

A peer-reviewed version of this preprint was published in PeerJ on 23 March 2017.

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Enriquez T, Colinet H. 2017. Basal tolerance to heat and cold exposure of the spotted wing drosophila, *Drosophila suzukii*. PeerJ 5:e3112
<https://doi.org/10.7717/peerj.3112>

Basal tolerance to heat and cold exposure of the spotted wing drosophila, *Drosophila suzukii*

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The spotted wing *Drosophila*, *Drosophila suzukii*, is a new pest in Europe and America which causes severe damages to stone fruits crops. Temperature and humidity are among the most important abiotic factors governing insect life. In many situations, temperature can become stressful thus compromising fitness and survival. The ability to cope with thermal stress depends on basal level of thermal tolerance. Basic knowledge on temperature-dependent mortality of *D. suzukii* is essential to facilitate management of this pest. The objective of the present study was to investigate *D. suzukii* basal cold and heat tolerance. Adults and pupae were submitted to six low (-5 to 7.5 °C) and seven high temperatures (30 to 37 °C) for various durations, and survival-time-temperature relationships were investigated. In addition, pupal thermal tolerance was analyzed under low vs. high relative humidity. Our results showed that males had higher cold survival than females, and pupae appeared less cold-tolerant than adults. Above 5 °C, adult cold mortality became minor, even after prolonged exposures (i.e. one month). Males were less heat tolerant than females, and pupae showed a better survival to extreme high temperatures than adults. Low relative humidity did not affect *D. suzukii* cold survival, but reduced survival under heat stress. Overall, this study shows that survival of *D. suzukii* under heat and cold conditions depends on both stress intensity and duration, and the methodological approach used here, which was based on thermal tolerance landscapes, provides a comprehensive description of *D. suzukii* thermal tolerance and limits.

1 **Basal tolerance to heat and cold exposure of the spotted wing drosophila,**

2 *Drosophila suzukii*

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11 Abstract

12 The spotted wing Drosophila, *Drosophila suzukii*, is a new pest in Europe and America which
13 causes severe damages to stone fruits crops. Temperature and humidity are among the most
14 important abiotic factors governing insect life. In many situations, temperature can become
15 stressful thus compromising fitness and survival. The ability to cope with thermal stress depends
16 on basal level of thermal tolerance. Basic knowledge on temperature-dependent mortality of *D.*
17 *suzukii* is essential to facilitate management of this pest. The objective of the present study was to
18 investigate *D. suzukii* basal cold and heat tolerance. Adults and pupae were submitted to six low
19 (-5 to 7.5 °C) and seven high temperatures (30 to 37 °C) for various durations, and survival-time-
20 temperature relationships were investigated. In addition, pupal thermal tolerance was analyzed
21 under low vs. high relative humidity. Our results showed that males had higher cold survival than
22 females, and pupae appeared less cold-tolerant than adults. Above 5 °C, adult cold mortality
23 became minor, even after prolonged exposures (i.e. one month). Males were less heat tolerant than
24 females, and pupae showed a better survival to extreme high temperatures than adults. Low relative
25 humidity did not affect *D. suzukii* cold survival, but reduced survival under heat stress. Overall,
26 this study shows that survival of *D. suzukii* under heat and cold conditions depends on both stress
27 intensity and duration, and the methodological approach used here, which was based on thermal
28 tolerance landscapes, provides a comprehensive description of *D. suzukii* thermal tolerance and
29 limits.

30

31 Introduction

32 Temperature and water availability are among the most important factors influencing animal
33 distribution, reproduction, and fitness (Chown & Nicolson, 2004; Angilletta, 2009). Temperature

34 has therefore also a great influence on the invasive success of alien species (Bellard *et al.*, 2013).
35 The spotted wing Drosophila (SWD), *Drosophila suzukii* (Matsumura), is a new insect pest in
36 Western Europe and North America that causes severe damages to stone fruits crops (Walsh *et al.*,
37 2011; Asplen *et al.*, 2015). While most *Drosophila* species oviposit in rotting fruits, SWD females
38 prefer to oviposit in ripe fruits (Kanzawa, 1939; Mitsui *et al.*, 2006). A sclerotized ovipositor
39 allows flies to pass through skin fruit (Hauser *et al.*, 2009), and lay their eggs into a very large host
40 range (Cini *et al.*, 2012; Poyet *et al.*, 2015). The damages that larvae cause to fruits can have great
41 economic impact (Goodhue *et al.*, 2011). This invasive species is native from Southeast Asia and
42 has been introduced in Spain, Italy, and North America in 2008 (Hauser *et al.*, 2009; Raspi *et al.*,
43 2011; Calabria *et al.*, 2012). It is now widely distributed in West Europa (Cini *et al.*, 2012) and
44 both in United States and in South Canada (Hauser, 2011). It is assumed that success of SWD
45 invasion is partly due to a series of adaptations to temperate climates (Rota-Stabelli *et al.*, 2013).
46 For instance, this species is freeze-intolerant and chill-susceptible (Kimura, 2004; Dalton *et al.*,
47 2011; Jakobs *et al.*, 2015; Ryan *et al.*, 2016; Plantamp *et al.*, 2016) but process a large thermal
48 tolerance plasticity which likely favors overwintering (Jakobs *et al.*, 2015). Another hypothesis
49 for explaining overwintering success in cold regions is that adults may take refuge into human-
50 made structures or migrate to suitable microclimates during cold periods (Kanzawa, 1939; Kimura,
51 2004; Cini *et al.*, 2012; Rota-Stabelli *et al.*, 2013; Zerulla *et al.*, 2015). SWD is supposed to
52 overwinter as adult dark winter morph (Kanzawa, 1936; Stephens *et al.*, 2015; Shearer *et al.*, 2016;
53 Toxopeus *et al.*, 2016; Wallingford *et al.*, 2016). This morph is characterized by an arrest of
54 reproduction and an increased cold tolerance (Stephens *et al.*, 2015; Toxopeus *et al.*, 2016; Shearer
55 *et al.*, 2016, Wallingford & Loeb, 2016), but it is not yet clear whether this morph entails a true
56 reproductive diapause or not (Toxopeus *et al.*, 2016; Wallingford *et al.*, 2016; Zhai *et al.*, 2016).

57 Most recent studies on SWD cold tolerance were designed to understand overwintering strategy in
58 new infested cold regions, in order to better predict invasion potential or winter survival probability
59 (e.g. Dalton *et al.*, 2011; Stephens *et al.*, 2015; Zerulla *et al.*, 2015; Shearer *et al.*, 2016;
60 Wallingford & Loeb, 2016). In most of these studies, cold survival was assessed by submitting
61 insects either to a single low temperature with different durations (e.g. Jakobs *et al.*, 2015;
62 Toxopeus *et al.*, 2016), or to a series of low temperatures but with a single duration of exposure
63 (e.g. Kimura, 2004; Ryan *et al.*, 2016; Wallingford *et al.*, 2016). From a conceptual perspective,
64 this can be questionable as the impact of any stress depends both on its intensity and duration.
65 Hence, investigating a single parameter may not be insufficient to describe a response that is
66 embedded in two dimensions (Rezende *et al.*, 2014). To fully appreciate the innate capacity of a
67 species to cope with cold and heat stress, an approach based on tolerance landscape was suggested
68 by Rezende *et al.*, (2014). The thermal tolerance landscape (TTL), describes the probability to
69 survive a thermal stress as a function of both the intensity and the duration of thermal stress. In the
70 present study, we adopted this approach to describe basal heat and cold tolerance of SWD at adult
71 and pupal stage. There is a lack of knowledge on SWD heat tolerance, as only Kanzawa (1939)
72 and Kimura (2004) have examined this aspect. However, describing upper thermal limits is also
73 important to understanding thermal biology of this invasive species. In *Drosophila melanogaster*,
74 the humidity during thermal stress modifies survival probability and the response can be
75 temperature-dependent (Bubliy *et al.*, 2012; Kobey & Montooth, 2013). Combining high
76 temperature with low humidity provides more stressful conditions to fruit flies than high
77 temperature with high humidity (Bubliy *et al.*, 2012). Furthermore, increasing humidity during
78 cold exposure increased survival at 6 °C, but not at -4 °C in *D. melanogaster* (Kobey & Montooth,
79 2013). This underlines that interaction among abiotic factors may differentially shape thermal

80 tolerance landscapes. In the present study, we also investigated the interaction between
81 temperature and humidity on SWD cold and heat survival of pupae. The general assumptions of
82 this study were that (1) SWD survival will be function of both temperature stress intensity and
83 time exposure. (2) Because thermal performance curves are nonlinear and asymmetric (Colinet *et*
84 *al.*, 2015a), we predicted uneven effects of increasing intensity of cold and heat stress (i.e. different
85 shapes of TTL). More specifically, we predicted that survival will decrease rather progressively
86 with cold stress intensity, and more abruptly with heat stress intensity. (3) We also expected
87 differential responses (i.e. different TTL) between sexes and stages. Based on previous data from
88 *D. melanogaster* (Jensen *et al.*, 2007), we predicted that pupae will be less thermotolerant than
89 adults. (4) Finally, we predicted that different humidity conditions will affect the shape of the TTL,
90 with desiccating condition during thermal stress further reducing survival probability compared to
91 thermal stress under high humidity.

92

93 **Materials and methods**

94 **Flies origin and rearing**

95 SWD flies were provided by the Insect Pest Control Laboratory (IPCL) from the FAO/IAEA
96 Division of Nuclear Techniques in Food and Agriculture (Austria). This strain was originally
97 captured in 2013 in San Michele all Adige (Trentino, Italia) and has been continuously reared in
98 IPCL. For our experimentations, SWD was reared in glass bottle (100mL) and supplied with
99 standard food (for 1 liter: agar: 15 g, sucrose: 50 g, brewer yeast: 40 g, cornmeal: 40 g, kalmus: 8
100 g, Nipagin: 8 mL). Bottles were kept in incubators (Model MIR-154-PE; PANASONIC,
101 Healthcare Co., Ltd. Gunma, Japan) at 25 °C, 12L : 12D. Adults and pupae randomly taken from
102 the rearing stock were used in experiments. All adults were between 5 and 7-day-old to avoid

103 effects of maturation at young age on stress tolerance (Colinet *et al.*, 2015b). Males were separated
104 from females visually (with an aspirator) without CO₂ to avoid stress due to anesthesia (Colinet &
105 Renault, 2012). For pupae, we used individuals that had pupated since maximum 48 h (i.e.
106 corresponding to 8 to 9 days after egg laying at 25 °C).

107

108 **Thermal tolerance assays**

109 We submitted flies and pupae to six low constant temperatures (-5, -2.5, 0, 2.5, 5 and 7.5 °C) and
110 seven high constant temperatures (30, 31, 32, 33, 34, 35 and 37 °C) for various durations. At least
111 seven specific durations were used for each temperature and these are provided in Supplemental
112 Information for each experiment (Table S1 to S5). We performed preliminary assays in order to
113 determine for each temperature the number of time points and the durations of exposure to get
114 survival data spanning from 0 to 100 % mortality. At the most stressful temperatures (both at heat
115 and cold), we added some time points because mortality occurred very quickly (within less than 2
116 h). Adults and pupae were isolated in groups of 10 independent individuals each coming from
117 different randomly chosen rearing stock bottles. For each sampling duration, three replicates of 10
118 flies or 10 pupae were used. Flies and pupae were exposed to the different thermal conditions
119 either using food vials placed in incubators (Model MIR-154-PE; PANASONIC, Healthcare Co.,
120 Ltd. Gunma, Japan) for the longer assays (2.5, 5, 7.5, 30, 31, 32 °C) or using glass vials immersed
121 in a glycol solution cooled by a cryostat (Cryostat Lauda ECO RE 630) for the shorter assays (-5,
122 -2.5, 0, 33, 34, 35, 36, 37 °C). Temperature was checked during all assays using thermocouple K
123 connected Testo thermometers (Model 175 T3; TESTO Limited, Hampshire, England) placed into
124 an empty vial. After stress exposure, SWD adults were allowed to recover in 40 mL food vials.
125 Adult survival was assessed by counting the proportion of dead and living individuals 24 h post

126 exposure. For pupae, the results were expressed as a percentage of emergence, considered here as
127 a proxy of survival. Flies were considered as alive when the adult totally emerged from the
128 puparium. Because isolation and manipulation of pupae in preparation to thermal assays might
129 cause some damage to the puparium, five sets of 20 untreated pupae were kept at 25 °C to estimate
130 possible mortality due to manipulation.

131

132 **Thermal stress under high and low relative humidity**

133 In this experiment, we used only pupae to assess the impact of humidity during thermal stress.
134 Groups of 15 pupae were exposed to four different low constant temperatures (0, 2.5, 5 and 7.5
135 °C) and five different high constant temperatures (32, 33, 34, 35 and 37 °C) either under a high
136 (80-100 %) or low (5-10 %) relative humidity (RH). To produce high RH condition, a cotton ball
137 saturated with water was placed at the bottom of 50 mL closed centrifugation tube. For low RH
138 condition, we used dehydrated silicagel placed at the bottom of a 50 mL tube. Foam slices were
139 added to the devices to prevent direct contact of SWD pupae with cotton or silicagel. RH and
140 temperatures were checked directly into experimental tubes using Ibutton's Hygrochron (Maxim
141 Integrated, San Jose, CA, U.S.A.), and thermocouples connected to Testo thermometers (Model
142 175 T3; TESTO Limited, Hampshire, England). As previously described, we performed
143 preliminary assays in order to determine for each temperature the number of time points and the
144 durations of exposure to get emergence data spanning from 0 to 100 %. Again, at least seven
145 durations were used for each tested temperature (Table S5). Flies were considered as alive when
146 the adult totally emerged from the puparium.

147

148 **Statistical analyses**

149 We modeled survival data in R (R Core Team, 2016) by specifying a generalized linear model
150 (GLM) with logistic link function for proportions outcome (i.e. number of dead/alive per vial).
151 The response variable was dependent on stress duration, temperature, thermal treatment, sex and
152 all the interactions. We used a full factorial model, and analyzed the effect of each variable through
153 an Analysis of Deviance (“Anova” function in “car” package, Fox & Weisberg, 2011). For males,
154 females and pupae we calculated the 50 % median lethal time (Lt_{50}) for each temperature as follow:

$$155 \quad Lt_{50} = \frac{\text{logit}(0.5) - a}{b} \text{ (Venables \& Ripley, 2002)}$$

156 Where a and b respectively corresponds to the intercept and the slope of each condition GLM’s
157 prediction. 95% confidence intervals around estimated Lt_{50} were estimated by resampling model
158 parameters (10000 iterations, “arm” package, Gelman & Su, 2014). Lt_{50} values represent standard
159 and useful proxies to describe thermal tolerance. However, it is important to consider the entire
160 range of probabilities and not only 50 % survival. Therefore, to complement this information, we
161 also plotted the predicted values acquired from GLMs as function of both stress intensity and
162 duration, following the thermal tolerance landscape (TTL) approach suggested by Rezende *et al.*,
163 (2014). Finally, to help interpreting all the terms of the GLMs, we used effect plots function in the
164 package “effects” (Fox, 2003). These effect plots show the conditional coefficients (“marginal
165 effects”) for all variables and interaction terms. All the effect plots are available in Supplemental
166 Information for each experiment separately (Fig. S1 to S6).

167

168 Results

169 SWD cold tolerance

170 Control mortality of untreated pupae didn't exceed 1%, thus we considered mortality due to
171 manipulation negligible. In both adults and pupae, 100 % mortality was reached for all tested
172 temperatures, except for adults at 7.5 °C. The multiple panels figure 1 illustrates cold survival data
173 in adults (males and females, Fig. 1A) and in pupae (Fig. 1B) according to the different tested
174 temperatures and durations. Temperature and duration had strong effects on adult cold survival (χ^2
175 = 856.36, $p < 0.001$; $\chi^2 = 502.59$, $p < 0.001$, respectively). Survival decreased with decreasing
176 temperature and with increasing exposure duration (Fig. 1A and Fig. S1). Furthermore, at lowest
177 temperatures, temporal reduction of survival was much faster (time x temperature interaction; χ^2
178 = 1075.71, $p < 0.001$; Fig. S1). Males were globally more cold-tolerant than females ($\chi^2 = 99.95$,
179 $p < 0.001$; Fig. 1A and Fig. S1). Sexes were however differentially affected by decreasing
180 temperatures (temperature \times sex interaction; $\chi^2 = 41.63$, $p < 0.001$), with females more affected
181 than males by the lowest tested temperatures (Fig. 1A and Fig. S1). Temporal changes of survival
182 were similar between sexes (no sex \times time interaction; $\chi^2 = 41.63$, $p > 0.05$).

183 Temperature and time also had a strong impact on pupal cold survival ($\chi^2 = 156.68$, $p < 0.001$; χ^2
184 = 463.94, $p < 0.001$, respectively) (Fig. 1B). Survival significantly decreased with decreasing
185 temperature and with increasing exposure duration (Fig. 1B and Fig. S2). The temporal decrease
186 in survival was dependent on temperature (time \times temperature interaction; $\chi^2 = 161.43$, $p < 0.001$),
187 it was much faster at lower temperatures (Fig. 1B and Fig. S2).

188 Lt_{50} for adults and pupae at the different tested low temperatures are provided in Fig. 2A. Based
189 on Lt_{50} values and their confidence intervals, pupae appeared much less cold-tolerant than adults

190 (Fig. 2A). Of note, for adults, models weren't able to calculate Lt_{50} at 7.5 °C, as after 30 days of
191 cold exposure, survival didn't decrease under 80 %. From TTLs (Fig. 2B,C,D), we observed that
192 during cold exposure females and males described rather similar surface patterns, but female's
193 survival to extreme low temperatures was lower than that of males. On the other hand, pupae
194 described a drastically different perspective, as they did not tolerate both short exposures to
195 extremes temperatures and long exposures to milder temperatures.

196

197 **SWD heat tolerance**

198 For both adults and pupae, 100 % mortality was reached for all tested temperatures, except for
199 pupae at 30 and 31°C, where respectively 5 and 4 individuals successfully emerged during the last
200 day of the experimentation. The multiple panels figure 3 illustrates survival data in adults (males
201 and females, Fig. 3A) and in pupae (Fig. 3B) according to the different tested temperatures and
202 durations. Temperature and duration had strong effect on adult heat survival ($\chi^2 = 819.69$, $p <$
203 0.001 ; $\chi^2 = 889.77$, $p < 0.001$, respectively). Survival decreased with increasing temperature and
204 with increasing exposure duration (Fig.2A and Fig. S3). Furthermore, at highest temperatures
205 temporal reduction of survival was much faster (time x temperature interaction; $\chi^2 = 1495.20$, $p <$
206 0.001 ; Fig. S3). There was no difference between males and females ($\chi^2 = 1.52$, $p > 0.05$). Yet
207 sexes were differentially affected by increasing temperature (temperature x sex interaction; $\chi^2 =$
208 94.43 , $p < 0.001$), with males more affected than females at the highest tested temperatures (Fig.
209 2A and Fig. S3). Temporal changes of survival were similar between sexes (no sex x time
210 interaction, $\chi^2 = 0.19$; $p > 0.05$).

211 Temperature and time also had a strong impact on pupal heat survival ($\chi^2 = 210.72, p < 0.001$; χ^2
212 $= 388.71, p < 0.001$, respectively). Survival significantly decreased with increasing temperature
213 and with increasing exposure duration (Fig. 3B and Fig. S4). The temporal decrease in survival
214 was dependent on temperature (time x temperature interaction; $\chi^2 = 662.25, p < 0.001$), it was
215 much faster at higher tested temperatures (Fig. 3B and Fig. S4).

216 Lt_{50} for adults and pupae at the different tested high temperatures are provided in Fig. 4A. Based
217 on Lt_{50} values and their confidence intervals, pupae appeared much less tolerant than adults to
218 temperatures under 33 °C but more tolerant than adults to temperatures above 33 °C (Fig. 4A).
219 From TTLs (Fig. 4B,C,D), we observed that during heat exposures males, females and pupae
220 described somewhat similar surface patterns, in the way that survival decrease very quickly with
221 time of exposure suggesting that SWD tolerate high temperatures for only short time. Nonetheless
222 pupae showed a greatly better survival than adults during extreme heat exposures (Fig. 4D),
223 comforting previous observations of Lt_{50} .

224

225 **Interaction between relative humidity and thermal stress**

226 Low and high temperature treatments were statistically analyzed separately. Under cold exposures,
227 100 % mortality was reached for all tested temperatures for both high and low RH. The multiple
228 panels figure 5 illustrates survival data in pupae according to the different tested temperatures
229 (cold and heat) and durations. Temperature and duration had strong effect on pupal cold survival
230 ($\chi^2 = 91.74, p < 0.001$; $\chi^2 = 649.88, p < 0.001$, respectively). Cold survival decreased with
231 decreasing temperature and with increasing exposure duration (Fig. 5 and Fig. S5). Furthermore,
232 at lowest temperatures, temporal reduction of survival was much faster (time x temperature

233 interaction; $\chi^2 = 68.07, p < 0.001$; Fig. S5). RH did not differentially affect cold survival ($\chi^2 =$
234 $0.02, p > 0.05$), but temporal changes were different between RH levels (RH x time interaction; χ^2
235 $= 6495.48, p < 0.05$) suggesting that temporal survival decrease at cold was slightly faster at low
236 RH (Fig. S5).

237 Under heat exposure, 100 % mortality was also reached for all tested temperatures for both high
238 and low RH. Temperature and duration had again strong effects on pupae heat survival ($\chi^2 =$
239 $306.20, p < 0.001$; $\chi^2 = 831.90, p < 0.001$, respectively). Survival decreased with increasing
240 temperature and with increasing exposure duration (Fig. 5 and Fig. S6). Furthermore, at the highest
241 temperatures, temporal reduction of survival was much faster (time x temperature interaction; χ^2
242 $= 83.46, p < 0.001$). RH greatly affected heat survival ($\chi^2 = 95.97, p < 0.001$), with survival being
243 significantly higher when pupae were exposed to high vs. low RH (Fig. S6). In addition, RH
244 interacted with both temperature and duration ($\chi^2 = 19.25, p < 0.001$; $\chi^2 = 14.17, p < 0.001$,
245 respectively). Survival decreased with increasing temperature and this thermal-dependent process
246 was more severe under low RH (Fig. 5 and Fig. S6). In addition, temporal decrease in survival
247 (across all temperatures) was globally faster under low RH.

248 Based on Lt_{50} values and their confidence intervals, it appeared that low RH greatly diminished
249 heat survival, but did not affect cold survival (Fig. 6A and B). From TTLs (Fig. 6C, D, E and F)
250 we observed that surface patterns were similar during cold exposures (Fig. 6C and D). On the other
251 hand, during heat exposure, low RH landscape were less extended than high RH landscape,
252 underlying that a low RH level slightly decreased heat survival (Fig. 6E and F).

253

254 Discussion

255 In the present study, we studied the basal thermal tolerance of SWD considering adult and pupal
256 survival as a function of both stress intensity (heat and cold) and exposure duration. We observed
257 a reduction in survival with increasing thermal stress intensity and duration, both under low and
258 high temperature. This decrease is consistent with the classical dose-response relationship where
259 survival declines with the “dose of stress” which is considered here as a combination of
260 temperature stress intensity and duration (Colinet *et al.*, 2011; Rezende *et al.*, 2014).

261 The cold tolerance of SWD estimated in the present study was rather consistent with previously
262 reported data. Indeed, we found that adult and pupal mortality occurred very rapidly at subzero
263 temperatures ($Lt_{50} < 2$ h at -5 °C and $Lt_{50} < 12$ h at -2.5 °C). This fits with early reports that also
264 found very short survival durations at subzero temperatures (e.g. Jackobs *et al.*, 2015; Stephens *et*
265 *al.*, 2015; Plantamp *et al.*, 2016; Ryan *et al.*, 2016). We thus corroborate that SWD is a chill
266 susceptible species that does not tolerate brief exposures to sub-zeros temperatures (Kimura, 2004;
267 Dalton *et al.*, 2011; Jakobs *et al.*, 2016; Ryan *et al.*, 2016; Plantamp *et al.*, 2016). At 0 °C, we
268 found that it required about one day to reach 50% mortality in adults and much less time was
269 needed (a few hours) in pupae. This is also in the range of previous data on SWD (Kimura, 2004;
270 Jakobs *et al.*, 2015; Plantamp *et al.*, 2016); however, our values appear slightly inferior to those
271 reported with other SWD strains. Slight variations in thermotolerance among strains could be
272 related to different rearing conditions. Indeed, we reared our flies at 25 °C not at 21 °C as in other
273 laboratories (Jakobs *et al.*, 2015; Plantamp *et al.*, 2016). Variations may also result from different
274 local adaptations of the tested strains (Hoffman *et al.*, 2002; Van Heerwaarden *et al.*, 2012). At
275 temperatures above 0 °C, several days were required before reaching Lt_{50} . Interestingly, there
276 appeared to be a sort of threshold between 5 and 7.5 °C where individuals shifted from detrimental

277 condition (at 5 °C) to non-injurious condition (at 7.5 °C). Indeed, at 5 °C, Lt_{50} was reached in only
278 5 days, while at 7.5 °C, mortality remained low (under 20 %) even after rather long exposure (one
279 month). Previous data reported that non-acclimated SWD adults start to fall into coma at
280 temperatures just below 5 °C (Jakobs, 2014). Therefore, we can assume that this temperature
281 represents a physiological limit under which chilling injuries, such as neuromuscular dysfunctions,
282 may start to accumulate (Hazell & Bale, 2011; MacMillan *et al.*, 2012). In temperate regions, cold
283 snaps with freezing events could be thus lethal to SWD. However, it seems that SWD overwinters
284 as adults by migrating into protected microclimates, in leaf litter or in human made structures
285 (Kanzawa, 1939; Kimura, 2004; Dalton *et al.*, 2011; Zerulla *et al.*, 2015; Rossi-Stacconi *et al.*,
286 2016). This avoiding strategy likely allows SWD to escape low winter temperatures *in natura*, and
287 even colonize new cool regions (Rota-Stabelli *et al.*, 2013; Asplen *et al.*, 2015).

288 Because thermal performance curves are nonlinear and asymmetric (skewed towards low
289 temperatures) (Martin & Huey, 2008; Colinet *et al.*, 2015a), we predicted uneven effects of
290 increasing the intensity of cold vs. heat stress. Essentially, we assumed that SWD survival will
291 decrease rather progressively with increasing cold stress intensity, and we expected a steep decline
292 in survival over certain limits under heat stress. Observation of Lt_{50} values and TTLs patterns
293 support this assumption. Under low temperature conditions, a progressive survival decrease was
294 observed (Fig. 2), while under high temperature stress, there was clearly a limit over which survival
295 crashed suddenly and became close to zero (Fig. 4). Indeed, at 32 °C, adult flies could sustain
296 continuous heat stress for several days (Lt_{50} of 3-4 days), whereas at 33 °C, most flies succumbed
297 within a couple of hours (Lt_{50} of about 2 h). Therefore 32 °C seem to be very close to critical
298 thermal maximum for survival of SWD. Characteristically, the drop in performance (i.e. survival
299 in our case) is generally more precipitous at supra-optimal temperatures than at sub-optimal

300 temperatures (Denlinger & Yocum, 1998). Arrhenius-like effects can explain progressive and
301 reversible changes of performance at sub-optimal temperatures, while the sharp decline at supra-
302 optimal temperature is generally ascribed to the destabilizing effects of heat on molecular
303 interactions such as irreversible protein denaturation (Schulte *et al.*, 2011). Our results are in
304 accordance with early studies performed on SWD populations. Kanzawa (1939) noticed a motor
305 activity decrease of SWD when exposed to 30°C, and Kimura (2004) estimated that the 50% lethal
306 temperature at heat was around 32°C. Also, the upper thermal limit for development is estimated
307 to be at 31.5 °C (Asplen *et al.*, 2015). Ryan *et al.*, (2016) found no adult hatching when Canadian
308 flies developed at 31 °C. Therefore, our survival data together with the previous literature suggest
309 that SWD is not a particularly heat-tolerant species. This likely explains the very low field survival
310 of SWD when temperatures exceed 30 °C (Dalton *et al.*, 2011; Tochen *et al.*, 2014). While
311 overwintering strategy is rather well studied (Kanzawa, 1936; Stephens *et al.*, 2015; Shearer *et al.*,
312 2016; Toxopeus *et al.*, 2016; Wallingford *et al.*, 2016), how flies cope with heat stress in the fields
313 and more generally how SWD manages to survive under summer conditions (i.e. heat coupled
314 with desiccation) is currently unknown.

315 We expected differences in thermotolerance between sexes and stages. Under low temperature, we
316 observed that SWD males were slightly more cold-tolerant than females. This contrasts with
317 previous SWD studies which reported that females were more cold-tolerant than males (Kimura,
318 2004; Dalton *et al.*, 2011; Jakobs *et al.*, 2015). However, lack of difference in cold tolerance
319 between sexes were also reported in SWD (Ryan *et al.*, 2016). In *D. melanogaster*, the sex effect
320 on cold tolerance can be sometimes in favor of males (Kelty & Lee, 2001; Sejerkilde *et al.*, 2003;
321 Jensen *et al.*, 2007), or females (David *et al.*, 1998; Condon *et al.*, 2015). These discrepancies may
322 result from various factors, such as different measures of cold tolerance, tested temperatures or

323 age of flies (Jensen *et al.*, 2007). In consequence, we suggest that in SWD, as in *D. melanogaster*,
324 sex dimorphism in various metrics of cold tolerance appears more as an idiosyncratic than a
325 general rule (Gibert & Huey, 2001). This view is also supported by our observations that, under
326 high temperature, we only detected an interaction (sex x temperature) that suggested that females
327 better tolerated heat exposure but at certain temperatures (i.e, at the greatest temperatures).

328 Based on previous data from *D. melanogaster* (Jensen *et al.*, 2007), we predicted that pupae would
329 be less cold-tolerant than adults. Indeed, we observed that pupal Lt_{50} values under cold conditions
330 were consistently much lower than values of adults at all tested temperatures; and this was clearly
331 visible on the shape of TTLs. Furthermore, exposition to 7.5°C induced high pupal mortality while
332 it hardly affected adults. Other studies suggest that SWD pupae are less cold-tolerant than adults.
333 For instance, Dalton *et al.*, (2011) reported that pupae died earlier than adults when exposed to
334 temperatures under 10°C, and Ryan *et al.*, (2016) found that SWD pupae could not survive 42 d
335 of cold exposure contrary to adults. This seems coherent with the observation that in SWD, the
336 overwintering stage is the adult under a reproductive diapause (Stephens *et al.*, 2015; Zerulla *et*
337 *al.*, 2015; Shearer *et al.*, 2016; Toxopeus *et al.*, 2016; Rossi-Stacconi *et al.*, 2016; Wallingford &
338 Loeb, 2016). Here, we show clear evidence of lower cold tolerance of pupae than adults (in
339 reproductive state). Ontogenetic differences under high temperature revealed different patterns
340 than under low temperature. Pupae appeared less tolerant than adults to prolonged mild heat stress
341 (temperatures < 33 °C), while under severe heat stress condition (temperatures > 33°C), pupae
342 could sustain heat stress for much longer than adults. Indeed, under acute heat stress, pupae
343 managed to survive exposures for several hours (from 10 to 50 h depending on the temperature),
344 while adults succumbed in less than 2h. In *Drosophila buzzatii*, pupae seem to be the most heat
345 resistant stage, surviving temperatures above those that would kill all the other life stages (Krebs

346 & Loeschcke, 1995). Higher tolerance to extreme high temperature of pupae is sound considering
347 that this stage is immobile and therefore may be particularly susceptible to lethal high temperatures
348 *in natura*.

349 Finally, we predicted that very contrasted RH levels will affect thermal tolerance patterns.
350 Specifically, we expected that highly desiccating condition during thermal stress will further
351 reduce survival compared to stress conditions under high RH. As predicted, RH had strong impact
352 on pupal survival, but this manifested only under heat stress. The Lt_{50} values illustrate these
353 marked differences. Under cold conditions, the shapes of TTLs were globally identical between
354 low and high RH, whereas under heat conditions, the TTLs were extended towards longer survival
355 under high RH. In *D. melanogaster*, the humidity during both heat (Bubliy *et al.*, 2012) and cold
356 exposure (Kobey & Montooth, 2013) alters survival rate. Combining two stressors like high
357 temperature with low RH provides more stressful conditions to fruit flies than high temperature
358 with high RH (Bubliy *et al.*, 2012). Here, we observed the same synergetic-like phenomena with
359 SWD pupae. Prince & Parsons (1977) showed that under low RH, *D. melanogaster* adults move
360 towards lower temperatures likely to mitigate water loss. It is reasonable to speculate that in natural
361 conditions, SWD adults also tend to avoid heat stress and low RH by searching for protected and
362 favorable microhabitats, but this remains to be tested. Recent mark-capture researches indicate that
363 SWD achieves short-distance migrations from field margins to cultivated crops (Klick *et al.*, 2016)
364 therefore migration towards favorable microclimates is completely conceivable. During the pupal
365 stage, however, flies are immobile and are thus potentially subjected to prolonged heat and
366 desiccation stress, particularly if pupation occurs outside of infested fruits, as is the case with SWD
367 (Asplen *et al.*, 2015). Despite being protected within the puparium, water loss by pupae can
368 strongly affect survival of drosophilids, and even moderately dry conditions can induce substantial

369 pupal mortality even at permissive temperature (Kojima & Kimura, 2003). A recent study from
370 Tochen *et al.*, (2016) indicates that low RH (e.g. 20 %) induced poor survival and lack of
371 reproduction in SWD, suggesting that this species is particularly sensitive to water loss. Under low
372 temperature, there was globally no effect of RH on cold survival. Death during prolonged cold
373 exposure may be due to a combination of stressors: low temperature, starvation and desiccation.
374 If pupae were suffering from desiccation at cold, then altering RH during low temperature
375 exposure should affect water loss, and therefore, the survival duration at cold. Lack of RH effect
376 at cold suggests that desiccation is not a primary cause of mortality under cold stress.

377 In this work, we studied basal thermal tolerance of SWD considering survival as a function of
378 temperature stress intensity (under heat and cold) and exposure duration in adults (males and
379 females) and in pupae. We found that survival under heat and cold conditions was dependent on
380 both stress intensity and duration, and we provide a comprehensive description and visualization
381 of SWD thermal tolerance and limits. We confirmed that SWD is a chill susceptible species, and
382 noted that at temperatures over 5 °C, adults managed to survive for rather long periods (one
383 month). Tolerance to thermal stress over a range of conditions, as illustrated by TTLs, showed
384 rather different perspectives: a sudden vs. a more progressive survival decline under heat vs. cold
385 conditions, respectively. In particular, 32 °C seemed to be very close to critical thermal maximum
386 for survival of SWD. A sex dimorphism in thermal tolerance was also found but was temperature-
387 dependent. Difference in thermal tolerance were also observed between stages, with pupae being
388 drastically more sensitive to cold stress but more resistant to extreme heat stress than adults.
389 Finally, we found that level of RH had strong impact on pupal survival under heat stress but not
390 under cold stress.

391 Recently, a consortium of scientists has published a useful review with the updated situation of
392 SWD all over the world (Asplen *et al.*, 2015). The authors suggested a few directions for future
393 research to improve the accuracy of SWD management. Acquisition of novel data on the biology
394 of SWD at low temperature was highlighted as a priority, and we believe the present dataset may
395 provide valuable elements in this regard. The present study is one of the first to provide a global
396 description of SWD basal thermal tolerance, especially bringing new information about heat stress
397 tolerance and the interaction between temperature and relative humidity. However, we wish to
398 draw attention to the fact that stress tolerance data acquired from field-collected populations may
399 contrast with those resulting from laboratory-adapted lines (e.g. Hoffmann *et al.*, 2001; Schou *et*
400 *al.*, 2015). In consequence, despite logistical constraints, the next needed step is the realization of
401 thermal studies on field-collected individuals.

402

403 **Acknowledgement**

404 The authors would like to thank the IPCL from the FAO/IAEA division for providing the
405 *Drosophila suzukii* flies, and Maxime Dahirel for his help with statistical analyses.

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571 9.
- 572

573 **Figures captions**

574 **Figure 1**

575 **SWD survival as a function of low temperature and duration exposure.**

576 A: Adults; B: Pupae. Points correspond to observed data, and lines to GLMs prediction (Binomial
577 GLM, link = logit). The tested temperatures are indicated in the left top corner of each plot.

578

579 **Figure 2**

580 **Lt₅₀ values and thermal tolerance landscapes for low temperatures.**

581 A: Males, females and pupal Lt₅₀ values \pm 95% confidence intervals. Lt₅₀ is the time at which 50%
582 of the population is dead. Lt₅₀ values are calculated from GLMs. B,C,D: males, females and pupal
583 thermal tolerance landscapes; points are observed values, and surfaces correspond to GLMs
584 predictions (Binomial GLM, link = logit).

585

586 **Figure 3**

587 **SWD survival as a function of high temperature and duration exposure.**

588 A: Adults; B: Pupae. Points correspond to observed data, and lines to GLMs prediction (Binomial
589 GLM, link = logit). The tested temperatures are indicated in the left top corner of each plot.

590

591 **Figure 4**

592 **Lt₅₀ values and thermal tolerance landscapes for high temperatures.**

593 A: Males, females and pupal Lt₅₀ values \pm 95% confidence intervals. Lt₅₀ is the time at which 50%
594 of the population is dead. Lt₅₀ values are calculated from GLMs. B,C,D: males, females and pupal

595 thermal tolerance landscapes; points are observed values, and surfaces correspond to GLMs
596 predictions (Binomial GLM, link = logit).

597

598 **Figure 5**

599 **SWD pupal survival as a function of temperature and exposure duration under two relative**
600 **humidity (RH) levels.**

601 Points: observed data, lines: model predictions (Binomial GLM, link = logit). The tested
602 temperatures are indicated in the left top corner of each plot. Black solid lines: low RH, grey
603 dashed lines: high RH.

604

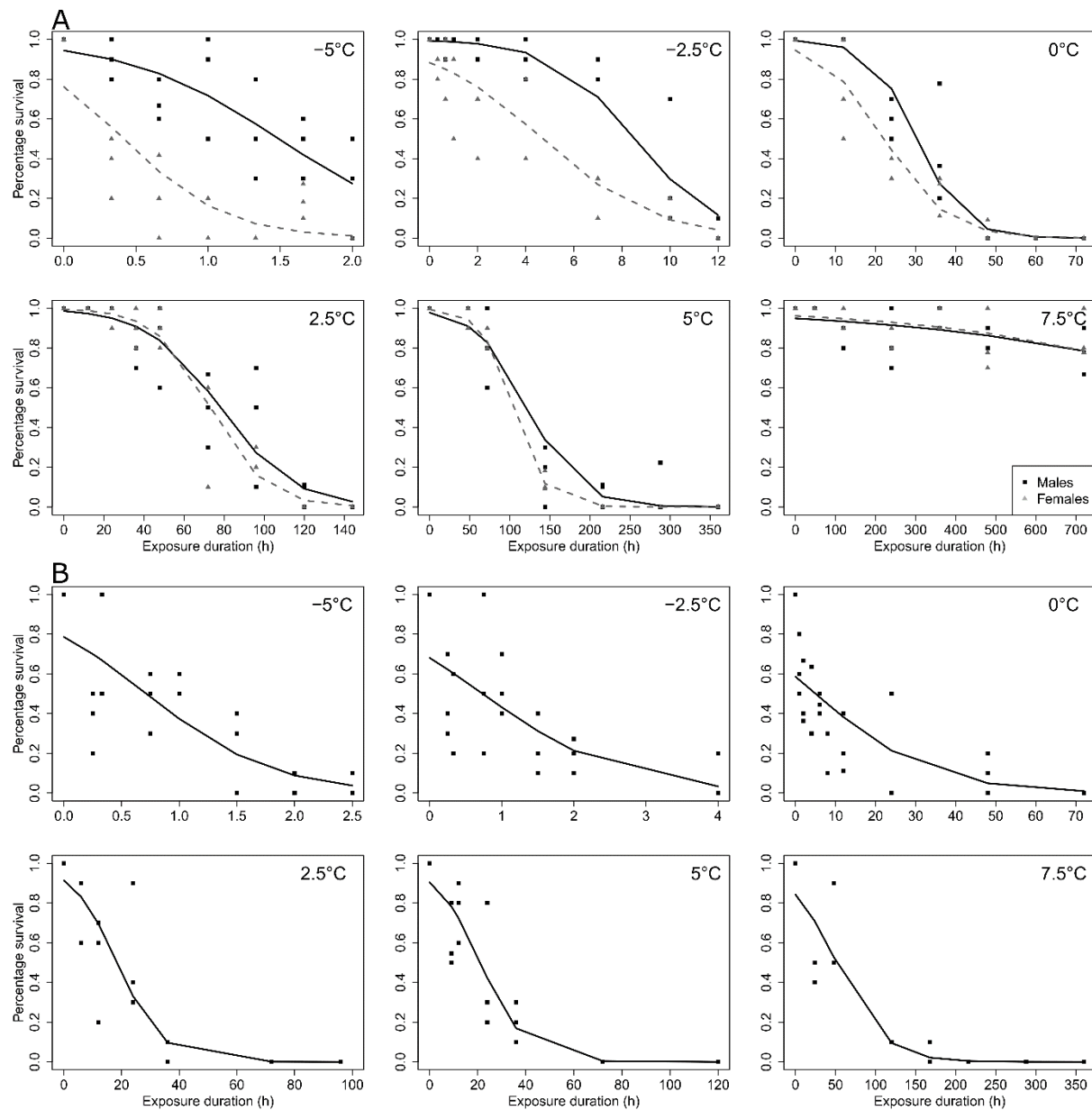
605 **Figure 6**

606 **Pupal Lt_{50} values and thermal tolerance landscapes for low and high temperatures under**
607 **two relative humidity (RH) levels.**

608 A,B: Lt_{50} values \pm 95% confidence interval values. Lt_{50} values are calculated from previous GLMs
609 predictions. C, D, E, F: thermal tolerance landscapes for low and high temperature; points
610 corresponds to observed values, and surfaces corresponds to GLMs predictions (Binomial GLM,
611 link = logit).

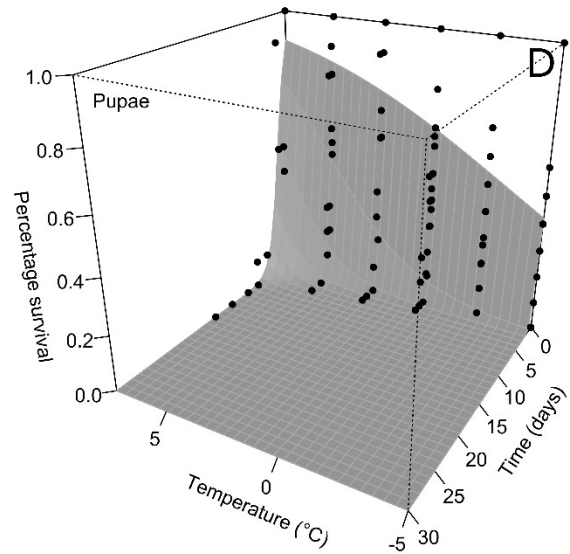
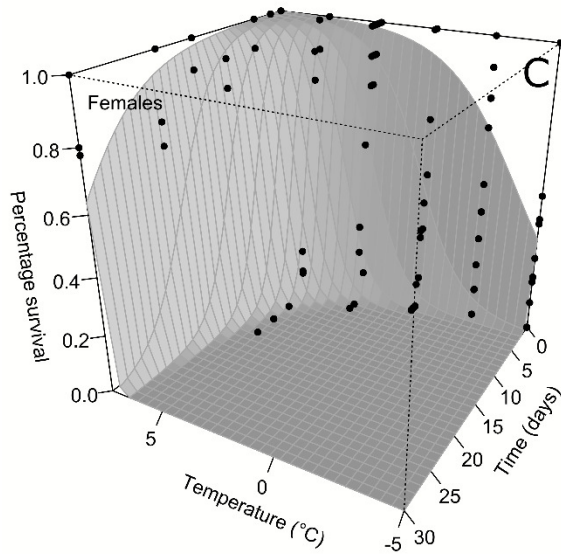
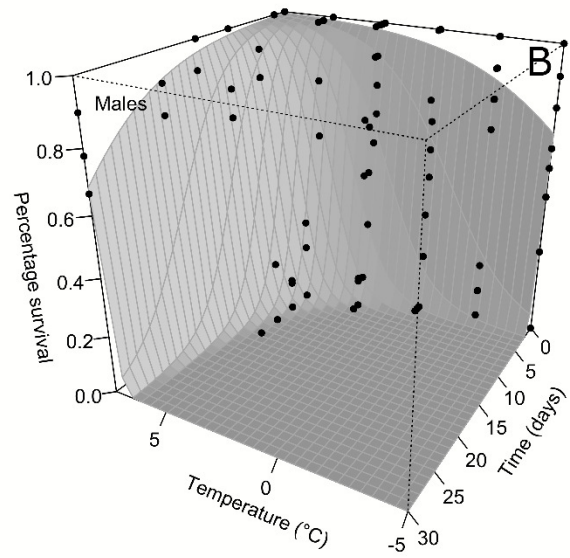
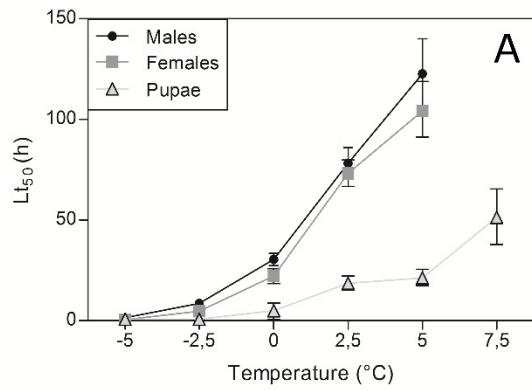
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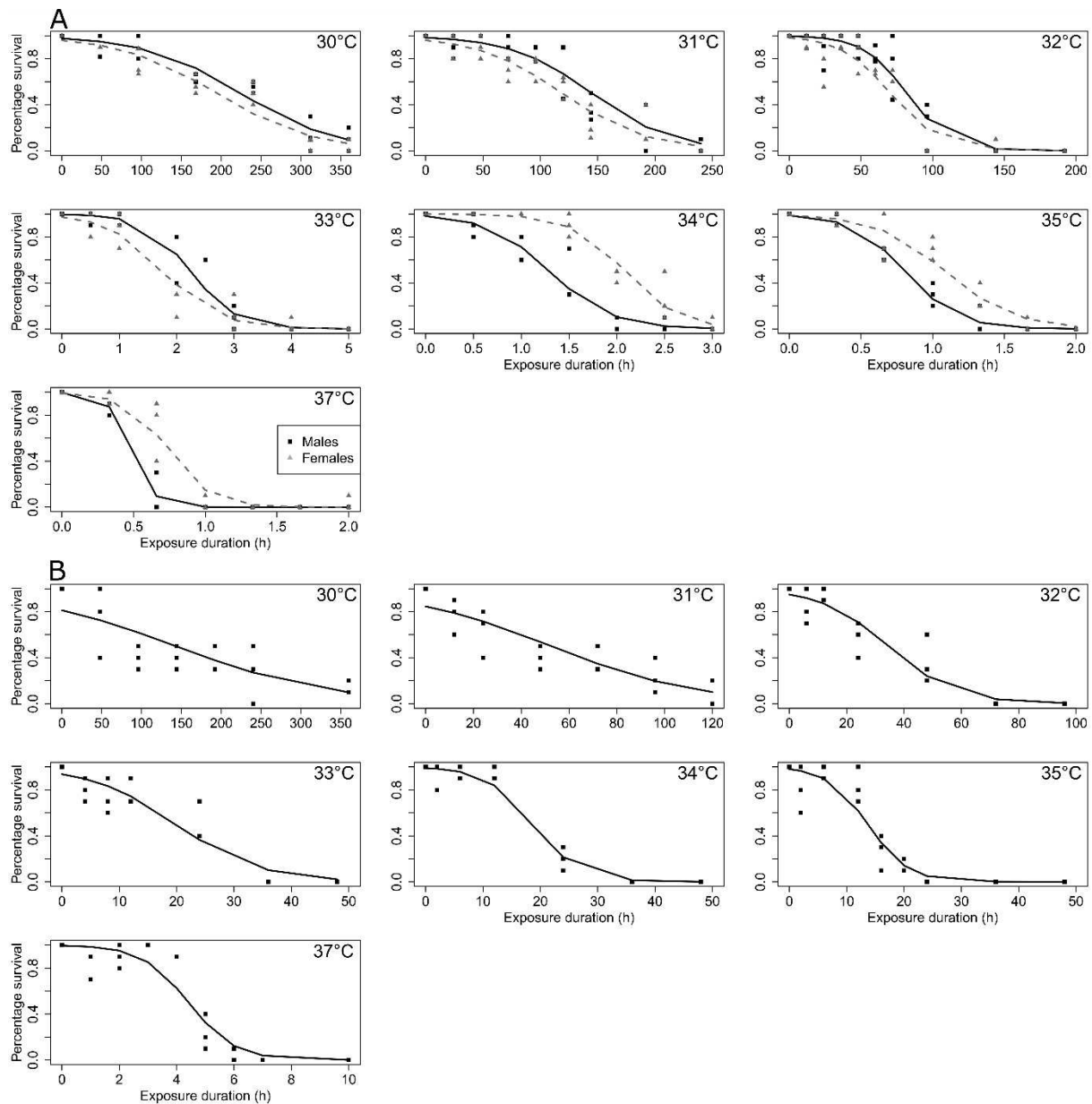
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617 **Figure 2:**

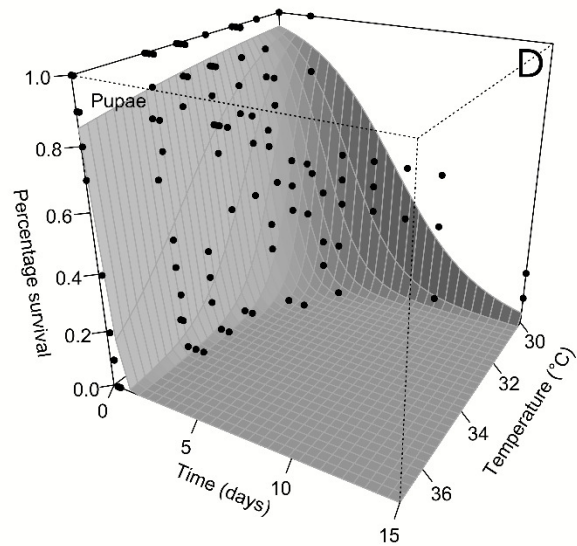
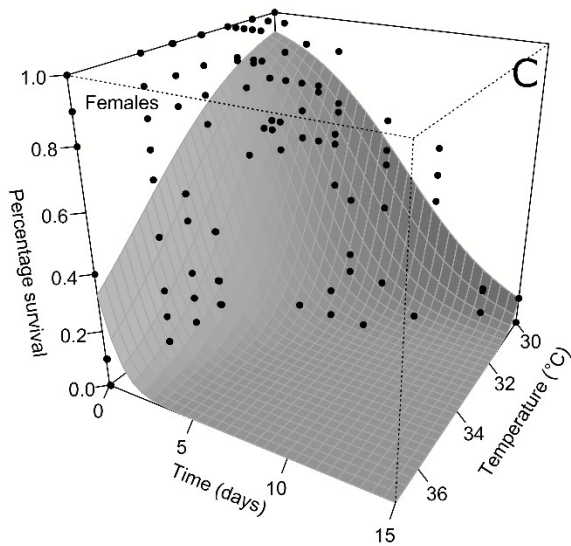
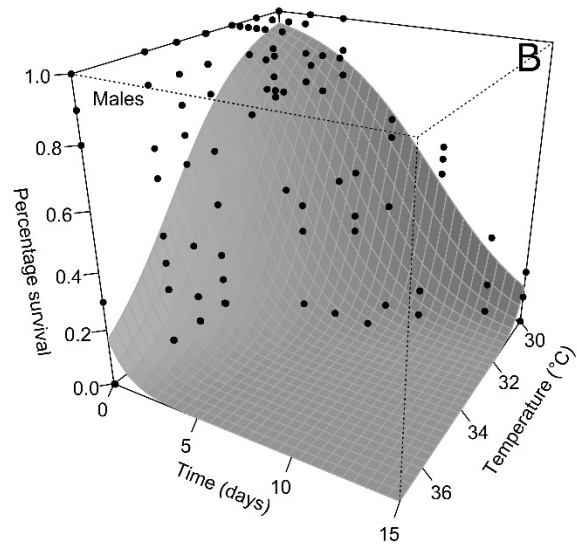
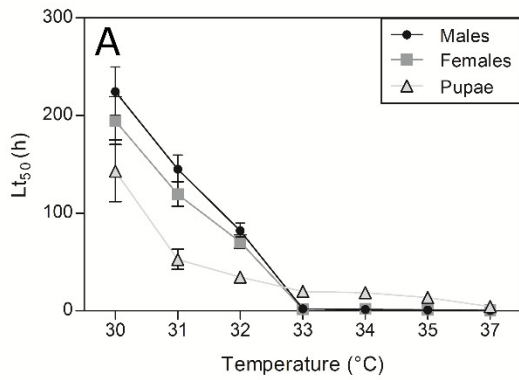
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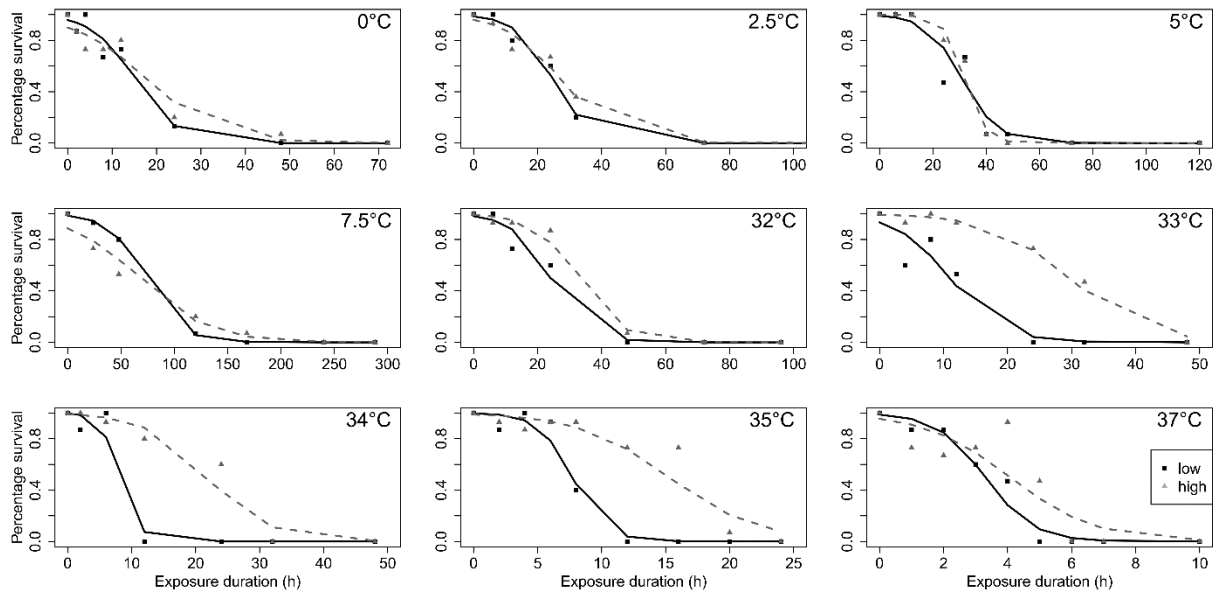
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623 **Figure4:**

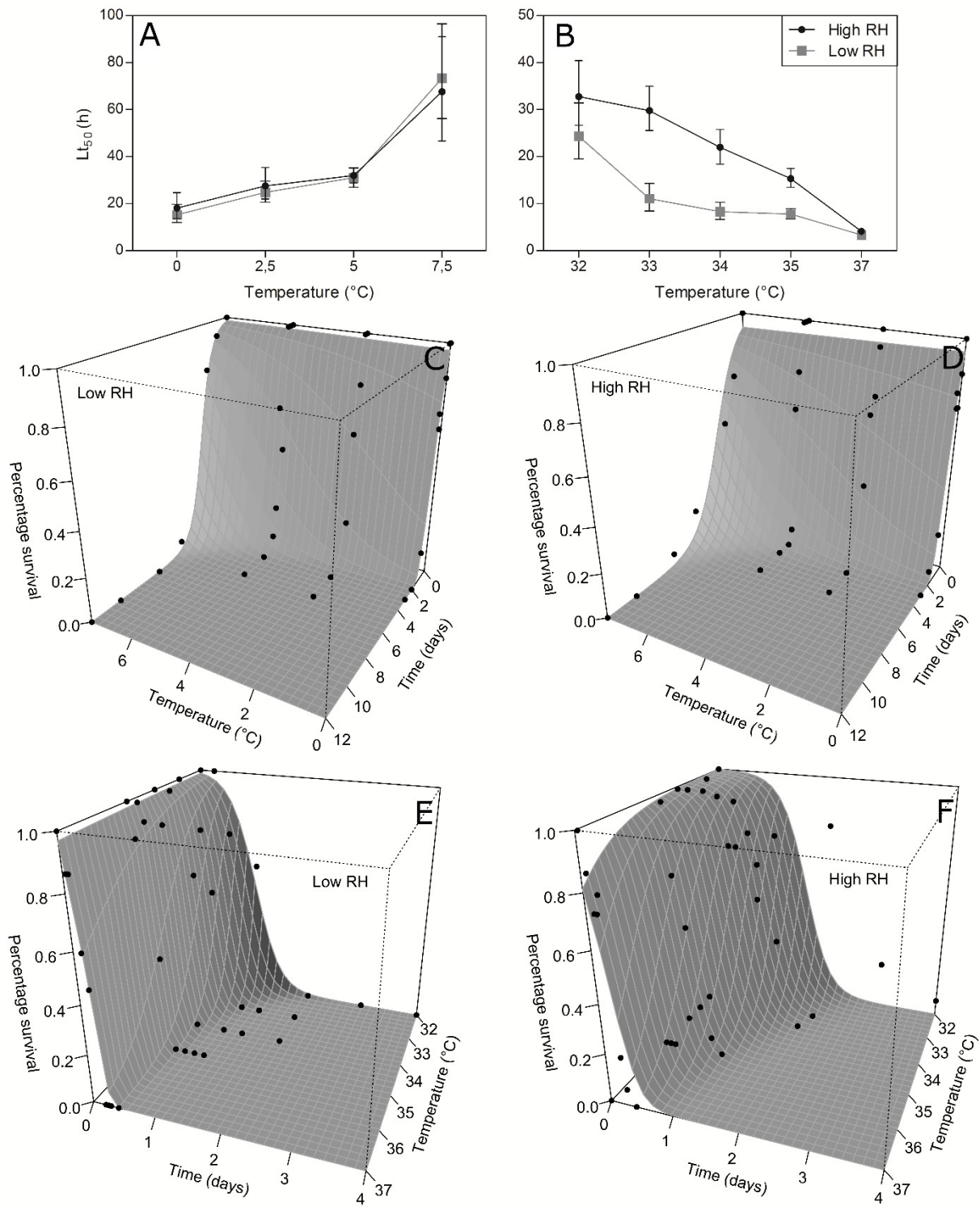
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626 **Figure5:**

627

628

629 **Figure6:**

630