growth chamber (one unit per block) to record temperature during the experiment and estimate spatial heterogeneity within and between growth chambers that is, for each temperature regime, also equivalent to within and between experimental date.

Statistical analyses were divided into two steps. Firstly, the effects of the food chain length, the temperature regimes, and their interactions on the aerial fresh mass of sweet peppers and aphid abundance were analyzed with mixed-model ANOVAs. Following Schank & Koehnle (2009), we included in our model blocks and growth chambers (equivalent to dates) as random factors nested within temperature regimes to avoid pseudoreplication issues. Secondly, when the temperature regimes or the interactions with the temperature regimes had a significant effect on the dependent variable, the regime with constant temperature was not taken into consideration and mixed-model ANOVAs were used to investigate the effects of the food chain length, temperature peak amplitude, peak frequency, and their interactions. *Post hoc* Tukey tests were used to determine differences among means. The effects of the five temperature regimes on *C. maculata* larval weight and developmental time were analyzed with one-way ANOVAs. As stated earlier, the constant temperature regime was omitted to analyze the effects of peak frequency, peak amplitude, and their interaction with two-way ANOVAs. Finally, we estimated temperature spatial heterogeneity within and between growth chambers to control for potential pseudoreplication. For each block and repetition, we measured, using Hobo temperature data, the average temperature across the experiment which was designed to be 23 °C for all temperature regimes. Because the number of replicates (i.e., data logger) was not sufficient to test the effects of temperature regimes, growth chambers, and blocks altogether, we divided statistical analyses into two steps. Firstly, the effects of temperature regimes and growth chambers (equivalent to dates) on the average temperature were analyzed using an ANOVA with growth chambers nested within temperature regimes. Secondly, the effects of growth chambers and blocks on the average temperature were analyzed using an ANOVA with blocks nested within growth chambers. Statistical analyses were performed with JMP v.8. (2008, SAS Institute, Cary, NC, USA).

Experiment 2. Effects of temperature peak amplitude on aphid fecundity

To examine the effect of temperature peak amplitude on aphid fecundity, four adults of *M. persicae* were transferred to a sweet pepper plant kept in a cylindrical cage (see above). The cages were exposed to three temperature regimes for 24 h: (1) constant 23 °C, (2) one 30 °C peak, and (3) one 40 °C peak. The average temperature of these three regimes was 23 °C and the peaks were similar to those of regimes 2 and 4 of experiment 1 (Fig. 1a and c). After 24 h, the numbers of newly hatched and adult aphids per plant were recorded. Because the number of offspring depends on adult fecundity and also on newborn mortality due to thermal stress, a treatment with 15 first instar aphids (less than 24-h-old) per plant was also conducted to assess their mortality. For each temperature regime, there were 26 replicates for estimating aphid fecundity and 10 replicates for estimating the mortality of first instar aphids. The effects of temperature regimes on adult fecundity and first instar mortality were analyzed with ANOVAs. Data were log(x + 1) transformed to satisfy normality and homoscedasticity requirements. Statistical analyses were performed with JMP v.8. (SAS Institute).

Results

Experiment 1. Effects of heat waves on organisms and trophic interactions

Spatial heterogeneity within and between growth chambers. The average temperature (± SE) throughout the experiment was 22.81 ± 0.22 °C. The first ANOVA with growth chambers nested in temperature regimes revealed that the average temperature was not affected by temperature regimes ($F_{4,16} = 1.75$, $P = 0.1878$) or by growth chambers ($F_{5,16} = 1.10$, $P = 0.3980$). The second ANOVA with blocks nested in growth chambers revealed that the average temperature was not affected by growth chambers ($F_{1,20} = 0.42$, $P = 0.5230$) but differ significantly between blocks ($F_{4,20} = 3.37$, $P = 0.0292$).

Plant biomass. Plant biomass was not affected by the temperature regimes ($F_{4,5.25} = 0.96$, $P = 0.50$) but was by food chain length (Fig. 2; $F_{2,10.94} = 18.19$, $P = 0.0003$). It decreased similarly in the Aphid and Predator treatments (Fig. 2) and across temperature regimes (temperature regimes × food chain length; $F_{8,10.61} = 0.76$, $P = 0.64$).

Aphid abundance. Aphid abundance differed between temperature regimes ($F_{4,4.97} = 8.62$, $P = 0.018$), but this

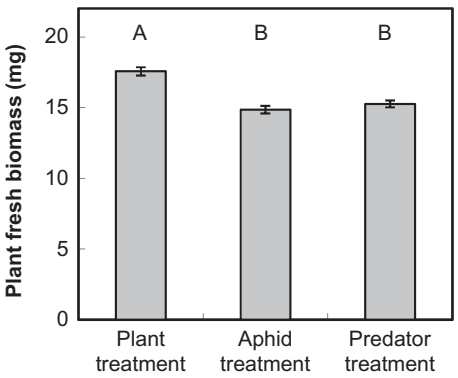

Fig. 2 Mean fresh biomass (± SE) of the sweet pepper plants in the three food chain length treatments. Histograms with different letters are significantly different ($P < 0.05$).

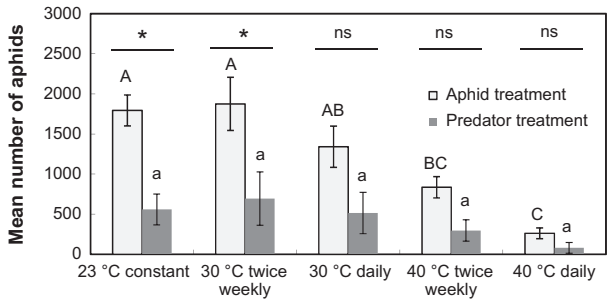

Fig. 3 Abundance of *Myzus persicae* per plant (mean ± SE) in the five temperature regimes for both the Aphid and Predator treatments. Different letters above bars denote differences between the temperature regimes while '*' and 'ns' apply to the differences between food chain length treatments within each temperature regime. Bars with different small or capital letters within the Predator and the Aphid treatments, respectively, are significantly different ($P < 0.05$). Within temperature regimes, 'ns' stands for not significant and '*' indicates significant differences ($P < 0.05$) between Predator and Aphid treatments. Significance levels are from *post hoc* Tukey tests.

depended on the length of the food chain (temperature regimes × food chain length, $F_{4,5.26} = 48.89$, $P = 0.0002$; histograms in Fig. 3). Although aphid number did not differ among temperature regimes for the Predator treatment, *M. persicae* abundance in the Aphid treatment was significantly lower when the aphids were exposed to a peak of 30 °C every day or to the two treatments with temperature reaching 40 °C (histograms in Fig. 3).

Concerning the effects of food chain length, the Predator treatment significantly decreased aphid abundance compared with the Aphid treatment ($F_{1,5.39} = 799.14$, $P < 0.0001$; Fig. 3), but this effect varied with the temperature regimes (temperature regimes × food chain length, $F_{4,5.26} = 48.89$, $P = 0.0002$; Fig. 3). When comparing aphid abundance within each temperature regime, the *post hoc* Tukey tests revealed that the impact of the Predator treatment on aphid abundance was only significant where the experimental system was exposed to a constant 23 °C or one peak of 30 °C twice a week (upper part of Fig. 3).

A mixed-model ANOVA was used to investigate the effects of food chain length, temperature peak amplitude, peak frequency, and their interactions on aphid abundance (Table 1). There were fewer aphids in the treatments with high amplitude peaks, but their abundance was not influenced by peak frequency or an interaction between peak amplitude and frequency (Table 1, column 2). Moreover, the effects of peak frequency and amplitude depended on length of the food chain (Table 1, column 2). In the Aphid treatment, aphid abundance decreased with peak amplitude and frequency but was not affected by their interaction (Table 1, column 3). In contrast, in the Predator treatment, aphid abundance was not significantly affected by peak frequency, peak amplitude, or the interaction between the two (Table 1, column 4).

Ladybeetle larvae significantly decreased aphid abundance (Table 1, column 2), but increasing peak frequency or amplitude reduced the impact of ladybeetle larvae on aphid abundance (Table 1, column 2).

Coleomegilla maculata developmental time and larval weight. The temperature regimes affected *C. maculata* larval weight ($F_{4,54} = 5.69$, $P = 0.0007$; Fig. 4), which was reduced by both peak amplitude ($F_{1,42} = 12.65$, $P = 0.0009$) and frequency ($F_{1,42} = 16.47$, $P = 0.0128$). There was no interaction between peak frequency and amplitude ($F_{1,42} = 2.89$, $P = 0.0965$).

Temperature regimes affected *C. maculata* larval developmental time ($F_{4,54} = 4.34$, $P = 0.0041$; Fig. 5), which was longer for larvae exposed to 40 °C peaks compared to 30 °C peaks (peak amplitude: $F_{1,42} = 6.65$, $P = 0.0135$). Larval developmental time was not affected by peak frequency ($F_{1,42} = 0.57$, $P = 0.45$) or by the interaction between peak frequency and amplitude ($F_{1,42} = 0.37$, $P = 0.54$).

Table 1 F and P values of the mixed-model ANOVAs for the effect of food chain length treatments, temperature peak amplitude, peak frequency, and their interactions on *Myzus persicae* abundance for Aphid and Predator treatments together, Aphid treatment alone, and Predator treatment alone. Significant effects are in bold

Effect	Full model (Aphid + Predator treatments)	Aphid treatment alone	Predator treatment alone
Frequency	$F_{1,3.98} = 4.76$, $P = 0.09$	$F_{1,3.99} = 9.67$, **$P = 0.036$**	$F_{1,3.95} = 1.06$, $P = 0.36$
Amplitude	$F_{1,3.98} = 18.36$, **$P = 0.013$**	$F_{1,3.99} = 33.33$, **$P = 0.004$**	$F_{1,3.95} = 7.37$, $P = 0.054$
Frequency × amplitude	$F_{1,3.98} = 0.01$, $P = 0.94$	$F_{1,3.99} = 0.0052$, $P = 0.95$	$F_{1,3.95} = 0.04$, $P = 0.85$
Food chain length	$F_{1,5.52} = 639.15$, **$P < 0.0001$**	–	–
Frequency × food chain length	$F_{1,5.76} = 53.20$, **$P = 0.0004$**	–	–
Amplitude × food chain length	$F_{1,5.9} = 129.84$, **$P < 0.0001$**	–	–

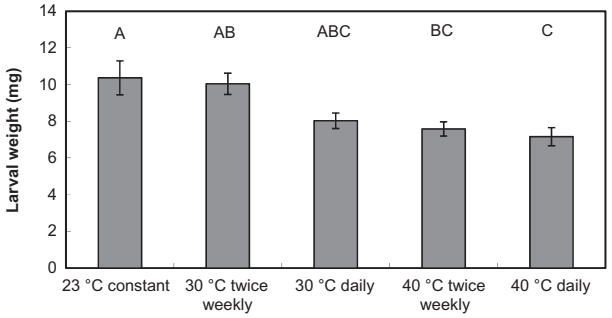

Fig. 4 Effect of the five temperature regimes on *Coleomegilla maculata* mean larval weight (± SE). Bars with different letters are significantly different ($P < 0.05$).

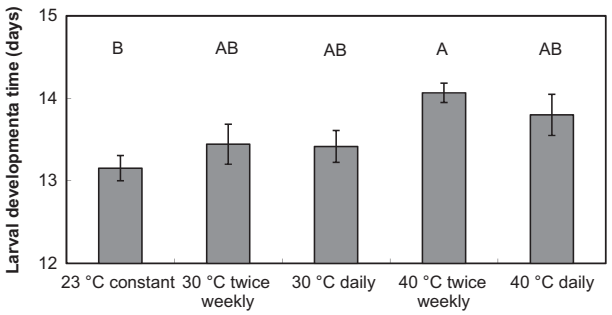

Fig. 5 Effect of the five temperature regimes on *Coleomegilla maculata* mean larval developmental time (± SE). Bars with different letters are significantly different ($P < 0.05$).

Experiment 2. Effects of temperature peak amplitude on aphid fecundity

The mortality of first instar aphids was very low (1.12%) and did not differ among the temperature regimes ($F_{2,22} = 0.0002$, $P = 0.99$); we therefore did not correct for mortality in the fecundity experiment. We did not record adult mortality during the experiment. Adult aphids experiencing a peak of 30 °C or 40 °C were significantly less fecund than individuals maintained at 23 °C ($F_{2,67} = 16.15$, $P < 0.0001$; Fig. 6).

Discussion

The frequency and amplitude of extreme climatic events are predicted to increase with global warming and have greater impacts on ecosystems than increasing mean temperatures (Easterling *et al.*, 2000). However, few studies have investigated the effects of extreme climatic events on organisms, trophic interactions, and food-web dynamics (Thibault & Brown, 2008; Harmon *et al.*, 2009; Bannerman *et al.*, 2011). Using a simple plant–herbivore–predator system, we explored the consequences on both organisms and their

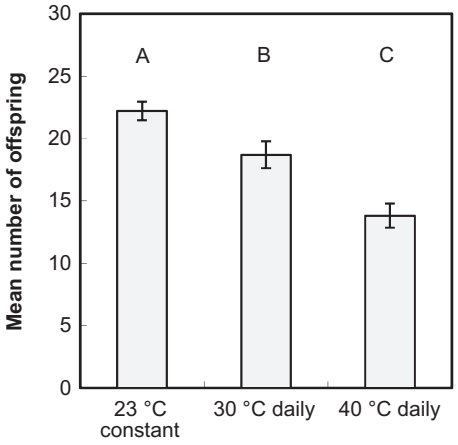

Fig. 6 Effect of three temperature regimes on *Myzus persicae* fecundity (mean ± SE). Bars with different letters are significantly different ($P < 0.05$).

interactions of heat waves that differed in the frequency and amplitude of thermal peaks but not in average temperature. We observed that (1) heat waves do not affect plant biomass, significantly reduce the abundance and fecundity of aphids, and only have mild effects on the biomass and developmental time of ladybeetles, (2) heat waves decrease the impact of ladybeetles on aphid populations but do not modify the effect of aphids on plant biomass, and (3) food chains including predatory ladybeetles are more resistant to heat waves than a simple plant–aphid association.

Potential limitations of our experimental design and statistical analyses

Because of logistic limitations, each temperature regime was only repeated twice over the experiment. This could be perceived as a 'pseudreplication issue' because we had multiple individual replicates within the same growth chamber. Experimental data should be independent and therefore not be 'pseudoreplicated' because pseudoreplication increases – but not imply – the likelihood of statistical nonindependence (Hurlbert, 1984). However, in this study, the average temperature over the experiment differed significantly among experimental blocks but was not affected by growth chambers or temperature regimes. This result indicates that growth chambers are not a uniform environment but exhibit spatial variations that, in our experiment, were larger within than between chambers. We can therefore argue that statistical dependence between individual replicates should be weak and thereby does not preclude inferential statistics. According to Schank & Koehnle (2009) among others, we used nested ANOVAS with growth chambers and blocks as nested factors to

avoid pseudoreplication issues. Such an approach takes into account the information within blocks and growth chambers and provides similar results for treatment effects than a simple ANOVA performed with averaged data using the mean for each experimental unit calculated with pseudoreplicates (Coss, 2009; Schank & Koehnle, 2009; Wiley, 2009). 'Averaging' is typically used to avoid pseudoreplication issues (Hurlbert, 1984, 2009) but obliterates information within experimental units and is thereby less powerful than hierarchical analyses as nested ANOVAS (Coss, 2009; Schank & Koehnle, 2009). Moreover, in our study, the estimated effects for temperature regimes and food chain lengths were, according to *P* values, sufficiently strong to discard a potential artifact induced by pseudoreplication. Finally, each temperature regime was replicated twice (once in each chamber), thereby balancing the potential growth chamber effect and precluding the possibility of a totally pseudoreplicated experiment. Although we cannot totally exclude pseudoreplication issues, we consider our experimental design, statistical analyses, and interpretation of the results to be sound and appropriate.

Effects of heat waves on organisms

Previous studies reported that heat waves generally decrease plant performance in nature because they are often associated with water stress, which is sometimes more important than the direct effect of temperature (Ciais *et al*., 2005; Porter & Semenov, 2005; Mittler, 2006; De Boeck *et al*., 2010). In our experiment, water was not a limiting factor and temperature variations were similar to those experienced by pepper plants in nature (Environment Canada, 2010). Moreover, the sweet pepper cultivar used (Bell Boy) was selected for greenhouse production, where temperatures are highly variable. This probably explains the lack of plant responses to temperature peaks in our study.

In agriculture, insect pests are predicted to be more abundant following climatic change because a slight increase in mean temperature generally enhances insect fecundity and developmental rate (Cannon, 1998; Bale *et al*., 2002; Newman, 2006). In this study, we observed that aphid populations were strongly reduced by extreme temperatures (Fig. 3), particularly in the Aphid treatment, which was more affected by peak amplitude and frequency (Fig. 3 and Table 1). Moreover, in a 24 h experiment, temperature peaks of 30 °C or 40 °C did not affect *M. persicae* mortality but decreased its fecundity (Fig. 6), which probably accounts for the reduction in aphid populations observed in experiment 1. Other studies also reported that aphids may produce fewer offspring, suffer from developmental damage, and lose their beneficial endosymbiosis under extreme temperatures (Bensadia *et al*., 2006; Davis *et al*., 2006; Hazell *et al*., 2010). In contrast with predictions based on a mean temperature increase of 1–5.8 °C (Cannon, 1998; Bale *et al*., 2002), we therefore hypothesize that heat waves associated with climatic change may lead to a reduction in aphid abundance if the frequency and amplitude are high enough to impact aphids.

The few studies investigating the effects of extreme high temperatures on natural enemies of aphids have reported contradictory results. The parasitoid *Aphidius avenae* Haliday (Hymenoptera: Braconidae) was less fecund when exposed to high temperatures (Roux *et al*., 2010), while the foraging ability and fecundity of *Aphidius matricariae* Haliday (Hymenoptera: Aphidiidae) were not affected by similar temperature regimes (Bannerman *et al*., 2011). In our study, heat waves decreased *C. maculata* larval weight and increased its developmental time (Figs 4 and 5). These effects may originate from the temperature regimes themselves or from an indirect effect associated with aphid density that was lower in extreme temperature regimes (Fig. 3). Although our experiment was not primarily designed to disentangle these two potential effects, it is unlikely that the quantity of resource available for ladybeetle larvae would have been a major issue because aphid densities were sufficiently high in all temperature regimes (Fig. 3) to support the daily consumption of a *C. maculata* larva, which is approximately 30 *M. persicae* per day (Sentis *et al*., 2012). Other potential indirect effects such as prey quality or aphid distribution might be involved but the observed effects are more likely linked to the ladybeetle energetic efficiency that decreases when temperature exceeds 30 °C (Sentis *et al*., 2012). Because ladybeetle weight is positively correlated with fecundity (Stewart *et al*., 1991), our results suggest that extreme temperature fluctuations can decrease ladybeetle fitness and thereby lower their abundance over the long term.

Plants, herbivores, and predators were differentially affected by extreme temperatures, with a large impact on aphid populations, a moderate impact on ladybeetle larvae, and no effect on plant biomass. The relative sensitivity of each trophic level to heat waves suggests that communities are unlikely to respond in a collective uniform manner to climatic changes, as has sometimes been assumed (see Schmitz *et al*., 2003). Over the long term, extreme temperatures are likely to disrupt trophic relationships, resulting in important changes in population dynamics and communities. However, given the stochastic nature of extreme climatic events and their great variability in frequency, amplitude, and duration (De Boeck *et al*., 2010), predictions remain difficult. As illustrated by our results, when heat waves were

less frequent or of smaller amplitude, we observed minor effects on organisms and few or no modifications in the dynamics of the simple three-level trophic system. The effects of extreme climatic events on organisms and communities thus largely depend on their frequency and amplitude.

Effects of extreme temperature fluctuations on trophic interactions

Myzus persicae infestations significantly decreased sweet pepper biomass independently of the temperature regimes and *C. maculata* presence (Fig. 2). Because aphid damage to plants – excluding virus transmission – is relative to their abundance (Dixon, 1998), which decreased with extreme temperatures and predation, we would have expected a relationship between temperature regimes, predation by *C. maculata,* and plant biomass reduction. However, our experiment was likely too short to reveal the benefits that pepper plants would gain from a reduction in aphid abundance.

Overall, *C. maculata* larvae significantly reduced aphid populations, but this effect was influenced by heat waves as indicated by a significant interaction between temperature regimes and food chain length. The impact of ladybeetles on the aphid population was weakened by both the amplitude and frequency of temperature peaks (Table 1). As a result, aphid abundance did not significantly differ with or without *C. maculata* in three temperature regimes (30 °C daily, 40 °C twice weekly, and 40 °C daily) (Fig. 3). These results challenge those of models and studies based on mean temperature that predict an increase in the strength of top-down control with climate change (Barton *et al*., 2009; Rall *et al*., 2010; Vucic-Pestic *et al*., 2011). According to Sentis *et al.* (2012), the reduction in prey abundance by predators increases with warming, reaches an optimum, and then decreases at higher temperatures. Because previous models and studies generally did not take into account or test high temperatures, part of the relationship between temperature and interaction strength is missing.

Fluctuating temperatures versus average temperatures

Studies based on an increase in mean temperatures generally lead to the conclusion or prediction that global warming will increase (1) plant and insect performances (i.e., fecundity, survival, generation time), (2) species distribution range, and (3) strength of trophic interactions (Cannon, 1998; Bale *et al*., 2002; Parmesan, 2006; Rall *et al*., 2010; Vucic-Pestic *et al*., 2011). In contrast, we found that extreme temperature variations decrease (1) organism performance in terms of aphid fecundity, growth rate of aphid colonies, and ladybeetle weight and developmental time, and (2) the strength of top-down control with a lower impact of ladybeetles on aphid populations. Moreover, our results suggest that extreme temperatures can decrease species distribution range by causing species extinction in habitats where extreme temperature episodes are frequent and intense. The differences between these two patterns of temperature changes (i.e., mean versus fluctuation) draw attention to the importance of assessing both scenarios in future studies.

Effects of ladybeetles on food-web resistance to extreme temperature fluctuations

The relationship between biodiversity and the resistance of natural communities to disturbance has emerged as an important ecological question, but few studies have investigated this issue within the context of climate change. Prior results suggest that interactions at higher trophic levels and top predators play an important role in the resistance of food webs to disturbance (Mccann, 2000; Wilmers & Post, 2006; Harmon *et al*., 2009). We observed that the decrease in aphid populations caused by extreme temperatures was more pronounced in the absence of *C. maculata* (Fig. 3). In other words, ladybeetles buffered the direct effects of extreme temperatures on aphid populations. In the absence of temperature variation, aphids had the highest fecundity but ladybeetles also had the strongest impact on aphid abundance. As the severity of the temperature regime increased, aphid fecundity decreased but ladybeetles also had a lower impact on aphid abundance. As a result, in the presence of *C. maculata*, aphid populations varied less as a function of temperature regime (Fig. 3) and were not significantly affected by peak amplitude and frequency (Table 1). In a scenario without predators, aphid populations would fluctuate in response to temperature variation while they would be more stable in the presence of predators. As reported by Wilmers & Post (2006), our results suggest that ecosystems with predators exerting a strong biotic control on prey populations should be more resistant to climate change than ecosystems lacking them. Therefore, if one's objective is to preserve communities and ecosystem functions, these predators should be protected to minimize the influence of climatic change on ecological communities.

In natural systems, food webs are generally complex, involving many species that interact in direct and indirect ways. General patterns for the influence of climate change on trophic interactions and community dynamics are therefore difficult to identify (Petchey *et al*., 1999; Van Der Putten *et al*., 2010; Walther, 2010). As is

the case with most laboratory studies, our small-scale, short-term experiments were conducted in an artificial environment that has little in common with natural conditions. However, our approach remains useful for determining mechanisms and processes behind the observed responses of species and communities as such studies would not be possible in nature given the stochasticity of extreme weather events. Overall, our study highlights the importance of assessing the effects of climatic change on each trophic level as well as on trophic interactions to further our understanding of the stability and resistance of ecological communities under climatic stress. The effects of extreme climatic events on organisms, populations, and communities are difficult to predict because (1) each species has its own sensitivity to thermal stress, (2) the effect of heat waves depends on their frequency and amplitude, which are highly variable in nature, (3) the response of communities depends not only on the sensitivity of individual species to environmental changes but also on trophic interactions.

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