Author/s:
Zhao, F;Xing, K;Hoffmann, AA;Ma, CS

Title:
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Date:
2019-07-01

Citation:
Zhao, F., Xing, K., Hoffmann, A. A. & Ma, C. S. (2019). The importance of timing of heat events for predicting the dynamics of aphid pest populations. Pest Management Science, 75 (7), pp.1866-1874. https://doi.org/10.1002/ps.5344.

Persistent Link:
https://hdl.handle.net/11343/285557

Zhao Fei (Orcid ID: 0000-0003-4158-3604)

The importance of timing of heat events for predicting the dynamics of aphid pest populations

Running title: Complex nature of thermal consequences

Fei Zhao [1,2*] *Kun Xing* [1] *Ary A. Hoffmann* [3] *and Chun-sen Ma* [2*]

[1] *Shanxi Key Laboratory of Integrated Pest Management in Agriculture, Institute of Plant Protection, Shanxi Academy of Agricultural Sciences, Taiyuan, Shanxi, China.*

[2] *Climate Change Biology Research Group, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China.*

[3] *Pest and Environmental Adaptation Research Group, School of BioSciences, Bio21 Institute, The University of Melbourne, Victoria, Australia.*

Authors for correspondence:

Chun-sen Ma:
Address: Yuan-Ming-Yuan West Road No.2, Beijing, 100193, P.R. China
Tel: (86) 10 6281 1430
E-mail: machunsen@caas.cn
Fei Zhao:
81 Longcheng Street, Xiaodian District,Taiyuan 030031, P. R. China
Tel: +86 0351-7136636,
E-mail: zhaofei12@sxagri.ac.cn

Author contributions

C.-S.M. and F.Z. designed the study, F.Z. and K.X. performed the experiments. F.Z., A.A.H. and K.X. performed the statistical analysis. F.Z., C.-S.M. and A.A.H. wrote the first draft of the manuscript and all authors contributed substantially to revisions.

ABSTRACT

BACKGROUND: Heat waves are increasing in frequency and there is growing interest in their impact on pest organisms. Previous work indicates that effects depend on timing of the stress event whose impact needs to be characterized across the full set of developmental stages and exposure periods of an organism. Here we undertake such a detailed assessment using heat stress (20-35 °C diurnal cycle) across the nymph and adult stages of the English grain aphid, *Sitobion avenae* (Fabricius).

RESULTS: Stress-related mortality increased with stress duration at all stages; effects were less severe at the late nymphal stage. Longevity of adults after stress showed a complex pattern with nymphal heat stress, increasing with stress duration at the late nymphal stage but decreasing with duration at the early nymphal stage. Longevity was also reduced by adult stress but to a lesser extent and patterns were not connected to duration. Post-stress productivity decreased following adult and nymphal stress and the decrease tended to be correlated with stress duration. The rate of offspring production was more affected by adult stress than nymphal stress. Productivity and longevity effects when combined showed that the largest effect of heat stress occurred at the early nymphal stage.

CONCLUSION: These findings highlight the complex ways in which heat stress at a particular life stage influences later fitness and they also emphasize the importance of considering multiple fitness components when assessing stress effects.

Key words: cereal aphid, climate change, fitness, heat selection, insect, life stage

1 INTRODUCTION

Global climate change is expected to increase not only mean temperature but also the intensity, frequency and duration of heat events [1], which are projected to accelerate in the future [2]. For instance, during the crop-growing season of May at Beijing (39°48 N, 116°28 E), a main winter wheat region in China, insect pests living in crop fields have been experiencing a marked increase in the number of hot days (daily maximum temperature exceeding 30 °C) over the last 45 years (Fig. S1A). Although mean temperatures in temperate areas are rarely expected to exceed optimum values for ectotherms [3], even small shifts in mean temperature will substantially increase the incidence of extreme temperatures [4, 5] and more intense, more frequent, and longer lasting heat waves have been predicted [6] resulting in stressful conditions for many ectotherms [7-10] which may affect demographic rates [11-13], phenology [14], population dynamics [15, 16] and community structure [17]. In this context, species with relatively short generation times including most agricultural insects are increasingly likely to suffer one or more heat events of variable duration (Fig. S1B) at some point of their life cycle [18, 19].

The impacts of high temperature on insects are likely to be stage-specific, because the behavior [20], thermal tolerance [21-23], physiological measures [24], developmental rates and metabolic rates [25] following heat exposures vary ontogenetically in many taxa with complex life stages including Lepidoptera [23, 26, 27], Diptera [28-30], Coleoptera [31, 32], Odonata [33] and Blattaria [34]. High temperature effects can also vary across stages in organisms with relatively simple life-histories including Hemiptera [35] and Homoptera [12, 18]. Furthermore, high temperature effects at early stages in a life cycle can be carried over to later stages [36] or even across generations[37]. Heat experienced in the egg stage may impact larval development [38, 39], as well as adult survival and body mass [40] while heat experienced during early developmental stages can impact adult activity [41, 42], size, coloration [32] and reproduction [13, 18, 26].

Despite recent progress in understanding stage-specific responses to high temperatures [43, 44], there is only limited information about the consequences of heat exposure when stress is applied in ecologically-relevant ways [45], such as a series of consecutively hot days [12, 13]. Indeed, the effect of different timescales - duration and pattern of thermal exposure - on ectotherms thermal performance and fitness [46] has been ignored (but see[47]. In most previous studies, life-stages only experienced a bout of brief heat with variable ramping rates [31, 35], intensities [23, 29] or exposure times [18, 26]. Repeat bouts of exposure may well produce results different from a single exposures given the potential for accumulated damage [48]. In many studies on thermal stress, constant temperatures are applied [19, 25, 41] which could incorrectly estimate behavior [49], thermal tolerance [38, 50-52] metabolic rate [53], development [39], and body mass [38] as well as fitness performance [54]. High constant temperatures prevent repair from heat lesions during mild intervening conditions, potentially underestimating thermal responses [55, 56]. On the other hand, the negative impacts of daily maximum temperatures might be overlooked if constant conditions minimize mortality [57, 58]. Therefore, studies on the effects of ecologically relevant variability in temperature including heat waves on thermal physiological performance and fitness are important [59]. Studies that incorporate more complex thermal regimens are necessary to predict acclimation capacities and/or evolutionary adaptation of thermal sensitivity in nature [60]. In evaluating the impact of heat events, temperatures need to vary in ecologically relevant ways [59] and the effects of heat events of varying duration and timing need to be considered across life stages [19].

Here we used the English grain aphid, *Sitobion avenae* (Fabricius), as a model species to investigate in detail the impact of different durations of heat stress during a life cycle, allowing temperatures to fluctuate and considering a range of fitness components. This aphid is an important global cereal pest common in temperate climates [61] with a short life cycle [62] which leads to overlapping generations and complex stage/age components in field populations. *S. avenae* populations usually peak in May around winter wheat crops of Beijing (39°48 N, 116°28 E) where a heat event involves consecutive hot days from one to five days duration (Fig. S1B). Given the increasing trend of heat events in the future, we created a series of heat events in which consecutive hot days from one to six days were simulated as daily cycles of sine-wave thermoperiods. The following questions are addressed. (1) What mortality patterns occur when heat stress occurs at different times and with different durations? (2) How are adult life history traits affected by heat stress periods at different times and of different durations, and are all components affected in the same way? (4) How do these findings translate into overall fitness effects at the population level? Our results demonstrate that mortality increased with the duration of heat events, but this also depends on the timing of the stress periods. Offspring numbers were decreased by heat events, but the effects on different fitness components varied. Overall the largest effects of heat events occurred when aphids were exposed at the early nymph stage.

2 MATERIALS AND METHODS

2.1 Stocks

English grain aphids were collected from a winter wheat field near Beijing (39°48 N, 116°28 E) as described by Zhao, Zhang, Hoffmann and Ma [56], and then were reared on winter wheat seedlings at 22 ± 0.5 °C, 50-60% relative humidity, and a photoperiod of 16 L : 8 D . Seedlings were replaced every week. Experiments were undertaken after this stock had been reared under these conditions for 2 years.

2.2 Temperature regimes

Daily temperature records for May in Beijing from 2014 to 2018 were downloaded from the China Meteorological Data Sharing Service System (Fig. S2A). The daily highest and lowest temperatures were used to set temperature fluctuations representing a hot day with a high minimum temperature and a normal day (Fig. S2A). These daily temperature changes of 20 - 35 °C and 13 - 28°C were mimicked in two growth chambers (RXZ-280B, Jiangnan Ltd., China) (Fig. S2B) designated as "hot" and "normal" growth chambers. The temperature regimen in each growth chamber involved changes at hourly intervals, which were recorded at 20-minute intervals using temperature/humidity data loggers (U23 - 001, Onset Ltd., USA). Relative humidity in the chambers fluctuated from about 40% (during the day) to 60% (during the night). Photoperiod was set to 16:8 (L: D) with lights on from 5.00 to 21.00 hours.

To clarify effects of the timing and duration of heat events, four timings [early nymph (NE), late nymph (NL), early adult (AE) as well as late adult (AL)] and six durations (1, 2, 3, 4, 5 and 6 consecutively hot days) were considered (Fig. 1).

2.3 Experiment protocol

At the beginning of the experiment, about 1250 newly born nymphs (<6h) were placed individually in a rearing tube consisting of a 5ml plastic tube (diameter 15mm, length 55mm) with 0.6% agar solution in one-third of the tube holding a newly-excised wheat leaf. They were then divided into 25 groups with about 50 nymphs/group.

Twelve groups were used immediately for exposure to heat at the nymphal stage. Six of these groups were put into the hot day chamber and another six were maintained in the normal day chamber (day 0, 21.00 h). On the next evening (day 1, 21.00 h), one group from the hot day chamber was transferred to the normal day chamber; at the same time, a group in the chamber of normal day was moved into the hot day chamber. This pattern was continued for six days and resulted in 12 nymphal treatments of timing × duration (Fig.1, NE and NL). On 21.00 h of the 7th day, all aphids were transferred into the normal day chamber and reared until the end of the experiment. The development and survival of nymphs were recorded twice a day at

09.00 and 21.00 h. When aphids had developed to the adult stage, the number of offspring and the survival of adults were checked daily at 21.00 h.

Another twelve groups were used for heat exposure at the adult stage. All aphids were reared in the normal day chamber until the eighth day (at 21.00 h), and then six groups were moved into the hot day chamber. At 21.00 h for the next six days, aphid groups in the two chambers were moved to obtain 12 adult treatments that varied in timing and duration (Fig. 1, AE and AL). At 21.00 h on the 15th day, all aphids were transferred into the normal day chamber and reared until the end of the experiment. The number of offspring and the survival of adults were checked daily at 21.00 h. As a control, a group of aphids was maintained in the normal day chamber until all individuals were dead (Fig. 1, CK). To minimize the effects of plant quality, plants in rearing tubes with aphids were renewed every day during the treatment period of hot days and every 3 days during the maintenance period of normal days.

2.4 Response variables and analysis

Apart from a few individuals that died before heat exposure, all aphids were used in the analysis. Lifespan of all individuals was defined as time from birth to death and immediate mortality after heat stress was defined as the death rate during a hot day treatment after a recovery of 24 h. Longevity (time from moulting to death), productivity (total number of nymphs produced) and productivity rate (number of nymphs per adult produced per day) were used as demographic variables to estimate effects of the hot events on survival individuals after 24h recovery. The effects of exposure on an overall fitness measure (r_m) based on mortality and longevity/reproduction was also calculated with PopTools 3.2.5 [63].

All statistical tests were performed using SPSS 20 (Chicago, Illinois, USA) or R 3.5.1. Fecundity variables (productivity and productivity rate) met assumptions for normality (Shapiro-Wilks test, $P>0.05$) and were therefore analyzed through parametric tests. Effects of timing and duration of heat events on each of the variables were analyzed using two-way ANOVA run with PROC GLM. Because of the significant interaction of timing and duration on traits (see below), we further compared the difference between stress duration periods within each timing treatment using one-way ANOVAs. Posthoc comparisons involved Dunnett or Games-Howell tests depending on whether treatment variances were equal or unequal. To define the impact of duration on the traits, we provide treatment – control means (i.e. control values were set to 0).

Overall survivorship (based on lifespan of all individuals including those that died after heat stress) against duration and timing of heat treatments was estimated and differences between timing treatments and the control were compared further by using the Cox regression procedure in the 'survival' and 'car' packages in R. To understand further the effects of heat treatments on survivorship, we analyzed immediate mortality after heat stress separately from adult longevity of surviving individuals (delayed mortality). The relationship between duration of hot days and immediate mortality was analyzed by fitting a linear regression model for each timing treatment. The difference between timing treatments in terms of regression slopes and intercepts was determined using one-way analysis of covariance (ANCOVA). Note that we also considered non-linear regressions in these analyses but duration effects were more appropriately treated as linear relationships. The same analysis was undertaken to consider duration effects on r_m.

3 RESULTS

3.1 Overall effects of heating treatments on survivorship

Duration had significant impacts on lifespan within the different life stages (Fig. 2; NE: $Z = 4.683$, $P < 0.001$; NL: $Z = 7.122$, $P < 0.001$; AE: $Z = 6.338$, $P < 0.001$; AL: $Z = 6.377$, $P < 0.001$). Compared with a mean lifespan 28.5 ± 8.6 days for the controls, mean lifespans were shortened the most by heat exposures at the NE stages (Fig2A, 12 - 15.5 days), but less so at the NL (Fig2B, 4 - 14.7 days), AE (Fig2C, 5 - 13.7 days) and AL (Fig2D, 5.5 - 11.5 days) stages. Mortality increased with an extended duration of hot days at all stages except NE; at this stage, even a single hot day was sufficient to sharply decrease survival (Fig. 2).

3.2 Immediate effects of heating treatments on survivorship

Overall, immediate mortality increased linearly with an increase in duration of hot days at all stages (Fig. 3; NE: R^2=0.939, P<0.001, NL: R^2=0.903, P=0.002, AE: R^2=0.928, P=0.001, AL: R^2=0.800, P=0.007). Comparisons between regression lines showed that there were significant differences between regression intercepts ($F_{3,19}$ = 12.958, P < 0.001) rather than slopes ($F_{3,16}$ = 1.633, P = 0.221). A posthoc comparison indicated that only the intercept of the NL treatment differed from the other treatments. Heat stress applied at the NL stage showed lower mortality than in the other treatments. Note that control mortality during the entire period was around 2%.

3.3 Delayed effects of heating treatments on adult lifespan

For longevity of surviving adults, there was a significant timing × duration interaction (Z = 3.542, P < 0.001). Duration of heat treatments had significant impacts on longevity within the life stages (Fig. 4; NE: Z = 5.660, P < 0.001; NL: Z = 4.928, P < 0.001; AE: Z= 5.661, P < 0.001; AL: Z= 6.220, P < 0.001). Compared with a mean longevity of 20.7 ± 8.6 days for the controls, mean longevities were reduced at the NE (Fig.4A, 1.8 - 7.7 days) and NL (Fig4B, 3.1 - 7.3 days) stages, and less so at the AE (Fig4C, < 4.7 days) and AL (Fig4D, < 3.7 days) stages. Longer heating treatments decreased lifespan at the NL stage, but showed the opposite trend at the NE stage (Fig. 4).

3.4 Fecundity costs of heating treatments: productivity and productivity rate

For productivity, there were significant effects of duration ($F_{5,573} = 3.131$, $P = 0.008$) and an interaction involving timing × duration ($F_{15,573} = 1.967$, $P = 0.016$) of hot days but no overall difference in timing treatments ($F_{15,573} = 1.453$, $P = 0.226$). Unlike for the longevity effects, negative effects of duration in heat stress were evident at all stages (Fig. 5A-5D; NE: $F_{6,178} = 3.491$, $P = 0.003$; NL: $F_{6,238} = 5.632$, $P < 0.001$; AE: $F_{6,170} = 4.709$, $P < 0.001$; AL: $F_{6,151} = 4.235$, $P = 0.001$). Compared with a mean productivity of 44.6 ± 17.5 nymphs/adult for the control, productivity was reduced at all stages (Fig.5A-5D; NE: 25.6 - 39.7 nymphs; NL: 27.7 - 43.2 nymphs; AE: 20.4 - 37.1 nymphs; AL: 26.9 - 39.1 nymphs). The negative effect increased with stress duration except in the case of NE.

There were significant effects of timing ($F_{3,573} = 32.816$, $P = 0.001$) and an interaction involving timing × duration ($F_{15,573} = 1.683$, $P = 0.050$) of heating treatments on productivity rate. In contrast to the other variables, increasing stress duration led to a decreasing productivity rate more strongly in the adult treatments (Fig. 6C and 6D; AE: $F_{6,170} = 12.245$, $P < 0.001$; AL: $F_{6,151} = 13.046$, $P < 0.001$) than in the nymph treatments (Fig. 6A and 6B; NE: $F_{6,178} = 3.649$, $P = 0.002$; NL: $F_{6,238} =$

1.066, P = 0.384).Compared with a mean productivity rate 2.5 ± 0.6 nymphs/adult/day for the control, productivity rate was reduced by stress particularly at the adult stages (AE:1.3 - 1.9 nymphs/adult/day ; AL: 1.5 - 2.0 nymphs/adult/day), and to a lesser extent at the nymph stages (NE :1.7 - 2.4 nymphs/adult/day; NL:2.1 - 2.6 nymphs/adult/day).

3.4 Effect of heat stress on fitness: intrinsic rate of population increase

Regression analyses indicated that r_m declined linearly with an increase of duration of hot days at all stages (Fig. 7; NEs: R^2=0.853, P=0.002, NLs: R^2=0.871, P=0.001, AEs: R^2=0.619, P=0.022, ALs: R^2=0.775, P=0.006). Comparisons between regression lines showed that there were significant differences between intercepts ($F_{3,23}$ = 6.759, P = 0.002) but not slopes ($F_{3,20}$ = 0.772, P = 0.523). A posthoc comparison indicated that only the intercept of the NL treatment differed from the other treatments. Heat stress applied at the NL stages showed higher r_m than when applied at the other stages.

4 DISCUSSION

Duration of heat treatments did have significant impacts on overall survival (lifespan) (Fig. 2) and immediate mortalities increased with the duration of heat events (Fig. 3) as expected from a likely increase in lesions with heat intensity/exposure time [48]. This pattern has been documented across taxa [64, 65]. The data also indicate differences in stage-specific heat tolerance with a relatively low lethality when only older nymphs were exposed, consistent with a previous study which also showed greater sensitivity in adults [18]. This pattern of stage-specific tolerance has been noted in many insects [66-68] including other aphids [12, 69, 70]. An increase of heat resistance at later instars might be related to the proliferation of facultative symbionts [71, 72] with host age [73]. The low level of adult tolerance might reflect trade-offs between heat tolerance and reproductive output [66, 74] or inherent differences in the capacity for thermo-regulation [75, 76].

Heat injuries can accumulate and be displayed at later stages which might result in delayed deaths [77, 78] or decreased adult performance [18, 19, 26]. In our experiment, a comparison of stress duration effects on survivors indicated that adult effects were contingent on exposed life-stage (Fig. 4 - Fig. 6). Heat stress reduced adult longevity when nymphs were exposed, but there were minor effects when adults were exposed (Fig.4). On the other hand, heat exposure at all timing treatments did have substantial effects on productivity (Fig. 5). Long-term heat exposure is known to depress reproduction through a shortened longevity [19, 56], a smaller body size [79] and/or decreased number of ovarioles [80, 81]. Given that the per day reproductive output was decreased following adult exposure, embryo production capacity may be decreased as a result of heat treatment.

Our results suggest that overall effects of heat stress on fitness are variable and likely involve a number of causes depending on the nature of the stress exposure. While effects from nymph exposures were driven at least partly by reductions in longevity/lifespan, those associated with adult exposure were due to other factors, even if the summed effects of these changes was similar. This highlights the usefulness of dissecting in detail such stress effects. Adult fecundity has previously been found negatively correlated with the total sum (across all life-stages) of heat stress which organisms experience during their entire life-cycle [19]. However, there is an underlying level of complexity that is not covered by such an approach. It is noteworthy that although productivities were reduced as expected with heat duration when adults or older nymphs were exposed, this was not a universal pattern across traits and stages, with increasing heat duration decreasing productivity when the exposed stage involved newly born nymphs, a life stage that is vulnerable to heat [18].

These results also raise the issue of whether resistant individuals could be selected in different ways when severe heat stress is imposed on sensitive immature stages. Previous studies have demonstrated that heat performance can be increased by local temperature environments [82-84] or laboratory selection [85-88] in which stress exposure is chronic [85, 89] or acute stress is imposed at some life-stage, particularly the adult stage [88, 90, 91]. Our results indicate that stress exposure at one stage has fitness consequences at a different stage, so that selection on fitness components at the adult stage could in fact be a consequence of heat events during an early stage in the life cycle of an organism. This suggests a level of complexity in evolutionary responses that needs to be considered when predicting the effects of thermal stress on populations.

In summary, we have shown that heat stress effects are not simply a consequence of exposure duration. While an increase in stress duration generally leads to an increase in negative effects on organisms, there is a level of complexity in this association depending on the life stage exposed and trait under consideration. We have shown that at least in one case an increase in duration improves subsequent performance of a fitness component. This means that measures of heat stress effects need to consider the way in which stress effects are characterized. It remains to be seen what underlying mechanisms drive these different effects.

ACKNOWLEDGMENTS

We thank Miss Xiu-qin Pei for assistance in completing experiments. This research was supported financially by the National Natural Science Foundation of China (31620103914 and 31272035), the Natural Science Foundation of Shanxi Province (2015011075) and the Foundation in Shanxi Academy of Agricultural Sciences (YBSJJ1512, yydzx16 and YCX2018D2BH5).

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Figure 1 Schematic diagram of experimental design (a 16-day long section). Four periods were identified with six duration patterns (NE1-6, NL1-6, AE1-6 and AL1-6) within each period. During the experiment, aphids were maintained as normal (blue bars) except for the periods of hot day treatments (red bars). Adult fitness components were measured on survivors.

Figure 2. Survival curves of all individuals in heating treatments applied at different stages of *S. avenae*. Exposure stages: NE=early nymph; NL= late nymph; AE= early adult; AL=late adult.

Figure 3. Association between heating treatments at each timing stage and immediately mortality during stress in *S. avenae*. Solid lines indicate linear regressions. Exposure stages: NE=early nymph; NL= late nymph; AE= early adult; AL=late adult.

Figure 4. Survival curves of longevity of survival individuals in heating treatments applied at different stages of *S. avenae*. Exposure stages: NE=early nymph; NL= late nymph; AE= early adult; AL=late adult.

Figure 5. Change in productivity (mean ± SE) in heating treatments at different stages of *S. avenae*. The "*" symbol represents a significant difference between each duration group and control group at the $P < 0.05$ level based on multiple comparisons (Dunnett or Games-Howell). Treated stages are plotted separately for NE = early nymph, NL = late nymph, AE = early adult, AL = late adult.

Figure 6. Change in productivity rate (mean ± SE) in heating treatments at different stages of *S. avenae*. See Figure 5 legend for explanation.

Figure 7. Linear regressions between intrinsic rate of population increase (r_m) and duration of hot days at different stages. Exposure stages: NE=early nymph; NL= late nymph; AE= early adult; AL=late adult.

The importance of timing of heat events for predicting the dynamics of aphid pest populations

Fei Zhao* Kun Xing Ary A. Hoffmann and Chun-sen Ma*

An interaction between timing and duration of heat wave is driving complex impacts on different fitness components and means its importance for predicting the dynamics of aphid pest populations.

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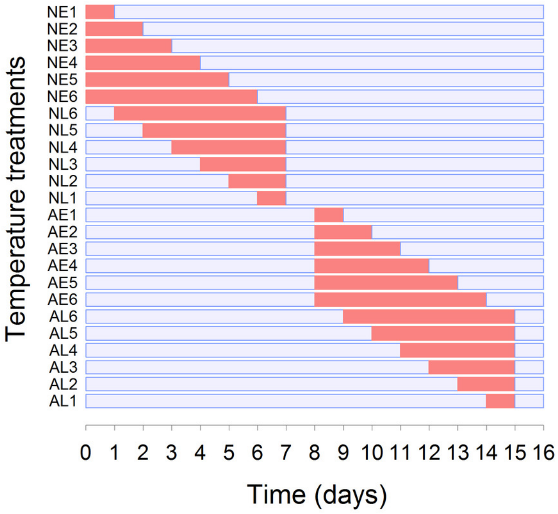

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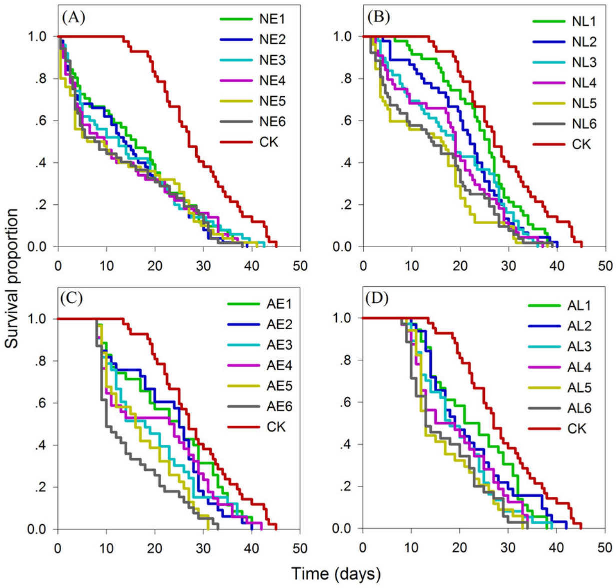

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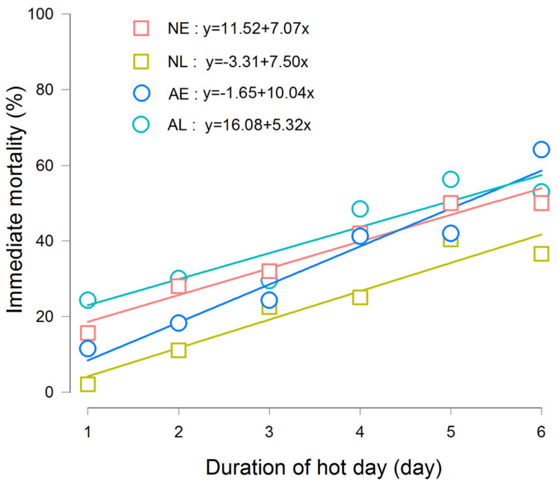

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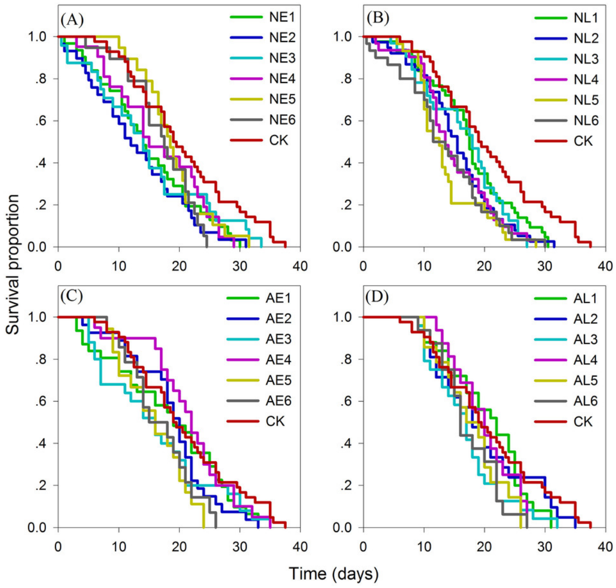

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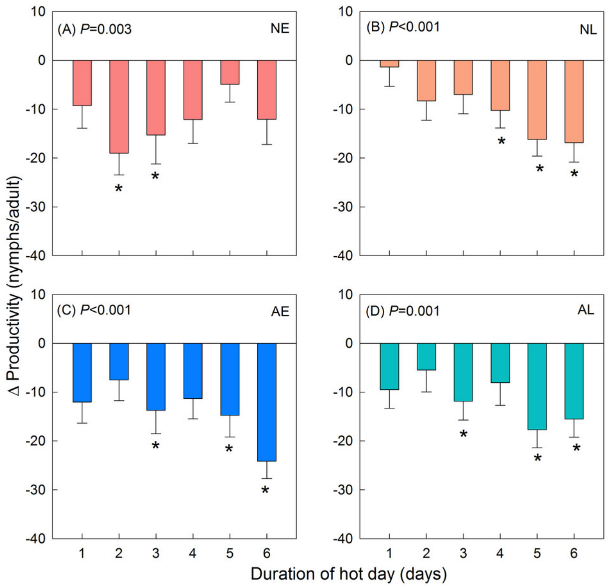

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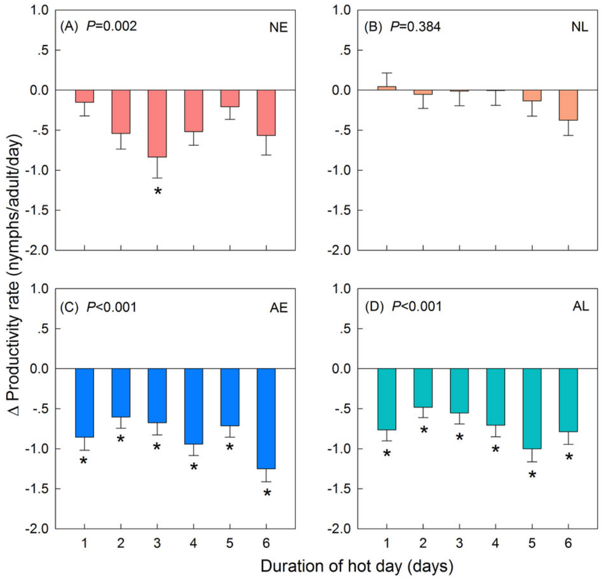

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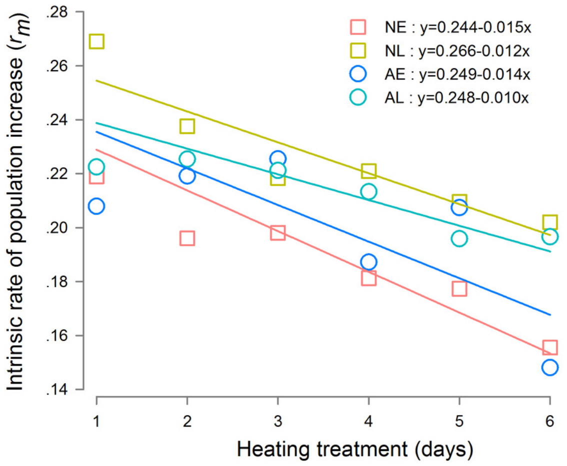

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