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Yuan-Jie Li, Si-Yang Chen, Lisa Bjerregaard Jørgensen, Johannes Overgaard, David Renault, H Colinet, Chun-Sen Ma

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**Authors' contributions**

**Yuan-Jie Li:** Investigation; Formal analysis; Writing – original draft; Writing – review & editing, **Si-Yang Chen:** Investigation, **Lisa Bjerregaard Jørgensen:** Formal analysis; Writing – review & editing; **Johannes Overgaard:** Formal analysis; Writing – review & editing, **David Renault:** Funding acquisition; Supervision; Writing – review & editing; **Hervé Colinet:** Conceptualization; Funding acquisition; Supervision; Writing – review & editing, **Chun-Sen Ma:** Conceptualization; Formal analysis; Funding acquisition; Supervision; Writing – original draft; Writing – review & editing

# 1 Interspecific differences in thermal tolerance landscape explain aphid community 2 abundance under climate change

3 Yuan-Jie Li<sup>a,b,c</sup>, Si-Yang Chen<sup>b</sup>, Lisa Bjerregaard Jørgensen<sup>d</sup>, Johannes Overgaard<sup>d</sup>, David  
4 Renault<sup>c</sup>, Hervé Colinet<sup>c</sup>, Chun-Sen Ma<sup>a,b,\*</sup>

5 <sup>a</sup> School of Life Science, Institute of Life Science and Green Development, Hebei University, Baoding  
6 071002, China

7 <sup>b</sup> Climate Change Biology Research Group, State Key Laboratory for Biology of Plant Diseases and  
8 Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No 2,  
9 Yuanmingyuan West Road, Haidian District, Beijing 100193, China

10 <sup>c</sup> UMR CNRS 6553 ECOBIO [(Ecosystèmes, biodiversité, évolution)], Université Rennes 1, avenue du  
11 Général Leclerc, 35042 Rennes cedex, France

12 <sup>d</sup> Zoophysiology, Department of Biology, Aarhus University, 8000 Aarhus C, Denmark

13 \*Corresponding author: machunsen@caas.cn

## 14 Abstract

15 A single critical thermal limit is often used to explain and infer the impact of climate change on  
16 geographic range and population abundance. However, it has limited application in describing the  
17 temporal dynamic and cumulative impacts of extreme temperatures. Here, we used a thermal  
18 tolerance landscape approach to address the impacts of extreme thermal events on the survival  
19 of co-existing aphid species (*Metopolophium dirhodum*, *Sitobion avenae* and *Rhopalosiphum*  
20 *padi*). Specifically, we built the thermal death time (TDT) models based on detailed survival  
21 datasets of three aphid species with three ages across a broad range of stressful high (34~40°C)  
22 and low (-3~-11°C) temperatures to compare the interspecific and developmental stage variations  
23 in thermal tolerance. Using these TDT parameters, we performed a thermal risk assessment by  
24 calculating the potential daily thermal injury accumulation associated with the regional  
25 temperature variations in three wheat-growing sites along a latitude gradient. Results showed that  
26 *M. dirhodum* was the most vulnerable to heat but more tolerant to low temperatures than *R. padi*  
27 and *S. avenae*. *R. padi* survived better at high temperatures than *Sitobion avenae* and *M.*  
28 *dirhodum* but was sensitive to cold. *R. padi* was estimated to accumulate higher cold injury than  
29 the other two species during winter, while *M. dirhodum* accrued more heat injury during summer.  
30 The warmer site had higher risks of heat injury and the cooler site had higher risks of cold injury  
31 along a latitude gradient. These results support recent field observations that the proportion of *R.*  
32 *padi* increases with the increased frequency of heat waves. We also found that young nymphs  
33 generally had a lower thermal tolerance than old nymphs or adults. Our results provide a useful

34 dataset and method for modelling and predicting the consequence of climate change on the  
35 population dynamics and community structure of small insects.

36 **Keywords:** Extreme temperature, Thermal death time curve, Cereal aphids, *Sitobion avenae*,  
37 *Rhopalosiphum padi*, *Metopolophium dirhodum*

## 38 Introduction

39 Climate change leads to more frequent extreme temperature events (Perkins-Kirkpatrick and  
40 Lewis 2020, Arias et al. 2021), which trigger consequences from individual physiology to  
41 ecosystem functions (Ma et al. 2021; Secretariat et al. 2021). Insects' body temperature is mainly  
42 determined by the microclimates of their habitats, making them highly sensitive to temperature  
43 changes (Pincebourde and Woods 2020). Exposure to a short-term extreme temperature that  
44 reaches or exceeds critical thermal limits can cause an immediate effect on fecundity, fertility and  
45 even mortality (Zhang et al. 2013; Liang et al. 2014; Sales et al. 2021). Therefore, thermal  
46 tolerance is often correlated with population abundance, species distribution, and phenology, and  
47 thus plays an important role in predicting the effect of climate change (Sunday et al. 2012;  
48 Amundrud and Srivastava 2020; Alruiz et al. 2022).

49 A single temperature estimated from static or dynamic assays has been widely adopted as an  
50 upper or lower thermal tolerance limit (Winther Bak et al. 2020). However, the critical thermal limit  
51 depends on the methodological context including the exposure time (Rezende et al. 2014;  
52 Terblanche et al. 2007). Moreover, this limit, which represents a static snapshot of thermal  
53 tolerance, is not able to describe the temporal dynamic and cumulative impact of environmental  
54 extreme temperatures on survival (Alruiz et al. 2022).

55 Thermal Death Time (TDT) curve, which describes that the tolerable duration at stressful  
56 temperatures exponentially decreases with increasing stress intensity, can fully and accurately  
57 characterize species' thermal tolerance (Bigelow 1921; Fry et al. 1946; Nedvěd et al. 1998;  
58 Colinet et al. 2011; Rezende et al. 2014; Jørgensen et al. 2019; Tarapacki et al. 2021). The TDT  
59 framework has been recently re-examined and parameterized to enable the analysis of the  
60 combined impact of extreme temperatures and exposure times on survival, which is now referred  
61 to as the "thermal tolerance landscape". (Rezende et al. 2014; Jørgensen et al. 2019; Jørgensen  
62 et al. 2021; Ørsted et al. 2022). Furthermore, the injury accumulation rate increases exponentially  
63 with temperature and the injury sustained at different static temperatures can be completely  
64 additive (Jørgensen et al. 2021; Ørsted et al. 2022). Thus, through the TDT model, we can not  
65 only get a holistic view of how thermal tolerance duration varies with temperatures (Tarapacki et  
66 al. 2021; Willot et al. 2022), but also link the thermal tolerance to the injury accrued under natural

67 temperatures. However, few studies employ this approach to extrapolate the thermal tolerance  
68 estimated in the laboratory to natural settings.

69 The same dose of exposure to thermal extremes might lead to different reactions among co-  
70 existing species (Ma et al. 2015a; Ma et al. 2015b) because some species can be more stress-  
71 resilient than others (Kaspari et al. 2015; Buckley and Huey 2016). The interspecific differences  
72 in thermal tolerance can shift the community structure through cascading effects (Harvey et al.  
73 2020; Filazzola et al. 2021), altering the relative abundance of coexisting insect species (Ma et al.  
74 2015b; Birkett et al. 2018; Zhu et al. 2021). This is particularly important for practice in agricultural  
75 pest management (Mohammed et al. 2019). In addition, the natural population of insects  
76 commonly consists of multiple life stages or age cohorts that have different thermal tolerance  
77 (Bowler and Terblanche 2008; Zhang et al. 2015; Zhao et al. 2019), even the stages living in the  
78 same microhabitat also show different heat tolerance (Zhao et al. 2017). When the mixed-stage  
79 population experiences extreme temperature events, the most sensitive stages are likely to be  
80 injured and die, whereas the most tolerant may survive and develop to the adult stage for  
81 reproduction during the following mild phases, thereby contributing to population recovery (Ma et  
82 al. 2021). Therefore, clarifying the thermal tolerance in different developmental stages is essential  
83 for understanding the ecological consequences of extreme temperature events.

84 Here we used three species of co-existing global pests (family Aphididae) in cereal crops, *Sitobion*  
85 *avenae* (Fabricius, 1775), *Rhopalosiphum padi* (Linnaeus, 1758), and *Metopolophium dirhodum*  
86 (Walker, 1849) as focus species. We aimed to answer the following questions: 1) can the change  
87 in aphid community abundance be related to interspecific differences in the basal thermotolerance  
88 of the three aphid species? 2) do older aphids suffer less than younger ones from heat/cold stress?  
89 To answer these questions, we conducted an elaborate study to characterize the interspecific  
90 variation in thermal tolerance among the three cereal aphid species using the TDT framework at  
91 both high and low temperatures. Then, we examined the effect of developmental stage/age on  
92 the intraspecific variations in thermal tolerance. Finally, we used the TDT model parameters to  
93 perform a risk assessment predicting the potential injuries under the natural environments for  
94 three aphid species in three sites along a latitude gradient in China.

## 95 **2. Material and methods**

### 96 **2.1 Aphid stock rearing**

97 Populations of three common aphid species developing on winter wheat, *S. avenae*, *M. dirhodum*  
98 and *R. padi* were originally collected in May 2010 from wheat fields near Beijing (39°48 N, 116°28  
99 E) (Zhao et al. 2014). The colonies were then refreshed annually with field-collected aphids. The

100 aphids were reared in screened cages (60 × 60 × 60 cm) at 22 ± 0.5 °C, RH 70%, and a  
101 photoperiod of 16 L: 8 D. Aphids were fed on 10 –20 cm tall winter wheat seedlings that were  
102 renewed weekly. Aphids used for experiments were prepared by placing newly molted adult  
103 aphids in a pot with wheat seedlings at 20 °C. After 24 hours, the apterous adult aphids were  
104 removed so that only newly-born nymphs remained. Subsequently, these nymphs were allowed  
105 to develop for 2, 6, 12 days to generate three different stages: 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup> instar (depending on  
106 species) and adult, respectively.

## 107 **2.2 Tests of heat and cold survival**

108 For heat tolerance tests, a multifactorial experiment involved the three aphid species × three ages  
109 (2, 6, 12 days) × seven temperature regimes (34, 35, 36, 37, 38, 39, 40°C) × ≥ five exposure  
110 times (ranging from 0 to 2000 min depending on the temperature) was conducted. Similarly, a  
111 multifactorial experiment for cold tolerance included the three aphid species × three ages (2, 6,  
112 12 days) × nine temperature regimes (-3, -4, -5, -6, -7, -8, -9, -10, -11°C) × ≥ five exposure times  
113 (ranging from 0 to 10162 min depending on the temperature) was conducted. All the treatments  
114 were replicated at least 3 times, and for each replicate, 7-12 aphids were tested (Table S1). To  
115 obtain a reliable survival curve at each temperature, we tried to find and test the exposure times  
116 that result in survival rates close to 0%, 25%, 50%, 75%, and 100%, respectively. To reduce  
117 handling stress and provide food as well as a water source, we cut off the wheat leaves with  
118 aphids and then transferred them directly into glass vials (10 mL). Then, we closed each vial with  
119 a dry sponge and placed a rack with vials into a glycol bath (Ministat 230-cc-NR; Huber Ltd.,  
120 Germany, accuracy ± 0.01 °C) pre-set at the experimental temperature. For each temperature  
121 and exposure time, the exposed aphids were removed from the bath and allowed to recover at  
122 20 °C for 24h before checking the survival of each individual. Aphids were considered alive if the  
123 individual moved following a gentle tactile stimulation with a brush. In total, 14980 and 14633  
124 individuals were tested, for heat and cold tolerance, respectively, corresponding to 1502 and 1536  
125 replicates.

## 126 **2.3 Survival curves, $Lt_{50}$ , and Thermal Death Time (TDT) curves**

127 For each tested temperature, the survival proportion was fitted to a logistic regression model using  
128 the *glm* function and the  $Lt_{50}$  (i.e. the temperature-specific exposure duration resulting in 50%  
129 mortality) was estimated using the *dose.p* function in the R package "MASS" (Ripley et al. 2013).  
130 TDT curves were generated by regressing  $\log_{10}(Lt_{50})$  against test temperature for each  
131 species/age-group combination, at both high and low temperatures. The output of the TDT

132 analysis is typically given by model estimates of temperature causing 50% mortality after a  
 133 specific time (e.g., the temperature that causes 50% mortality after 1 hour), as well as the thermal  
 134 sensitivity, which is often expressed from  $z$  ( $-1/\text{slope}$ ). The latter parameter reveals how many  
 135 degrees are required to change tolerance duration 10-fold (Rezende et al. 2014). Note that for  
 136 cold tolerance, thermal sensitivity is expressed by  $z'$  which corresponds to  $1/\text{slope}$ .

#### 137 **2.4 Examining effects of species and age**

138 The effect of test temperature, species, and age on  $Lt_{50}$  was examined in a three-way ANCOVA  
 139 including all interaction terms, with test temperature as a continuous variable and species and  
 140 age as categorical variables. Interspecific differences in heat or cold tolerance were examined  
 141 independently for each age group by comparing differences in the parameters of the TDT curves.  
 142 Specifically, slopes of the TDT curves (thermal sensitivity) were first compared pairwise using  
 143 the functions *emtrends()* and *pairs()* in the R package “emmeans” (Lenth, 2019), which are based  
 144 on least-squares means. Further, we tested whether the intercepts (Log-transformed  $Lt_{50}$  estimate)  
 145 differed between species by comparing the intercept at 37 °C for heat tolerance and -7 °C for cold  
 146 tolerance using the function *emmeans()* in “emmeans” package (Lenth, 2019). These  
 147 temperatures were chosen as they represented intermediate values in the range of temperatures  
 148 used to assess heat and cold tolerance, respectively. A similar approach, comparing slopes and  
 149 intercepts, was used to examine the effects of aphid age on both heat and cold tolerance for each  
 150 species. All data analyses were performed in R version 4.1.2.

#### 151 **2.5 Relating TDT curves to natural temperature regimes**

152 Following the method described by Jørgensen et al. 2021, we used parameters of TDT curves  
 153 (slope and intercept) to estimate the cumulative daily thermal injury experienced by aphids during  
 154 natural thermal fluctuations. This basic risk analysis has of course some limits, for instance, it  
 155 ignores the importance of microhabitat choice and behavioral thermoregulation by using the  
 156 simple assumption that aphid body temperature is equal to air temperature. Further, this analysis  
 157 does not consider if cold tolerance changes seasonally due to acclimatization to winter or summer  
 158 conditions. Despite these caveats, this simple approach can provide a raw proportional risk  
 159 assessment to compare species or regions based on both the intensity and duration of thermal  
 160 extremes. Specifically, it calculates, minute by minute, the accumulation of thermal stress over 24  
 161 hours (1440 min) using the formula:

$$162 \quad \text{Accumulated injury} = \sum_{i=1}^{i=1440} \frac{100 \cdot (t_{i+1} - t_i)}{10^{(\beta \cdot \max(T_i; T_{i+1}) + \alpha)}}$$



163 where  $\alpha$  is the slope and  $\beta$  is the intercept of the TDT curve,  $t_i$  and  $t_{i+1}$ , are the  $i$  minute and the  
 164  $i+1$  minute, respectively,  $\max(T_i; T_{i+1})$  is the maximum temperature between the  $i$  and  $i+1$  minute;  
 165 for winter, it is the  $\min(T_i; T_{i+1})$ , the minimum temperature between the  $i$  and  $i+1$  minute. Note that  
 166 thermal injuries only accumulate in the stressful temperature range (above a critical temperature  
 167  $T_c$  for heat stress or below a critical temperature  $T_c'$  for cold stress) (Jørgensen et al. 2021, Ørsted  
 168 2022). We defined  $T_c$  or  $T_c'$  as the temperature estimated to cause 50% mortality ( $Lt_{50}$ ) in one  
 169 week. Specifically, we calculated this  $T_c$  or  $T_c'$  from the TDT curves. For 6-day-old *M. dirhodum*,  
 170 *R. padi* and *S. avenae*,  $T_c$  was estimated as 25.29°C, 29.34 °C and 28.82 °C, respectively and  
 171 cold  $T_c'$  was -5.15°C, -1.81 °C and -4.79°C, respectively.

172 To associate thermal tolerance measures to natural climate conditions, we downloaded hourly  
 173 temperature data for winter (from November to February, 2016/2017) and summer (from May to  
 174 August, 2016) months from three sites in China where grow winter wheat infested by cereal aphids:  
 175 southern site (Wuhan, N 30.78°, E 114.21°), intermediate site (Xinxiang, N 35.30°, E 113.92°) and  
 176 northern site (Beijing, N 40.07°, E 116.58°) (Figure 4A). Data were downloaded from a global  
 177 weather API (<https://www.visualcrossing.com/weather-data>) in May 2022. We calculated minutely  
 178 temperature by linear interpolation from the hourly temperature using the `na.approx` function in  
 179 the “zoo” package (Zeileis et al. 2014). The accumulated daily heat stress dose was calculated  
 180 from midnight to midnight and the accumulated daily cold stress dose from noon to noon. In total,  
 181 this analysis produced 122 estimates of daily heat stress doses and 120 estimates of daily cold  
 182 stress doses for each species and region.

183 We used Kruskal-Wallis Rank Sum Tests followed by Pairwise Wilcoxon Rank Sum Tests with a  
 184 Benjamini-Hochberg correction to compare the differences in the calculated daily accumulated  
 185 injury between species within sites and sites with the same species. Note that to avoid comparing  
 186 a lot of days without any injury accumulation, only days with injury accumulation were compared.

### 187 3. Results

#### 188 3.1 Survival curves, TDT curves: estimation of critical thermal limits

189 The survival curves generally fitted the logistic function well, with a median  $R^2$  of 0.81 (Fig. 1A  
 190 and B, 74% of assays had McFadden’s pseudo- $R^2 > 0.70$ , See Table S2). For both heat and cold  
 191 tolerance, the estimated  $Lt_{50}$  decreased exponentially with increasing stress intensity  
 192 (temperature) as predicted by the TDT curves (Figure 1C and D). Linear regressions of  $\log_{10}(Lt_{50})$   
 193 against temperature generated nine TDT curves (three species  $\times$  three ages) for high and low  
 194 temperatures respectively. All curves had a high determination coefficient ( $R^2$ ) ranging from 0.78  
 195 to 0.99 ( $P < 0.01$ ), especially for those at high temperatures (see Table 2 and Table 3).



196 Here, we used the temperature causing 50% mortality after 1 hour ( $CT_{\max(1h)} / CT_{\min(1h)}$ ) as the  
197 upper or lower critical thermal limit. *R. padi* ranked first in  $CT_{\max(1h)}$  (36.76°C ~ 37.29°C in different  
198 ages), 0.97~1.94°C higher than *M. dirhodum* (Table 1). The thermal sensitivity at high  
199 temperatures ranged from 3.19 to 4.17 (Table 1).  $CT_{\min(1h)}$  fell between -8.24 ~ -2.84 °C (Table 2).  
200 Aphids had high cold sensitivity as the lower  $z'$  values indicated greater thermal sensitivity (i.e., a  
201 larger change in  $Lt_{50}$  with a change in exposure temperature).

### 202 **3.2 Interspecific variation in thermal tolerance**

203 The effects of temperature, species, and age on  $Lt_{50}$  were examined in a three-way ANCOVA  
204 including all interaction terms. This analysis found that species interacted with both age and  
205 temperature at both high and low temperatures (Table S3 and S4). Accordingly, we split the  
206 dataset according to age so that TDT slopes and intercepts from different species could be  
207 compared. *R. padi* generally had the highest heat tolerance, as depicted by the significantly higher  
208  $\log_{10}(Lt_{50})$  value (1.71~1.86) at 37 °C for all age groups, followed by *S. avenea*, and then *M.*  
209 *dirhodum* (Table 1, see p-values in Table S5). The thermal sensitivity ( $z$ ) showed few interspecific  
210 differences; *M. dirhodum* was characterized by a lower thermal sensitivity (higher  $z$ ) than *R. padi*  
211 and *S. avenea* (Table 1, See p-values in Table S6).

212 For cold tolerance, *R. padi* showed a significantly lower  $\log_{10}(Lt_{50})$  at -7°C for all age groups than  
213 the other two species (Table 2, see p-values in Table S7). The difference between *M. dirhodum*  
214 and *S. avenae* was relatively small. The thermal sensitivity ( $z'$ ) did not differ significantly among  
215 species, except for 12-day adults of *R. padi* that had a statistically higher  $z'$  (lower thermal  
216 sensitivity) than *M. dirhodum*. (Table 2, See p-values in Table S8).

### 217 **3.3 Developmental stage variation in thermal tolerance**

218 Adults (12-d-old) or old nymphs (6-d-old) generally had higher thermal tolerance than young  
219 nymphs(2-d-old). For *M. dirhodum* and *S. avenea*, adults (12-d-old) had a lower  $z$ -value (higher  
220 thermal sensitivity) and longer  $\log_{10}(Lt_{50})$  at 37 °C than younger life stages, although this was only  
221 statistically significant for *M. dirhodum* (Table 1). For *R. padi*, old nymphs (6-d-old) were the most  
222 tolerant among all life stages (Table 1).

223 For cold tolerance, none of the species displayed a significant difference in thermal sensitivity ( $z'$ )  
224 between ages. Adult (12-d-old) *R. padi* showed a significantly lower  $\log_{10}(Lt_{50})$  at -7 °C than  
225 nymphs (Table 2, see p-values in Table S7).

### 226 **3.4 Relating TDT curves to natural temperature regimes**

227 To associate thermal tolerance measures with natural climate conditions, we calculated the  
 228 accumulated thermal injury of three aphid species in three age groups using the TDT model and  
 229 regional temperatures. Results were similar for all three age groups (2-d-old, 6-d-old, 12-d-old,  
 230 see Figure S3-6), thus here we only present the result of 6-day-old aphids (Figure 2-4).

231 In each site, the estimated heat injury differed among species ( $\chi^2 = 53.3$ ,  $df = 2$ ;  $p < 1*10^{-11}$  in  
 232 Beijing;  $\chi^2 = 36.0$ ,  $df = 2$ ;  $p < 1*10^{-7}$  in Xinxiang;  $\chi^2 = 9.0$ ,  $df = 2$ ;  $p < 0.05$  in Wuhan, Figure 2B). *M.*  
 233 *dirhodum* always accumulated more heat injury than the two other species in Beijing and Xinxiang  
 234 (Pairwise Wilcoxon Rank Sum Tests,  $p < 0.05$ ).

235 According to the calculation, *R. padi* might suffer cold injury across the whole winter months while  
 236 the two other species might be affected only during the coldest days (Figure 3A). Estimated  
 237 accumulated cold injury differed among species in Beijing ( $\chi^2 = 8.5$ ,  $df = 2$ ;  $p < 0.05$ ; Figure 3B).  
 238 Specifically, *R. padi* had higher daily injury accumulation than *S. avenae* in Beijing (Pairwise  
 239 Wilcoxon Rank Sum Tests,  $p < 0.05$ ), while no significant interspecific difference was found in  
 240 Xinxiang. *M. dirhodum* and *S. avenae* might not accumulate any injury in Wuhan during winter  
 241 (Figure 3B).

242 According to the calculation, heat injury of three species might differ with sites ( $\chi^2 = 6.4$ ,  $df = 2$ ;  $p$   
 243  $< 0.05$  for *M. dirhodum*;  $\chi^2 = 9.9$ ,  $df = 2$ ;  $p < 0.01$  for *S. avenae*;  $\chi^2 = 10.6$ ,  $df = 2$ ;  $p < 0.01$  for *R.*  
 244 *padi*, Figure 4C). In Beijing, the northernmost site, aphids accumulated less injury during summer  
 245 (Pairwise Wilcoxon Rank Sum Tests,  $p < 0.05$ , Figure 4C) but more injury during winter than the  
 246 other two sites (Pairwise Wilcoxon Rank Sum Tests,  $p < 0.05$ , Figure 4D).

### 247 **Discussion**

248 The previous studies often inferred the impact of climate change on the coexisting species based  
 249 on a single estimate of thermal tolerance, such as  $CT_{max}$  and  $CT_{min}$ , or  $LT_{50}$  and  $Lt_{50}$  (Warren and  
 250 Chick 2013; Birkett et al. 2018). High-tolerant species will increase their population or expand  
 251 their range, while low-tolerant species will decrease their population or shrink their range under  
 252 extreme climate scenarios. However, thermal tolerance is not constant but varies with the dose  
 253 determined by temperature and exposure duration as shown in our study. In nature, extreme high  
 254 and low temperatures occur regularly in a diurnal and seasonal cycle, and irregularly across time  
 255 and space, thereby altering the mortality of insects both dynamically and cumulatively. The  
 256 thermal tolerance represented with a single temperature value can be hardly used to calculate  
 257 the dynamic and cumulative thermal injury. Studies that assess demographic performances of co-  
 258 existing species under limited temperature scenarios (e.g., Ma et al. 2015b) may not fully reflect

259 the abundance responses to arbitrary fluctuating temperatures in nature. Here we measured the  
260 mortality under a broad range of extreme high/low temperatures and multiple exposure durations,  
261 thus providing a solid dataset to build the TDT model or to do other analyses for the impact of  
262 extreme climate on cereal aphid species. Through the group of survival curves and TDT model,  
263 we can have a holistic and comprehensive view of the interspecific and ontogenetic variations in  
264 thermal tolerance. Moreover, using the values of TDT parameters, we made a thermal stress risk  
265 assessment of three aphid species in natural temperature regimes. Our data and approach  
266 provide a framework for calculating the cumulative thermal mortality of aphids at different ages at  
267 a given time (from one day to a year), thus could quantitatively estimate the relative abundance  
268 of co-existing species.

### 269 ***TDT curves, interspecific and ontogenetic variations in thermal tolerance***

270 Here we aimed to examine if/how both thermal stress intensity and duration affected the survival  
271 of three cereal aphid species. We found strong linear relationships between high/low  
272 temperatures and log-transformed  $Lt_{50}$  across different ages of three aphid species as in other  
273 ectotherms (Jørgensen et al. 2022). The steep slope (low  $z'$  value) of TDT curves from our data  
274 at low temperatures is similar to the typical freezing time-temperature curve (Salt 1966), which  
275 indicates that the cold tolerance we measured might reflect the susceptibility to freezing rather  
276 than chill injury (Tarapacki et al., 2021).

277 It is intuitive to compare interspecific tolerance using a single value, such as  $CT_{max}$ ,  $CT_{min}$ ,  $LT_{50}$ ,  
278 or  $Lt_{50}$ . However, we should realize that interspecific differences in thermal tolerance are not  
279 constant but change with the thermal dose (intensity and exposure duration). The TDT curves of  
280 three aphid species at high temperatures converge due to their different slopes (thermal sensitivity)  
281 (Figure 1C), which means the  $Lt_{50}$  values of the three species are getting closer with increasing  
282 intensity. Even, the interspecific differences in tolerate duration can reverse depending on the  
283 temperature, for example, at temperatures below  $-9^{\circ}C$ , *S. avenae* could sustain a longer duration  
284 than *M. dirhodum* (Figure 1D). However, our results generally showed that *R. padi* survived longer  
285 at high but shorter at low temperatures, indicating it was the most heat-tolerant but cold-vulnerable  
286 species. Whereas *M. dirhodum* performed as the most heat-vulnerable but cold-tolerant species.  
287 These results are supported by the previous studies under lifetime constant temperature  
288 exposures or the static thermal tolerance experiments (Asin and Pons 2001; Alford et al. 2014;  
289 Alford et al. 2016).

290 We found that the effect of the developmental stage was similar at both high and low temperatures.  
291 The old nymphs survived longer than young nymphs at either high or low temperatures in the

292 three aphid species. However, the thermal tolerance of adults was species-specific. For *S. avenae*  
293 and *M. dirhodum*, adults (12-d-old) showed high tolerance, but the tested adults of *R. padi* were  
294 extremely sensitive to cold stress. This pattern was also found by Alford et al 2014. Typically,  
295 thermal tolerance is highest at eclosion and then declines during the early days of adult life  
296 (Bowler and Terblanche 2008; Colinet et al. 2013). In our case, the development rate of *R. padi*  
297 was faster than the other two species, thus 12-d-old *R. padi* was at the 3<sup>rd</sup> or 4<sup>th</sup> day of adult life  
298 (but 1<sup>st</sup> or 2<sup>nd</sup> day for *S.avenae* and *M.dirhodum*). Therefore, the intolerant 12-d-old *R. padi* might  
299 be resulting from a rapid decline of thermal tolerance in adult life, as observed in other insects  
300 (Bowler 1967, Davison 1969, Colinet et al. 2013).

### 301 ***Relating TDT curves to natural temperature regimes***

302 The injury risk analysis showed that *R. padi* was the least affected by high temperatures during  
303 summer, which seems to fit the field observation showing that the relative abundance of *R. padi*  
304 increased with extremely high-temperature frequency in south area (Ma et al. 2015b, Zhu et al.  
305 2021). In Wuhan, *S. avenae* has the largest proportional abundance (78%) in 2008 when hot-day  
306 (daily mean temperature  $\geq 30$  °C) frequency was low (Ma et al. 2015b). However, *R. padi*  
307 became the most abundant species (79%) in the aphid community in 2011 when hot days  
308 occurred more frequently (Ma et al. 2015b). In the middle region (near Xinxiang), the proportional  
309 abundance of *R. padi* has increased from 13% in 1998 to 83% in 2012 (Zhu et al. 2021). While in  
310 north sites (near Beijing), *S. avenae* was still the most abundant species which accounted for  
311 89.8% in 2015 and 73.0% in 2016, followed by *R. padi* (9.26% and 14.3% in 2015 and 2016) and  
312 *M. dirhodum* (0.01% and 11.81% in 2015 and 2016) (Yang et al. 2017). *R. padi* might become  
313 the most abundant species, outcompeting *S. avenae* in a warmer future (Zhu et al. 2021).  
314 Conversely, *M. dirhodum* is the least abundant species (Yang et al. 2017) owing to its lower  
315 tolerance to high temperatures. It appears in the early spring and leaves the wheat field when the  
316 temperature warms up (Chen et al. 1994).

317 It is also interesting to see the differences in the calculated accumulated injuries of aphids  
318 between the three sites, which represent a climatic gradient from cold to hot climates. Aphids  
319 accumulated more heat injuries in Wuhan, the southmost site, than Xingxiang and Beijing owing  
320 to the longest duration of exposure to high temperatures during summer months (the durations  $\geq$   
321 30 °C in Wuhan, Xinxiang, and Beijing were 868h, 736h, and 611h, respectively, see supporting  
322 information Table S9). To some extent, this result underlines the importance of exposure duration  
323 to stressful temperatures when assessing the impact of thermal stress.

324 Winter temperature varied greatly in the three sites: Beijing, the northmost site, had a minimum  
325 temperature of  $-13^{\circ}\text{C}$  and an average daily temperature of  $0.6^{\circ}\text{C}$  whereas winter in Wuhan was  
326 mild with an average daily temperature of  $8.7^{\circ}\text{C}$  (Table S9). Aphids could suffer from cold injury,  
327 manyfold the dose resulting in 50% mortality in Beijing, which implied that the three species may  
328 not be able to overwinter as nymphs in the winter wheat field here. Indeed, it had been reported  
329 that the geographical overwinter limit for *S. avenae* and *R. padi* was the  $0^{\circ}\text{C}$  isotherm in January  
330 ( $\text{N } 35^{\circ}$ , near Xinxiang) (Yang and Yang 1991, Luo et al. 1994). Instead, they might be able to  
331 overwinter in Wuhan where they hardly get injured during winter and then migrate to the north  
332 region at the start of spring (Li et al. 2014).

333 Over the past 5 decades (1961–2013), China's temperature increased by  $1.44^{\circ}\text{C}$  (90%  
334 confidence interval  $1.22\text{--}1.66^{\circ}\text{C}$ ) (Sun et al. 2016). There was a general increase in extreme  
335 warm indices (such as warm days, warm nights,  $T_{\text{max}}$ ) and a significant reduction of cool nights  
336 and cool days during winter (Sun et al. 2016). Thus, the changes in aphid community abundance  
337 that had taken place in the south area would probably push northward in the future.

### 338 **Limitations**

339 Although the risk analysis shows very coherent patterns that seem to match with field  
340 observations, it is, however, important to recognize that such a model has some limitations, in  
341 particular, it does not consider i) the difference between air and insects' body temperature and ii)  
342 the capacity of insects to relocate into more permissive microclimates (i.e. avoidance) (Ma and  
343 Ma 2012a, b, Woods et al. 2015, Pincebourde and Suppo 2016, Ma et al. 2018b). In addition, the  
344 approach does not consider if thermal tolerance changes seasonally with acclimation (Overgaard  
345 et al. 2008, Bujan et al. 2020), nor the possibility that changes in feeding behavior could be altered  
346 to provide on-time nutritional adjustments aiding physiological changes (for instance: synthesis of  
347 compatible solutes, acquisition of antioxidant molecules). Furthermore, we calculated the daily  
348 injury accumulation with the assumption that aphids that survived the thermal stress could recover  
349 quickly during non-stressful temperature regimes (cooler nights for heat injury and warm noon for  
350 cold injury). While this recovery phase is true and well described (see discussion in Colinet et al.  
351 2015), the capacity to fully recover depends on the level of thermal injury and the repair  
352 temperature and duration (Colinet et al. 2011, Ma et al. 2018a, Bai et al. 2019, Zhu et al. 2019,  
353 Ørsted et al. 2022). Advanced models that include the temperature-dependent repair function  
354 have recently been developed to incorporate this recovery capability (Ørsted et al. 2022). Despite  
355 these pitfalls, this simple approach based on basal thermal tolerance and cumulative daily thermal  
356 stress proved to be rather efficient in discriminating both the most susceptible species and regions.

357 It could be further developed into a fine model to predict the population dynamics of aphid species  
358 in the field if integrating the effect of thermal dose on fertility in the future.

### 359 **Conclusion**

360 Our results answered the three questions: 1) interspecific differences in basal thermal tolerance  
361 can be a factor driving the community abundance change in aphid communities. The risk  
362 assessment under the natural temperature profile seems to fit the field observations that the more  
363 tolerant species is becoming more abundant with increasing temperature in the context of climate  
364 change. 2) Although the effect of age was not consistently observed in the three aphid species,  
365 we found that younger nymphs tended to have a lower thermal tolerance.

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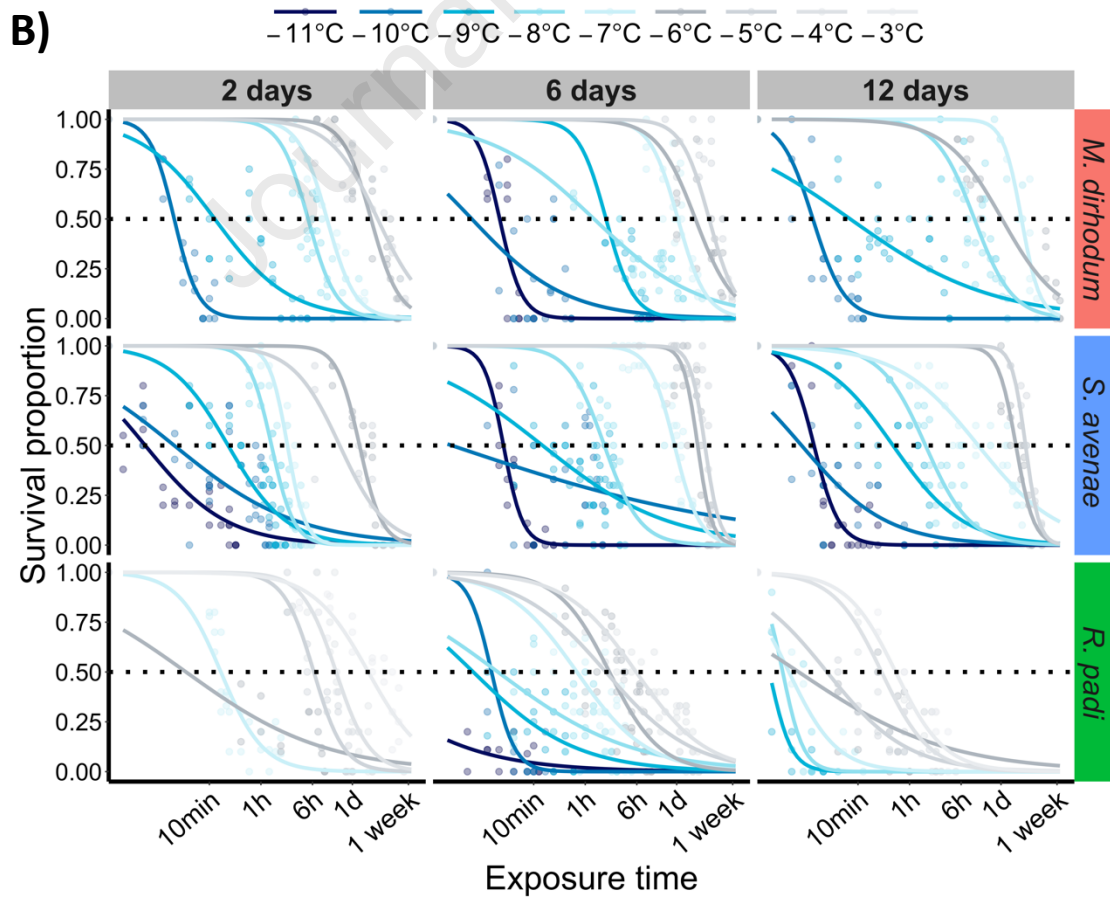
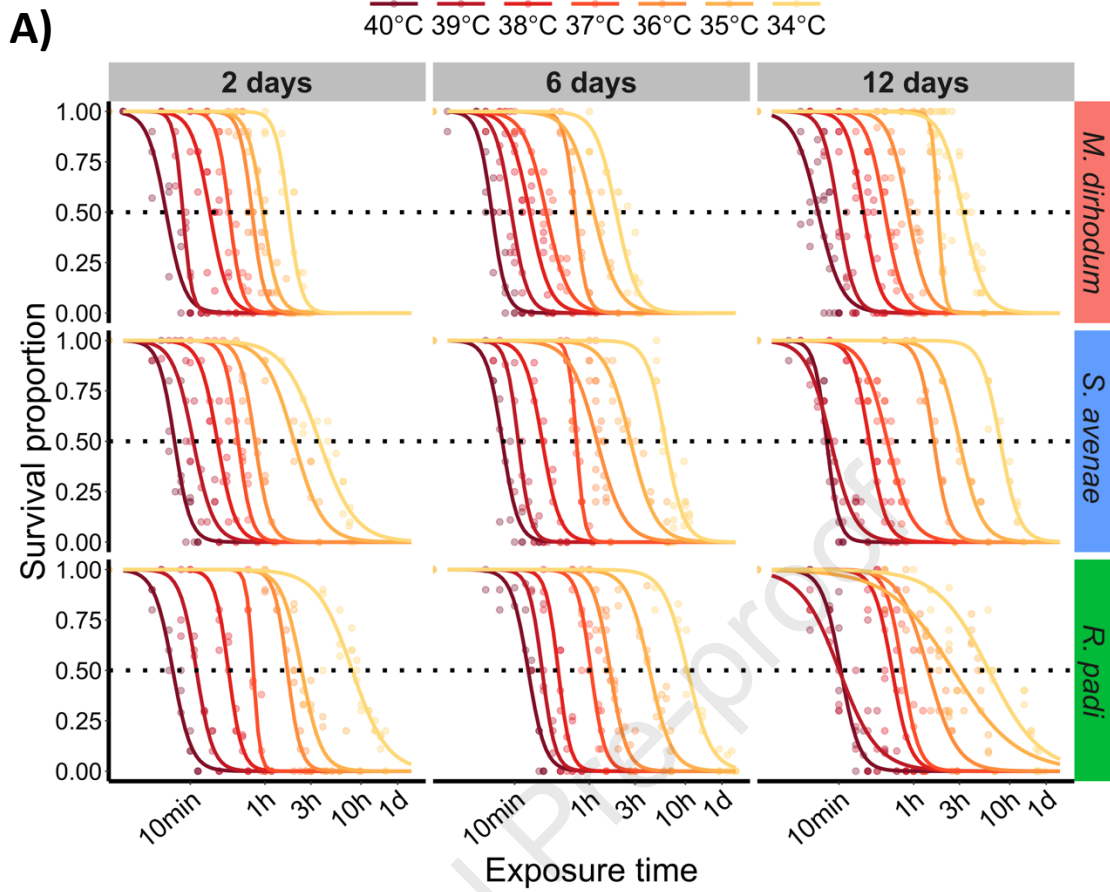


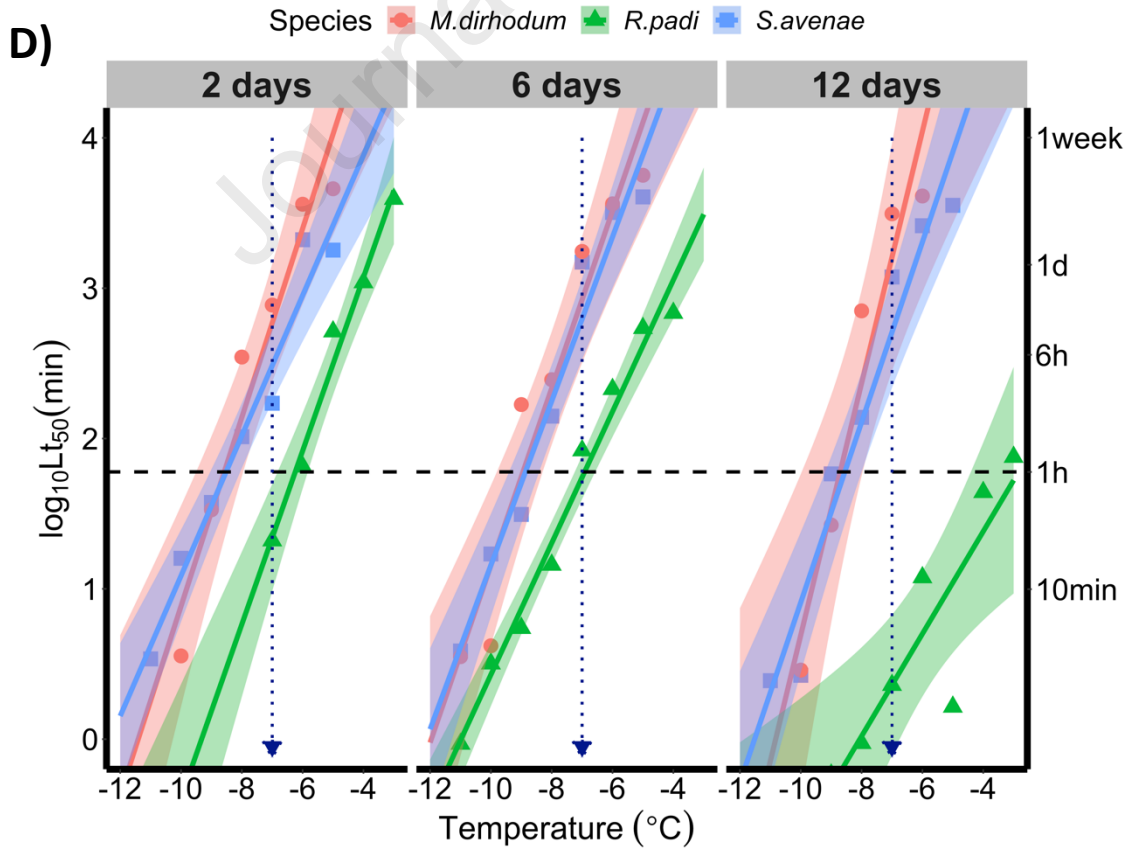
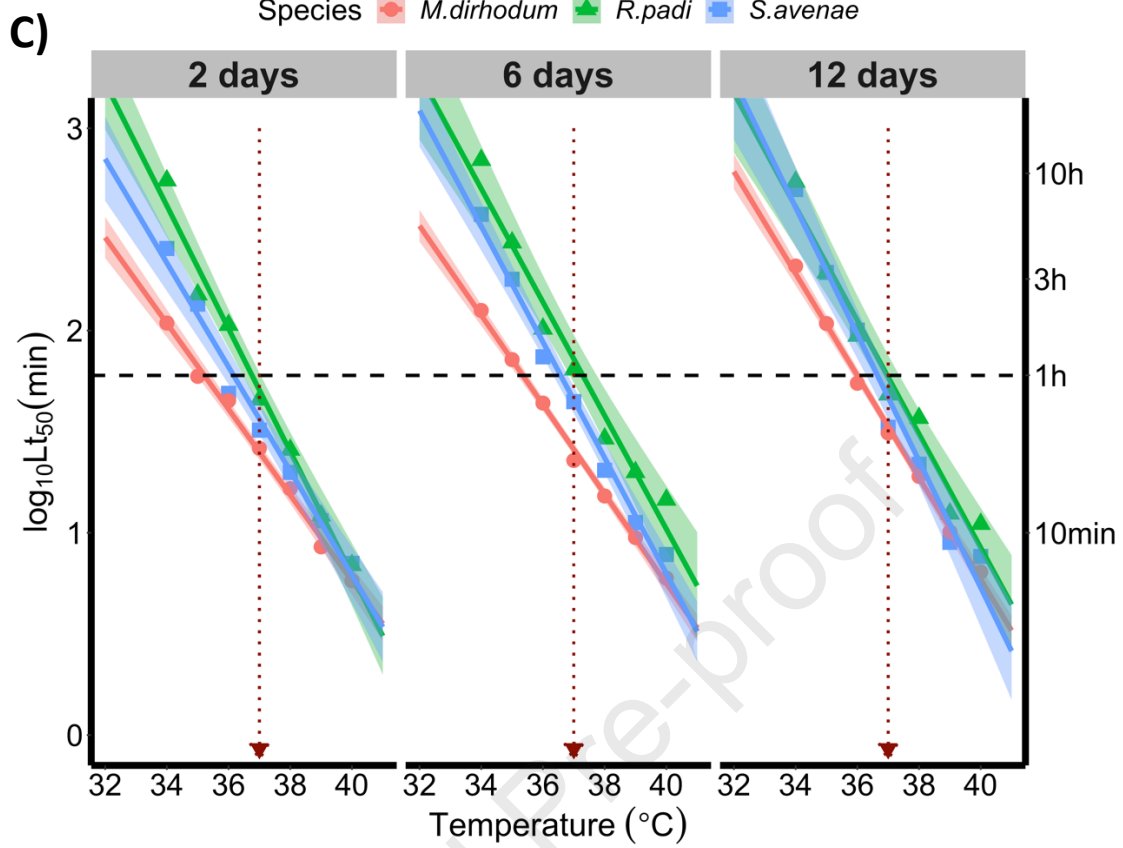
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**Figure 1. Survival curves (panel A for heat, B for cold) and thermal death time curves (panel C for heat, D for cold) for the three ages (2, 6, and 12 days) of *M. dirhodum*, *S. avenae*, and *R. padi*.** The curves in different colors (A and B) were fitted by the logistic regression model at each temperature. Each point in panels A and B represented the survival proportion of aphids in a single replicate and each point in panels C and D represented the  $\log_{10}(Lt_{50})$  value estimated from the survival curve at a tested temperature. Lines and shades in C and D illustrated the linear regression lines and the corresponding 95% confidence intervals.  $CT_{max}$  and  $CT_{min}$  were the intercepts with the black dashed line ( $Lt_{50} = 1$  hour). Thermal sensitivity ( $z$  or  $z'$ ) was derived from the slope of the line ( $z/z' = -1/\text{slope}$ ). The  $\log_{10}(Lt_{50})$  at  $37^{\circ}\text{C}$  and  $\log_{10}(Lt_{50})$  at  $-7^{\circ}\text{C}$  where red and blue dashed arrows pointed were pairwise compared between species and ages.

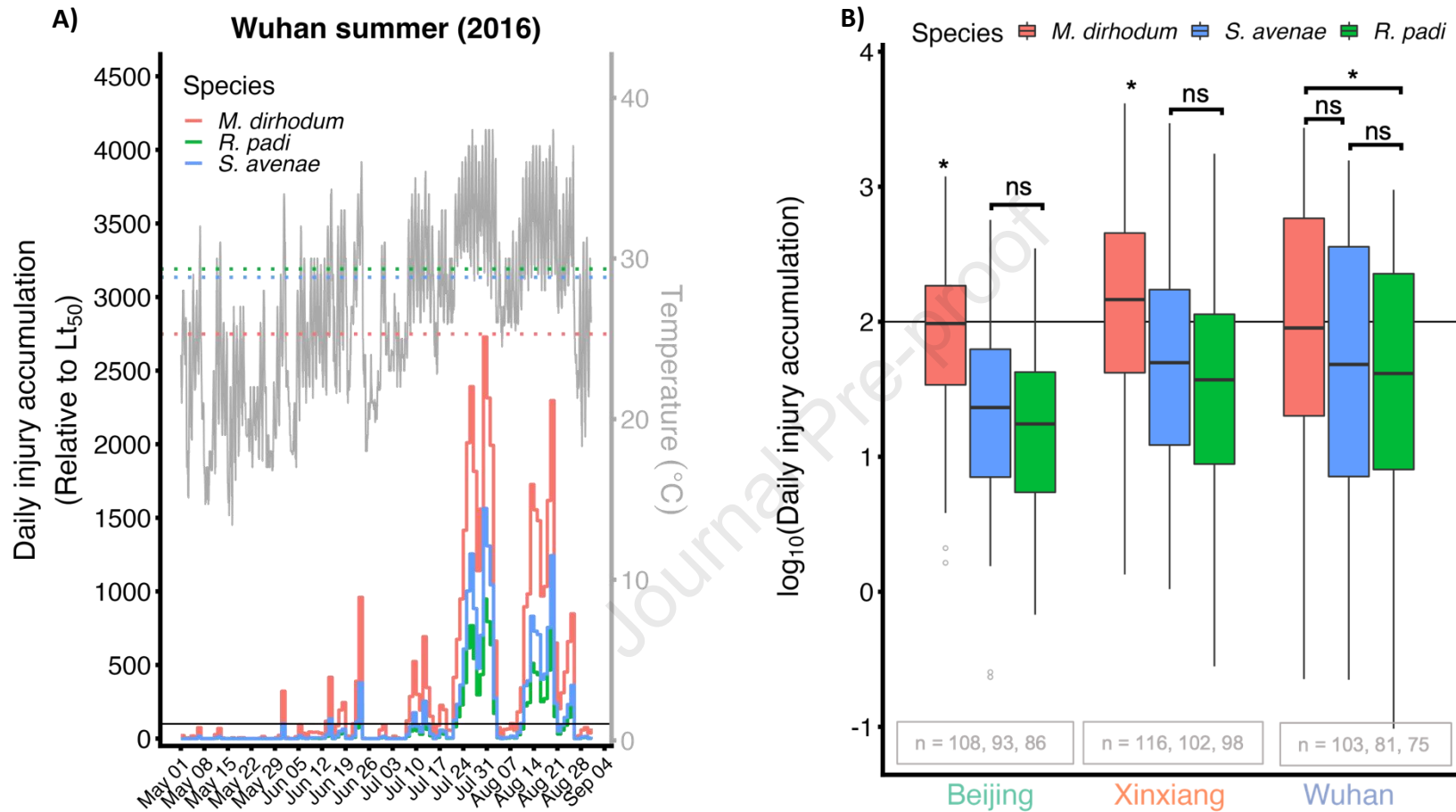


**Table 1. Parameters of thermal death time curves for each age group of the three species at high temperatures.** Superscript lowercase letters in thermal sensitivity (z) and  $\log_{10}(Lt_{50})$  at 37°C marked significant differences in slope and intercept between species with the same age ( $p < 0.05$ ). Superscript uppercase letters marked significant differences between age groups for the same species ( $p < 0.05$ ).

Species	R <sup>2</sup>	P value	Thermal sensitivity (z)	CT <sub>max</sub> (°C)	log <sub>10</sub> (Lt <sub>50</sub> ) at 37°C
<b>2 days</b>					
<i>M. dirhodum</i>	0.99	<1*10 <sup>-4</sup>	4.71 <sup>B, b</sup>	35.21	1.40 <sup>A, a</sup>
<i>S. avenae</i>	0.98	<1*10 <sup>-4</sup>	3.89 <sup>A, a</sup>	36.16	1.56 <sup>A, b</sup>
<i>R. padi</i>	0.99	<1*10 <sup>-4</sup>	3.29 <sup>A, a</sup>	36.76	1.71 <sup>A, c</sup>
<b>6 days</b>					
<i>M. dirhodum</i>	0.99	<1*10 <sup>-4</sup>	4.52 <sup>B, b</sup>	35.35	1.41 <sup>A, a</sup>
<i>S. avenae</i>	0.99	<1*10 <sup>-4</sup>	3.49 <sup>A, a</sup>	36.58	1.66 <sup>A, b</sup>
<i>R. padi</i>	0.97	<1*10 <sup>-4</sup>	3.57 <sup>A, a</sup>	37.29	1.86 <sup>B, c</sup>
<b>12 days</b>					
<i>M. dirhodum</i>	0.99	<1*10 <sup>-4</sup>	3.97 <sup>A, a</sup>	36.00	1.53 <sup>B, a</sup>
<i>S. avenae</i>	0.98	<1*10 <sup>-4</sup>	3.19 <sup>A, a</sup>	36.65	1.67 <sup>A, b</sup>
<i>R. padi</i>	0.97	<1*10 <sup>-4</sup>	3.56 <sup>A, a</sup>	36.97	1.77 <sup>A, c</sup>

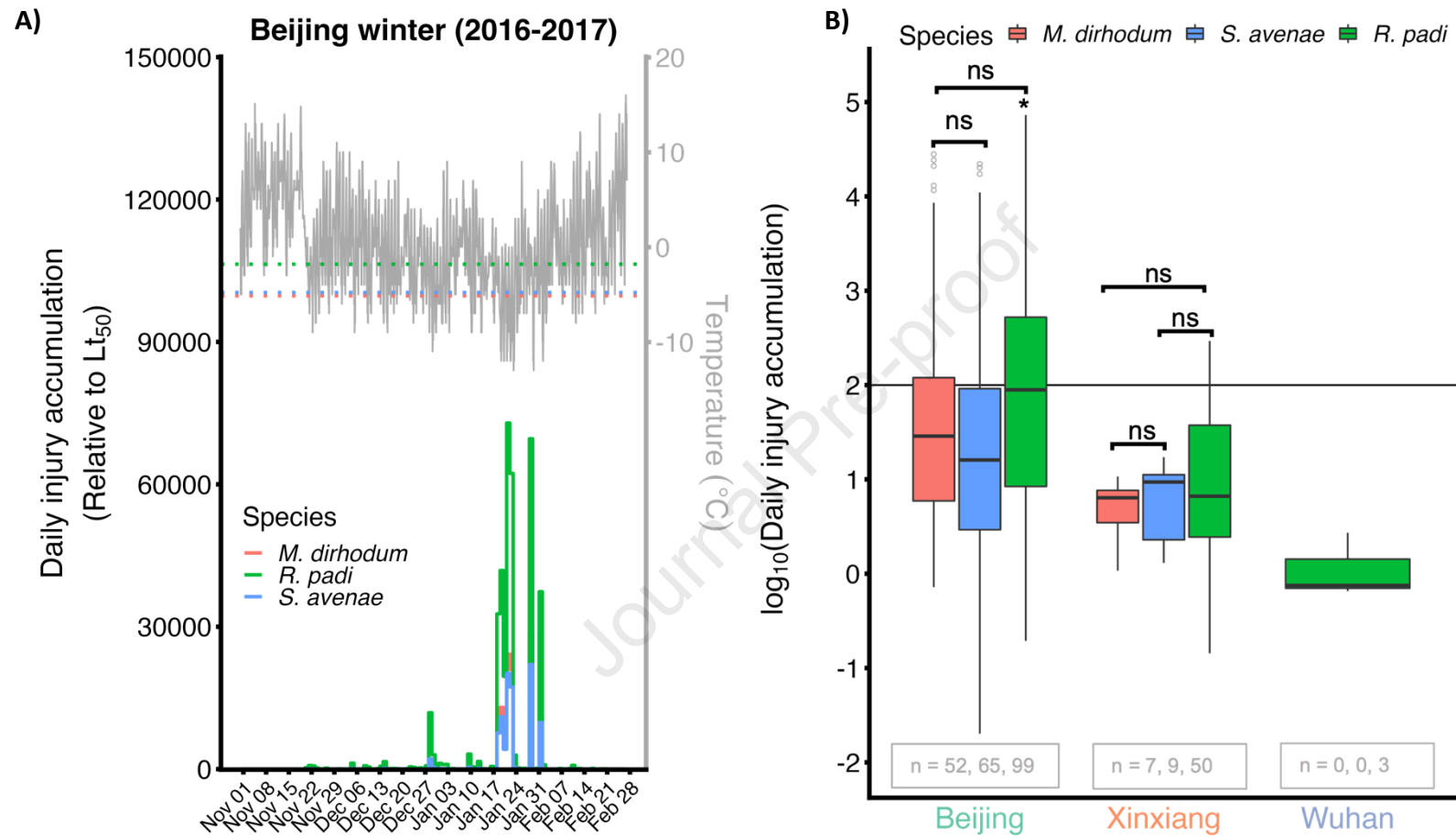
**Table 2. Parameters of thermal death time curves for each age group of the three species at low temperatures.** Superscript lowercase letters in thermal sensitivity ( $z'$ ) and  $\log_{10}(Lt_{50})$  at  $-7^{\circ}\text{C}$  marked significant differences in slope and intercept between species with the same age ( $p < 0.05$ ). Superscript uppercase letters marked significant differences between age groups for the same species ( $p < 0.05$ ).

Species	R <sup>2</sup>	P value	Thermal sensitivity ( $z'$ )	CT <sub>min</sub> (°C)	$\log_{10}(Lt_{50})$ at $-7^{\circ}\text{C}$
<b>2 days</b>					
<i>M. dirhodum</i>	0.94	$<1*10^{-2}$	1.59 <sup>A, a</sup>	-8.58	2.77 <sup>A, b</sup>
<i>S. avenae</i>	0.96	$<1*10^{-3}$	2.14 <sup>A, a</sup>	-8.52	2.49 <sup>A, b</sup>
<i>R. padi</i>	0.98	$<1*10^{-2}$	1.73 <sup>A, a</sup>	-6.24	1.34 <sup>B, a</sup>
<b>6 days</b>					
<i>M. dirhodum</i>	0.93	$<1*10^{-3}$	1.70 <sup>A, a</sup>	-8.94	2.92 <sup>A, b</sup>
<i>S. avenae</i>	0.96	$<1*10^{-4}$	1.83 <sup>A, a</sup>	-8.86	2.80 <sup>A, b</sup>
<i>R. padi</i>	0.98	$<1*10^{-4}$	2.29 <sup>A, a</sup>	-6.92	1.74 <sup>B, a</sup>
<b>12 days</b>					
<i>M. dirhodum</i>	0.93	$<1*10^{-2}$	1.19 <sup>A, a</sup>	-8.70	3.21 <sup>A, b</sup>
<i>S. avenae</i>	0.98	$<1*10^{-3}$	1.67 <sup>A, ab</sup>	-8.55	2.71 <sup>A, b</sup>
<i>R. padi</i>	0.78	$<1*10^{-2}$	2.94 <sup>A, b</sup>	-2.84	0.36 <sup>A, a</sup>



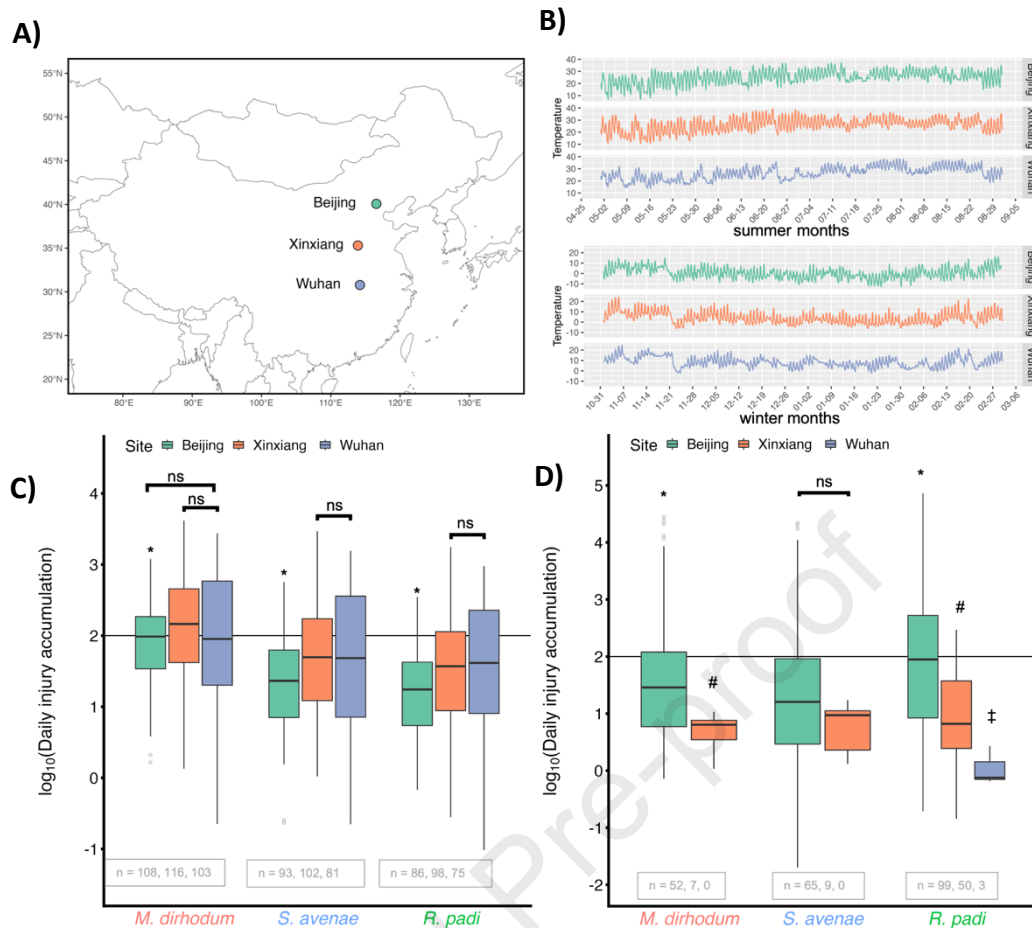
**Figure 2. Predicted daily injury accumulation based on the TDT parameters for the three species (age 6 days) under natural temperature fluctuations in the summer (May-August) of 2016. A)** The temperature profile (grey, secondary y-axis) was used to project the daily

accumulation of injury by use of the TDT parameters (see the formula in the method part). The species-colored lines/steps at the bottom of the graph indicated the amount of injury accumulated *per day* relative to the amount of injury that would result in half the aphid dying (here set to 100 with the black line). In the current run of this model, only temperatures above a  $T_c$  of 25.29°C (red dotted line), 29.34 °C (green dotted line) and 28.82 °C (blue dotted line) were set to result in injury for *M. dirhodum*, *R. padi* and *S.avenae*, respectively. **B)** Boxplot summarizing the data from (A) and similar analyses for Beijing and Xinxiang in the summer (see figure S1A and B). Note that the y-axis was  $\log_{10}$ -transformed and the injury accumulation resulting in median lethal (100) was marked by the line at  $\log_{10}(\text{Daily injury accumulation}) = 2$ . To avoid comparing a lot of days without any injury accumulation, only days with injury accumulation (indicated as n in the graph) were summarized in this plot. Comparisons among species within sites were examined using Kruskal-Wallis Rank Sum Tests followed by Pairwise Wilcoxon Rank Sum Tests with a Benjamini-Hochberg correction – results were indicated on the plot. Asterisks marked significant differences ( $p < 0.05$ ) between two groups.



**Figure3.** Predicted daily injury accumulation based on the TDT parameters for the three species of cereal aphids (*M. dirhodum*, *S. avenae*, *R. padi*) (age 6 days) under natural temperature fluctuations found in the winter (November-February) of 2016-2017. A) The

temperature profile (grey, secondary y-axis) was used to project the daily accumulation of injury by use of the TDT parameters (see the formula in the method part). The species-colored lines/steps at the bottom of the graph indicated the calculated amount of injury accumulated *per day* relative to the amount of injury that would result in half the aphids dying (here set to 100 with the black line). Injuries only accumulated when temperature dropped below a  $T_c'$  of  $-5.15^{\circ}\text{C}$  (red dotted line),  $-1.81^{\circ}\text{C}$  (green dotted line) and  $-4.79^{\circ}\text{C}$  (blue dotted line) for *M. dirhodum*, *R. padi* and *S. avenae*, respectively **B**) Boxplot summarizing the data from (A) and similar analyses for Wuhan and Xinxiang in the winter (see figure S2 A and B). Note that the y-axis was  $\log_{10}$ -transformed and the injury accumulation resulting in median lethal (100) was marked by the line at  $\log_{10}(\text{Daily injury accumulation}) = 2$ . To avoid comparing a lot of days without any injury accumulation, only days with injury accumulation (indicated as n in the graph) were summarized in this plot. Comparisons between species within sites were examined using Kruskal-Wallis Rank Sum Tests followed by Pairwise Wilcoxon Rank Sum Tests with a Benjamini-Hochberg correction. Asterisks marked significant differences ( $p < 0.05$ ) between two groups.



**Figure 4. Temperature profiles and predicted daily injury accumulation based on the TDT parameters for the three species of cereal aphids (*M. dirhodum*, *S. avenae*, *R. padi*) (age 6 days) under natural temperature fluctuations found in three sites in the summer months (May-August) of 2016 and winter months (November-February, 2016-2017). A) Map of the geographic locations of three sites indicated in different colors. B) Overview of temperature profiles in the three sites during summer (May-August 2016) (top) and winter months (Nov-Feb, 2016-2017) (bottom). C) and D) showed a comparison of log<sub>10</sub>(daily injury accumulation) between sites within species during summer months and winter months respectively. The comparisons were conducted using Kruskal-Wallis Rank Sum Tests followed by Pairwise Wilcoxon Rank Sum Tests with a Benjamini-Hochberg correction. \*, #, and ‡ marked significant differences ( $p < 0.05$ ) among sites. To avoid comparing a lot of days without any injury accumulation, only days with injury accumulation (indicated as n in the graph) were summarized in the plot.**



## Highlights

- Thermal tolerance landscape reflected the survivals of cereal aphids at extreme temperatures.
- *Rhopalosiphum padi* was more heat-tolerant and cold-vulnerable than *Sitobion avenae* and *Metopolophium dirhodum*.
- Young nymphs generally had lower thermal tolerance than old nymphs or adults.
- According to estimated thermal injury, *R. padi* was severely injured by cold while *M. dirhodum* was by heat.
- Interspecific differences in thermal tolerance may drive the aphid community abundance change.

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### **Competing interests**

The authors declare that they have no conflict of interest.

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