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

Thomas ENRIQUEZ 1 and Hervé COLINET 1

1 Université de Rennes 1, UMR CNRS 6553 ECOBIO, 263 avenue du Général-Leclerc, 35042 Rennes, France.

Corresponding Author:

Hervé COLINET 1

Email address: herve.colinet@univ-rennes1.fr
Abstract

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.

Introduction

Temperature and water availability are among the most important factors influencing animal distribution, reproduction, and fitness (Chown & Nicolson, 2004; Angilletta, 2009). Temperature
has therefore also a great influence on the invasive success of alien species (Bellard et al., 2013).

The spotted wing Drosophila (SWD), *Drosophila suzukii* (Matsumara), is a new insect pest in Western Europe and North America that causes severe damages to stone fruits crops (Walsh et al., 2011; Asplen et al., 2015). While most *Drosophila* species oviposit in rotting fruits, SWD females prefer to oviposit in ripe fruits (Kanzawa, 1939; Mitsui et al., 2006). A sclerotized ovipositor allows flies to pass through skin fruit (Hauser et al., 2009), and lay their eggs into a very large host range (Cini et al., 2012; Poyet et al., 2015). The damages that larvae cause to fruits can have great economic impact (Goodhue et al., 2011). This invasive species is native from Southeast Asia and has been introduced in Spain, Italy, and North America in 2008 (Hauser et al., 2009; Raspi et al., 2011; Calabria et al., 2012). It is now widely distributed in West Europa (Cini et al., 2012) and both in United States and in South Canada (Hauser, 2011). It is assumed that success of SWD invasion is partly due to a series of adaptations to temperate climates (Rota-Stabelli et al., 2013).

For instance, this species is freeze-intolerant and chill-susceptible (Kimura, 2004; Dalton et al., 2011; Jakobs et al., 2015; Ryan et al., 2016; Plantamp et al., 2016) but process a large thermal tolerance plasticity which likely favors overwintering (Jakobs et al., 2015). Another hypothesis for explaining overwintering success in cold regions is that adults may take refuge into human-made structures or migrate to suitable microclimates during cold periods (Kanzawa, 1939; Kimura, 2004; Cini et al., 2012; Rota-Stabelli et al., 2013; Zerulla et al., 2015). SWD is supposed to overwinter as adult dark winter morph (Kanzawa, 1936; Stephens et al., 2015; Shearer et al., 2016; Toxopeus et al., 2016; Wallingford et al., 2016). This morph is characterized by an arrest of reproduction and an increased cold tolerance (Stephens et al., 2015; Toxopeus et al., 2016; Shearer et al., 2016, Wallingford & Loeb, 2016), but it is not yet clear whether this morph entails a true reproductive diapause or not (Toxopeus et al., 2016; Wallingford et al., 2016; Zhai et al., 2016).
Most recent studies on SWD cold tolerance were designed to understand overwintering strategy in new infested cold regions, in order to better predict invasion potential or winter survival probability (e.g. Dalton et al., 2011; Stephens et al., 2015; Zerulla et al., 2015; Shearer et al., 2016; Wallingford & Loeb, 2016). In most of these studies, cold survival was assessed by submitting insects either to a single low temperature with different durations (e.g. Jakobs et al., 2015; Toxopeus et al., 2016), or to a series of low temperatures but with a single duration of exposure (e.g. Kimura, 2004; Ryan et al., 2016; Wallingford et al., 2016). From a conceptual perspective, this can be questionable as the impact of any stress depends both on its intensity and duration. Hence, investigating a single parameter may not be insufficient to describe a response that is embedded in two dimensions (Rezende et al., 2014). To fully appreciate the innate capacity of a species to cope with cold and heat stress, an approach based on tolerance landscape was suggested by Rezende et al., (2014). The thermal tolerance landscape (TTL), describes the probability to survive a thermal stress as a function of both the intensity and the duration of thermal stress. In the present study, we adopted this approach to describe basal heat and cold tolerance of SWD at adult and pupal stage. There is a lack of knowledge on SWD heat tolerance, as only Kanzawa (1939) and Kimura (2004) have examined this aspect. However, describing upper thermal limits is also important to understanding thermal biology of this invasive species. In Drosophila melanogaster, the humidity during thermal stress modifies survival probability and the response can be temperature-dependent (Bubliy et al., 2012; Kobey & Montooth, 2013). Combining high temperature with low humidity provides more stressful conditions to fruit flies than high temperature with high humidity (Bubliy et al., 2012). Furthermore, increasing humidity during cold exposure increased survival at 6 °C, but not at −4 °C in D. melanogaster (Kobey & Montooth, 2013). This underlines that interaction among abiotic factors may differentially shape thermal
tolerance landscapes. In the present study, we also investigated the interaction between temperature and humidity on SWD cold and heat survival of pupae. The general assumptions of this study were that (1) SWD survival will be function of both temperature stress intensity and time exposure. (2) Because thermal performance curves are nonlinear and asymmetric (Colinet et al., 2015a), we predicted uneven effects of increasing intensity of cold and heat stress (i.e. different shapes of TTL). More specifically, we predicted that survival will decrease rather progressively with cold stress intensity, and more abruptly with heat stress intensity. (3) We also expected differential responses (i.e. different TTL) between sexes and stages. Based on previous data from D. melanogaster (Jensen et al., 2007), we predicted that pupae will be less thermotolerant than adults. (4) Finally, we predicted that different humidity conditions will affect the shape of the TTL, with desiccating condition during thermal stress further reducing survival probability compared to thermal stress under high humidity.

Materials and methods

Flies origin and rearing

SWD flies were provided by the Insect Pest Control Laboratory (IPCL) from the FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (Austria). This strain was originally captured in 2013 in San Michele all Adige (Trentino, Italia) and has been continuously reared in IPCL. For our experimentations, SWD was reared in glass bottle (100mL) and supplied with standard food (for 1 liter: agar: 15 g, sucrose: 50 g, brewer yeast: 40 g, cornmeal: 40 g, kalmus: 8 g, Nipagin: 8 mL). Bottles were kept in incubators (Model MIR-154-PE; PANASONIC, Healthcare Co., Ltd. Gunma, Japan) at 25 °C, 12L : 12D. Adults and pupae randomly taken from the rearing stock were used in experiments. All adults were between 5 and 7-day-old to avoid
effects of maturation at young age on stress tolerance (Colinet et al., 2015b). Males were separated from females visually (with an aspirator) without CO₂ to avoid stress due to anesthesia (Colinet & Renault, 2012). For pupae, we used individuals that had pupated since maximum 48 h (i.e. corresponding to 8 to 9 days after egg laying at 25 °C).

Thermal tolerance assays

We submitted flies and pupae to six low constant temperatures (-5, -2.5, 0, 2.5, 5 and 7.5 °C) and seven high constant temperatures (30, 31, 32, 33, 34, 35 and 37 °C) for various durations. At least seven specific durations were used for each temperature and these are provided in Supplemental Information for each experiment (Table S1 to S5). We performed preliminary assays in order to determine for each temperature the number of time points and the durations of exposure to get survival data spanning from 0 to 100 % mortality. At the most stressful temperatures (both at heat and cold), we added some time points because mortality occurred very quickly (within less than 2 h). Adults and pupae were isolated in groups of 10 independent individuals each coming from different randomly chosen rearing stock bottles. For each sampling duration, three replicates of 10 flies or 10 pupae were used. Flies and pupae were exposed to the different thermal conditions either using food vials placed in incubators (Model MIR-154-PE; PANASONIC, Healthcare Co., Ltd. Gunma, Japan) for the longer assays (2.5, 5, 7.5, 30, 31, 32 °C) or using glass vials immersed in a glycol solution cooled by a cryostat (Cryostat Lauda ECO RE 630) for the shorter assays (-5, -2.5, 0, 33, 34, 35, 36, 37 °C). Temperature was checked during all assays using thermocouple K connected Testo thermometers (Model 175 T3; TESTO Limited, Hampshire, England) placed into an empty vial. After stress exposure, SWD adults were allowed to recover in 40 mL food vials. Adult survival was assessed by counting the proportion of dead and living individuals 24 h post
exposure. For pupae, the results were expressed as a percentage of emergence, considered here as
a proxy of survival. Flies were considered as alive when the adult totally emerged from the
puparium. Because isolation and manipulation of pupae in preparation to thermal assays might
cause some damage to the puparium, five sets of 20 untreated pupae were kept at 25 °C to estimate
possible mortality due to manipulation.

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

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

$$L_{t_{50}} = \frac{\log(0.5) - a}{b}$$ (Venables & Ripley, 2002)

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

SWD cold tolerance

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

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

$L_{t50}$ for adults and pupae at the different tested low temperatures are provided in Fig. 2A. Based on $L_{t50}$ values and their confidence intervals, pupae appeared much less cold-tolerant than adults.
Of note, for adults, models weren’t able to calculate Lt_{50} at 7.5 °C, as after 30 days of cold exposure, survival didn’t decrease under 80 %. From TTLs (Fig. 2B,C,D), we observed that during cold exposure females and males described rather similar surface patterns, but female’s survival to extreme low temperatures was lower than that of males. On the other hand, pupae described a drastically different perspective, as they did not tolerate both short exposures to extremes temperatures and long exposures to milder temperatures.

**SWD heat tolerance**

For both adults and pupae, 100 % mortality was reached for all tested temperatures, except for pupae at 30 and 31°C, where respectively 5 and 4 individuals successfully emerged during the last day of the experimentation. The multiple panels figure 3 illustrates survival data in adults (males and females, Fig. 3A) and in pupae (Fig. 3B) according to the different tested temperatures and durations. Temperature and duration had strong effect on adult heat survival ($\chi^2 = 819.69, p < 0.001; \chi^2 = 889.77, p < 0.001$, respectively). Survival decreased with increasing temperature and with increasing exposure duration (Fig.2A and Fig. S3). Furthermore, at highest temperatures temporal reduction of survival was much faster (time x temperature interaction; $\chi^2 = 1495.20, p < 0.001 ;$ Fig. S3). There was no difference between males and females ($\chi^2 = 1.52, p > 0.05$). Yet sexes where differentially affected by increasing temperature (temperature x sex interaction; $\chi^2 = 94.43, p < 0.001$), with males more affected than females at the highest tested temperatures (Fig. 2A and Fig. S3). Temporal changes of survival were similar between sexes (no sex x time interaction, $\chi^2 = 0.19; p > 0.05$).
Temperature and time also had a strong impact on pupal heat survival ($\chi^2 = 210.72, p < 0.001$; $\chi^2 = 388.71, p < 0.001$, respectively). Survival significantly decreased with increasing temperature and with increasing exposure duration (Fig. 3B and Fig. S4). The temporal decrease in survival was dependent on temperature (time x temperature interaction; $\chi^2 = 662.25, p < 0.001$), it was much faster at higher tested temperatures (Fig. 3B and Fig. S4).

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

Interaction between relative humidity and thermal stress

Low and high temperature treatments were statistically analyzed separately. Under cold exposures, 100 % mortality was reached for all tested temperatures for both high and low RH. The multiple panels figure 5 illustrates survival data in pupae according to the different tested temperatures (cold and heat) and durations. Temperature and duration had strong effect on pupal cold survival ($\chi^2 = 91.74, p < 0.001$; $\chi^2 = 649.88, p < 0.001$, respectively). Cold survival decreased with decreasing temperature and with increasing exposure duration (Fig. 5 and Fig. S5). Furthermore, at lowest temperatures, temporal reduction of survival was much faster (time x temperature...
interaction; \( \chi^2 = 68.07, p < 0.001 \); Fig. S5). RH did not differentially affect cold survival (\( \chi^2 = 0.02, p > 0.05 \)), but temporal changes were different between RH levels (RH x time interaction; \( \chi^2 = 6495.48, p < 0.05 \)) suggesting that temporal survival decrease at cold was slightly faster at low RH (Fig. S5).

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

Based on Lt50 values and their confidence intervals, it appeared that low RH greatly diminished heat survival, but did not affect cold survival (Fig. 6A and B). From TTLs (Fig. 6C, D, E and F) we observed that surface patterns were similar during cold exposures (Fig. 6C and D). On the other hand, during heat exposure, low RH landscape were less extended than high RH landscape, underlying that a low RH level slightly decreased heat survival (Fig. 6E and F).
Discussion

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

The cold tolerance of SWD estimated in the present study was rather consistent with previously reported data. Indeed, we found that adult and pupal mortality occurred very rapidly at subzero temperatures (Lt50 < 2 h at -5 °C and Lt50 < 12h at -2.5 °C). This fits with early reports that also found very short survival durations at subzero temperatures (e.g. Jackobs et al., 2015; Stephens et al., 2015; Plantamp et al., 2016; Ryan et al., 2016). We thus corroborate that SWD is a chill susceptible species that does not tolerate brief exposures to sub-zeros temperatures (Kimura, 2004; Dalton et al., 2011; Jakobs et al., 2016; Ryan et al., 2016; Plantamp et al., 2016). At 0 °C, we found that it required about one day to reach 50% mortality in adults and much less time was needed (a few hours) in pupae. This is also in the range of previous data on SWD (Kimura, 2004; Jakobs et al., 2015; Plantamp et al., 2016); however, our values appear slightly inferior to those reported with other SWD strains. Slight variations in thermotolerance among strains could be related to different rearing conditions. Indeed, we reared our flies at 25 °C not at 21 °C as in other laboratories (Jakobs et al., 2015; Plantamp et al., 2016). Variations may also result from different local adaptations of the tested strains (Hoffman et al., 2002; Van Heerwaarden et al., 2012). At temperatures above 0 °C, several days were required before reaching Lt50. Interestingly, there appeared to be a sort of threshold between 5 and 7.5 °C where individuals shifted from detrimental
condition (at 5 °C) to non-injurious condition (at 7.5 °C). Indeed, at 5 °C, Lt50 was reached in only 5 days, while at 7.5 °C, mortality remained low (under 20 %) even after rather long exposure (one month). Previous data reported that non-acclimated SWD adults start to fall into coma at temperatures just below 5 °C (Jakobs, 2014). Therefore, we can assume that this temperature represents a physiological limit under which chilling injuries, such as neuromuscular dysfunctions, may start to accumulate (Hazell & Bale, 2011; MacMillan et al., 2012). In temperate regions, cold snaps with freezing events could be thus lethal to SWD. However, it seems that SWD overwinters as adults by migrating into protected microclimates, in leaf litter or in human made structures (Kanzawa, 1939; Kimura, 2004; Dalton et al., 2011; Zerulla et al., 2015; Rossi-Stacconi et al., 2016). This avoiding strategy likely allows SWD to escape low winter temperatures in natura, and even colonize new cool regions (Rota-Stabelli et al., 2013; Asplen et al., 2015).

Because thermal performance curves are nonlinear and asymmetric (skewed towards low temperatures) (Martin & Huey, 2008; Colinet et al., 2015a), we predicted uneven effects of increasing the intensity of cold vs. heat stress. Essentially, we assumed that SWD survival will decrease rather progressively with increasing cold stress intensity, and we expected a steep decline in survival over certain limits under heat stress. Observation of Lt50 values and TTLs patterns support this assumption. Under low temperature conditions, a progressive survival decrease was observed (Fig. 2), while under high temperature stress, there was clearly a limit over which survival crashed suddenly and became close to zero (Fig. 4). Indeed, at 32 °C, adult flies could sustain continuous heat stress for several days (Lt50 of 3-4 days), whereas at 33 °C, most flies succumbed within a couple of hours (Lt50 of about 2 h). Therefore 32 °C seem to be very close to critical thermal maximum for survival of SWD. Characteristically, the drop in performance (i.e. survival in our case) is generally more precipitous at supra-optimal temperatures than at sub-optimal
temperatures (Denlinger & Yocum, 1998). Arrhenius-like effects can explain progressive and
reversible changes of performance at sub-optimal temperatures, while the sharp decline at supra-
optimal temperature is generally ascribed to the destabilizing effects of heat on molecular
interactions such as irreversible protein denaturation (Schulte et al., 2011). Our results are in
accordance with early studies performed on SWD populations. Kanzawa (1939) noticed a motor
activity decrease of SWD when exposed to 30°C, and Kimura (2004) estimated that the 50% lethal
temperature at heat was around 32°C. Also, the upper thermal limit for development is estimated
to be at 31.5 °C (Asplen et al., 2015). Ryan et al., (2016) found no adult hatching when Canadian
flies developed at 31 °C. Therefore, our survival data together with the previous literature suggest
that SWD is not a particularly heat-tolerant species. This likely explains the very low field survival
of SWD when temperatures exceed 30 °C (Dalton et al., 2011; Tochen et al., 2014). While
overwintering strategy is rather well studied (Kanzawa, 1936; Stephens et al., 2015; Shearer et al.,
2016; Toxopeus et al., 2016; Wallingford et al., 2016), how flies cope with heat stress in the fields
and more generally how SWD manages to survive under summer conditions (i.e. heat coupled
with desiccation) is currently unknown.

We expected differences in thermotolerance between sexes and stages. Under low temperature, we
observed that SWD males were slightly more cold-tolerant than females. This contrasts with
previous SWD studies which reported that females were more cold-tolerant than males (Kimura,
2004; Dalton et al., 2011; Jakobs et al., 2015). However, lack of difference in cold tolerance
between sexes were also reported in SWD (Ryan et al., 2016). In D. melanogaster, the sex effect
on cold tolerance can be sometimes in favor of males (Kelty & Lee, 2001; Sejerkilde et al., 2003;
Jensen et al., 2007), or females (David et al., 1998; Condon et al., 2015). These discrepancies may
result from various factors, such as different measures of cold tolerance, tested temperatures or
age of flies (Jensen et al., 2007). In consequence, we suggest that in SWD, as in D. melanogaster, sex dimorphism in various metrics of cold tolerance appears more as an idiosyncretic than a general rule (Gibert & Huey, 2001). This view is also supported by our observations that, under high temperature, we only detected an interaction (sex x temperature) that suggested that females better tolerated heat exposure but at certain temperatures (i.e., at the greatest temperatures).

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

Finally, we predicted that very contrasted RH levels will affect thermal tolerance patterns. Specifically, we expected that highly desiccating condition during thermal stress will further reduce survival compared to stress conditions under high RH. As predicted, RH had strong impact on pupal survival, but this manifested only under heat stress. The Lt50 values illustrate these marked differences. Under cold conditions, the shapes of TTLs were globally identical between low and high RH, whereas under heat conditions, the TTLs were extended towards longer survival under high RH. In D. melanogaster, the humidity during both heat (Bubliy et al., 2012) and cold exposure (Kobey & Montooth, 2013) alters survival rate. Combining two stressors like high temperature with low RH provides more stressful conditions to fruit flies than high temperature with high RH (Bubliy et al., 2012). Here, we observed the same synergetic-like phenomena with SWD pupae. Prince & Parsons (1977) showed that under low RH, D. melanogaster adults move towards lower temperatures likely to mitigate water loss. It is reasonable to speculate that in natural conditions, SWD adults also tend to avoid heat stress and low RH by searching for protected and favorable microhabitats, but this remains to be tested. Recent mark-capture researches indicate that SWD achieves short-distance migrations from field margins to cultivated crops (Klick et al., 2016) therefore migration towards favorable microclimates is completely conceivable. During the pupal stage, however, flies are immobile and are thus potentially subjected to prolonged heat and desiccation stress, particularly if pupation occurs outside of infested fruits, as is the case with SWD (Asplen et al., 2015). Despite being protected within the puparium, water loss by pupae can strongly affect survival of drosophilids, and even moderately dry conditions can induce substantial
pupal mortality even at permissive temperature (Kojima & Kimura, 2003). A recent study from Tochen et al., (2016) indicates that low RH (e.g. 20 %) induced poor survival and lack of reproduction in SWD, suggesting that this species is particularly sensitive to water loss. Under low temperature, there was globally no effect of RH on cold survival. Death during prolonged cold exposure may be due to a combination of stressors: low temperature, starvation and desiccation. If pupae were suffering from desiccation at cold, then altering RH during low temperature exposure should affect water loss, and therefore, the survival duration at cold. Lack of RH effect at cold suggests that desiccation is not a primary cause of mortality under cold stress.

In this work, we studied basal thermal tolerance of SWD considering survival as a function of temperature stress intensity (under heat and cold) and exposure duration in adults (males and females) and in pupae. We found that survival under heat and cold conditions was dependent on both stress intensity and duration, and we provide a comprehensive description and visualization of SWD thermal tolerance and limits. We confirmed that SWD is a chill susceptible species, and noted that at temperatures over 5 °C, adults managed to survive for rather long periods (one month). Tolerance to thermal stress over a range of conditions, as illustrated by TTLs, showed rather different perspectives: a sudden vs. a more progressive survival decline under heat vs. cold conditions, respectively. In particular, 32 °C seemed to be very close to critical thermal maximum for survival of SWD. A sex dimorphism in thermal tolerance was also found but was temperature-dependent. Difference in thermal tolerance were also observed between stages, with pupae being drastically more sensitive to cold stress but more resistant to extreme heat stress than adults. Finally, we found that level of RH had strong impact on pupal survival under heat stress but not under cold stress.
Recently, a consortium of scientists has published a useful review with the updated situation of SWD all over the world (Asplen et al., 2015). The authors suggested a few directions for future research to improve the accuracy of SWD management. Acquisition of novel data on the biology of SWD at low temperature was highlighted as a priority, and we believe the present dataset may provide valuable elements in this regard. The present study is one of the first to provide a global description of SWD basal thermal tolerance, especially bringing new information about heat stress tolerance and the interaction between temperature and relative humidity. However, we wish to draw attention to the fact that stress tolerance data acquired from field-collected populations may contrast with those resulting from laboratory-adapted lines (e.g. Hoffmann et al., 2001; Schou et al., 2015). In consequence, despite logistical constraints, the next needed step is the realization of thermal studies on field-collected individuals.

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Figures captions

Figure 1
SWD survival as a function of low temperature and duration exposure.
A: Adults; B: Pupae. Points correspond to observed data, and lines to GLMs prediction (Binomial GLM, link = logit). The tested temperatures are indicated in the left top corner of each plot.

Figure 2
Lt$_{50}$ values and thermal tolerance landscapes for low temperatures.
A: Males, females and pupal Lt$_{50}$ values ± 95% confidence intervals. Lt$_{50}$ is the time at which 50% of the population is dead. Lt$_{50}$ values are calculated from GLMs. B,C,D: males, females and pupal thermal tolerance landscapes; points are observed values, and surfaces correspond to GLMs predictions (Binomial GLM, link = logit).

Figure 3
SWD survival as a function of high temperature and duration exposure.
A: Adults; B: Pupae. Points correspond to observed data, and lines to GLMs prediction (Binomial GLM, link = logit). The tested temperatures are indicated in the left top corner of each plot.

Figure 4
Lt$_{50}$ values and thermal tolerance landscapes for high temperatures.
A: Males, females and pupal Lt$_{50}$ values ± 95% confidence intervals. Lt$_{50}$ is the time at which 50% of the population is dead. Lt$_{50}$ values are calculated from GLMs. B,C,D: males, females and pupal
thermal tolerance landscapes; points are observed values, and surfaces correspond to GLMs predictions (Binomial GLM, link = logit).

Figure 5
SWD pupal survival as a function of temperature and exposure duration under two relative humidity (RH) levels.

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

Figure 6
Pupal Lt50 values and thermal tolerance landscapes for low and high temperatures under two relative humidity (RH) levels.

A,B: Lt50 values ± 95% confidence interval values. Lt50 values are calculated from previous GLMs predictions. C, D, E, F: thermal tolerance landscapes for low and high temperature; points corresponds to observed values, and surfaces corresponds to GLMs predictions (Binomial GLM, link = logit).
Figure 1:
Figure 2:
Figure 3:
Figure 4:
Figure 5:
Figure 6: