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Combined effect of temperature and *Wolbachia* infection on the fitness of *Drosophila suzukii*

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Abstract

Maternally inherited *Wolbachia* is prevalent among insects and is often linked with modifications of host fitness. The result and evolution of host symbiont interaction depend on environmental limits but are difficult to predict since they arise from complex interactions among host, parasite, and environment. In this study, we evaluated whether fitness traits of *Wolbachia*-free and *Wolbachia*-infected individuals of *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae) were affected when flies were exposed to temperature stress (29 °C versus 22 °C) as well as to nutritional and starvation stress. Two lines of *D. suzukii* were prepared for the experiments: a *Wolbachia*-free line, treated with antibiotics, and a *Wolbachia*-infected line, hosting the endoparasite *w*Suz. Survival and fecundity of the two lines were assessed as well as the effect of nutritional stress on time of development and starvation stress on survival. Results showed that *Wolbachia* had a positive effect on egg hatch rates and viability at 29 °C, while at the same temperature there was a negative effect on adult survival. *Wolbachia*-free and *Wolbachia*-infected flies did not show any difference regarding nutritional and starvation stress. It is concluded that temperature associated changes in *w*Suz did not cause cytoplasmic incompatibility but significantly affected some fitness traits. This result may help to understand the performance of *D. suzukii*-*Wolbachia*-host-symbiont interaction in nature.

Key words: temperature, nutrition, cytoplasmic incompatibility, survival, performance.

Introduction

Drosophila suzukii (Matsumura) (Diptera Drosophilidae) is an invasive species that threatens soft fruit industries in North America and Europe (Walsh *et al*., 2011; Cini *et al*., 2014) through feeding on unripe and undamaged fruits (Lee *et al*., 2011; Atallah *et al*., 2014). It has become the 8th pest of this genus introduced into Europe and is considered a highly damaging pest of agricultural areas (Asplen *et al*., 2015), as it attacks various ornamental and wild fruits (Poyet *et al*., 2015). The species is spreading rapidly, becoming a significant pest and a cause for concern to fruit handling industries, but existing traditional control methods (chemical, cultural, monitoring) have been neither efficient nor sustainable. Combined bio-control strategies building on knowledge of the pest ecology and biology might provide an answer to the problem. Among these bio-control strategies (including entomopathogens, parasitoids, parasites), the endosymbiont *Wolbachia* might play an important role. *Wolbachia* (Rickettsiaceae) are obligate intracellular parasites that have been recorded in the reproductive systems of 20 to 76% of insects (Hilgenboecker *et al*., 2008; Zug and Hammerstein *et al.*, 2015), are transmitted from infected females to their offspring and linked to a variety of reproductive abnormalities in the host (Carrington *et al*., 2010; Guruprasad *et al*., 2011). These abnormalities include male-killing (Hurst *et al*., 1999), feminization (Rigaud *et al*., 1991), parthenogenesis (Stouthamer *et al*., 1990), and cytoplasmic incompatibility (CI) (Hoffmann *et al*., 1990; Werren *et al*., 2008). CI is a sperm-egg incompatibility that occurs in crosses involving a male that harbours at least one *Wolbachia* strain that the female lacks, all other crosses being fertile (Poinsot *et al*., 2003). The understanding of the molecular mechanism of *Wolbachia*-induced CI has recently improved (LePage *et al*., 2017; Beckmann *et al*., 2017), supporting the modification-rescue framework, with two CI-inducing genes that appear to work together causing modification that can be rescued by *Wolbachia*-infected host females.

Some benefits of *Wolbachia*-infection include increased fecundity, survival, nutritional supply (Zug and Hammerstein, 2015), protection against pathogens (Hedges *et al*., 2008; Teixeira *et al*., 2008) and thermal tolerance (Chen *et al*., 2000; Montllor *et al*., 2002). Reproductive manipulation for the conservation of *Wolbachia* can be explained by positive impacts upon fitness traits, for instance longer survival of *Wolbachia*-infected flies in nature (Fry and Rand, 2002). In the mosquito *Aedes albopictus* (Skuse), *Wolbachia*-infected females survive longer, produce more eggs and have higher hatch rates than *Wolbachia*-free females (Dobson *et al*., 2002). Unfavourable effects include a decrease in lifespan for the *w*MelPop strain in *Drosophila melanogaster* Meigen (Chrostek and Teixeira, 2015).

Wolbachia was also found to be associated with *D. suzukii* (*w*Suz) by Cordaux *et al*. (2008), who first genotyped the *Wolbachia* genome and found that it was a distinctive strain and also recorded with the genome of *w*Ri strain allied to *Drosophila simulans* Sturtevant (Siozios *et al.*, 2013). These findings were confirmed by Mazzetto *et al*. (2015), who found mutualistic association between *Wolbachia* and *D. suzukii* through increased fecundity whereas due to polymorphic infection, little CI or other reproductive manipulation was reported in *Drosophila* sp. (Hoffmann *et al*., 1996; Hamm *et al*., 2014). Geographical diversity of *w*Suz was also identified by Kaur *et al*. (2017) in *D. suzukii* populations from different continents. Recently, transfer ability of *w*Ri was also checked

by Conner *et al*. (2017) between two sister species, *D. suzukii* (*w*Suz) and *Drosophila subpulchrella* Takamori et Watabe (*w*Spc). The transmission efficiency of *Wolbachia* is reduced at high temperature (Hurst *et al*., 2001), demonstrating that this has a large impact on its prevalence. In Australian lines of *D. melanogaster*, the frequency of *Wolbachia* suggested that the endosymbiont endows fitness benefits that totally depend on the environment (Hoffmann *et al*., 1994; 1998). Several findings have confirmed that temperature leads to major impacts on the effects of associations between host and microorganism remarkably by disturbing the virulence of microorganisms (Thomas and Blanford, 2003). This is why at extreme temperature *Wolbachia* could be the main reason for CI (Bordenstein and Bordenstein, 2011). Indeed, temperature effects on a wide variety of heritable symbionts have recently been studied by Corbin *et al*. (2017).

Furthermore, some *Wolbachia* strains have shown to be nutritionally mutualistic such as F supergroup *Wolbachia* found in bedbug *Cimex lectularius* Latreille, where symbionts reside in specialized bacteriome cells and provide vitamin B to insect hosts (Hosokawa *et al*., 2010). So, novel metabolic ways provided to hosts in the form of nutrition and starvation stress have also been offered as a main route to endosymbiosis of bacteria (Douglas, 1994). Protein deficiency decreases fecundity and development in *D. melanogaster* (Wang, 1995). In contrast, diet limit or mild starvation can enhance longevity as well as tolerance to stressors such as heat (Wenzel, 2006; Smith *et al*., 2007), indicating the complication of organismal nutrient acquirement and utilization.

As there is no indication that *Wolbachia* can induce strong reproductive effects, such as CI, in relation to temperature in European populations of *D. suzukii*, we explored whether *Wolbachia* provides temperature-linked fitness benefits. The effects of temperature on *Wolbachia*-free and *Wolbachia*-infected lines of *D. suzukii* were assessed by considering survival, fecundity, and viability whereas nutritional and starvation stress was examined by observing developmental time and survival under temperature stress for both lines.

Materials and methods

Insect culture

The *Wolbachia*-infected culture of *D. suzukii* used for the study was obtained from Department DAFNAE-Entomology, from adults collected from strawberries and organic cherry orchards in Verona province, (North-Eastern Italy) in autumn 2014. The flies were fed in plastic vials (Falcon type of 50 ml capacity, 30 mm diameter, 115 mm length) with specific *D. suzukii* rearing medium prepared according to Tonina *et al*. (2016). The medium contained 75 g cornmeal, 17 g yeast, 15 g sucrose, 12 g soybean meal, 5.6 g agar, 5 ml propionic acid and water to 1000 ml. All ingredients were mixed and heated for about 25 min at 100 °C, excluding propionic acid that was added at a temperature below 50 °C before pouring 15 ml of medium into vials. Insects were reared in climatic chamber at 23 ± 2 °C and 70-80% relative humidity with a photoperiod of 16L:8D.

DNA isolation and *Wolbachia* detection

Wolbachia presence in the population was assessed on 30 adults (15 males and 15 females) by specific PCR assays. The microbial DNA was extracted from individual following the protocol described in Palmano *et al*. (2000). Detection of *Wolbachia* was accomplished by amplifying 16S rDNA gene using specific primers 16S-F (TTGTAGC(C/T)TGCTATGGTATAACT) and 16S-R (GAATAGGTATGATTTTCATGT) (O'Neill *et al*., 1992). All PCR reactions were conducted in a 20 µl volume containing 4 µl PCR of 5x colorless GoTaq Flexi Buffer (Promega), 2.5 mM MgCl, 0.1 mM dNTPs, 0.5 µM of each primer, 1U of GoTaq Flexi DNA polymerase (Promega) and 2 µl of extracted DNA. Cycling conditions consisted of an initial denaturation step at 94 °C for 5 min followed by 35 cycles with a denaturation step at 95 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 5 min.

Preparation of *Wolbachia*-free lines

After maintaining 1,000 flies culture on normal rearing media, two lines of flies were created; *Wolbachia*-free line (hereafter: WF) following a commonly used antibiotic procedure (Poinsot and Mercot, 1997; Bordenstein and Werren, 1998) and *Wolbachia*-infected line (hereafter: WI) on the medium without antibiotic. We created WF lines adding tetracycline [final concentration 0.2 mg ml^{-1} (0.02%)] (Min and Benzer, 1997) to the medium mentioned above. This procedure was continued for four discrete generations to ensure that *Wolbachia* were totally removed. After tetracycline treatments, *Wolbachia* elimination was confirmed by PCR analysis. Consecutively, the new descendants were fed for two discrete generations on the normal food medium without antibiotic before the beginning of crossing experiments, to avoid any possible effect of the antibiotic on the flies' fitness (Fry *et al*., 2004). The WI lines received identical food and environment as the WF lines except for tetracycline. The presence of *Wolbachia* was confirmed by PCR assessment.

The preparation and maintenance of WF and WI lines were conducted at two different temperatures, 22 ± 1 °C and 29 ± 1°C, 70-80% relative humidity with a photoperiod of 16L:8D.

Fecundity

At the start of the seventh generation, four crosses were made between WF and WI flies as follows: (1) $WI_{♂} \times WI_{♀}$, (2) $WF_{♂} \times WI_{♀}$, (3) $WI_{♂} \times WF_{♀}$, (4) $WF_{♂} \times WF_{♀}$. Each cross was replicated five times. For each cross, a newly enclosed single female and male (3 days old) were placed in the same vials with medium to mate and lay eggs. The flies were transferred to new vials containing 15 ml of food each day and the number of eggs deposited in the vials during the previous 24 h was recorded. For easy recognition of eggs, red food dye was used in diet preparation as described by Uchizono *et al*. (2017). The number of eggs laid was then counted under a compound stereomicroscope. Fecundity was checked every day for up to 12 days. Vials were maintained at two temperatures, 22 ± 1 °C and 29 ± 1 °C.

Cytoplasmic incompatibility and viability

CI occurs when the ratio of eggs hatching from the incompatible cross (3) WI × WF is less than compatible crosses (1) WI × WI and (2) WF × WI. CI was estimated by holding the vials from the fecundity assays at both temperatures for an additional 35-40 h, when the number of unhatched eggs in each vial was recorded and compared with the previous egg counts. This was repeated five times for all vials containing eggs during 12 days from the fecundity assay (Mazzetto *et al*., 2015). Larval to adult viability was assessed from eggs deposited in vials during a 24 h period. The protocol was to hold vials containing the eggs of each day for 14 days after laying and count the number of emerging adults from the vials set at the two temperatures. The number of emerging adults was then compared with the number of hatched eggs recorded previously for that vial.

Survival

At the start of the seventh generation, survival of WF and WI lines was measured by placing 30 adults (3-4 days old, 15 males and 15 females) in three replicate boxes (20 × 20 × 20 cm). The boxes were placed in climate chambers at two temperatures (22 ± 1 °C and 29 ± 1 °C), 70-80% relative humidity, and 16L:8LD photoperiod. The food media was replaced every 1-2 days as indicated by Strunov *et al*. (2013); dead flies were removed and both sexes counted daily for both WF and WI flies. The boxes were monitored in this way until all the flies had died.

Nutritional stress

At the start of the seventh generation, the impact of nutrition was investigated by rearing larvae of WF and WI flies on yeast poor food media. Nutritional stress was imposed using a lower amount of yeast (0.17 g yeast, 1%) as poor medium compared to the normal medium (17 g yeast, 100%) (Harcombe and Hoffmann, 2004). Five plastic jars (60 mm diameter, 80 mm length) were set up for both media (poor and normal) and WF and WI lines. Twenty eggs were placed in each jar containing 20 ml food medium. The trial was conducted at 22 ± 1 ºC and 29 ± 1 °C. To determine nutritional effects, the time necessary for the flies to emerge was assessed.

Starvation stress

At the start of seventh generation, starvation tolerance was determined by putting 30 flies (15 males and 15 females, 3-4 days old) into 40 ml glass vials with no food. Humidity in vials was kept high by using cotton wool soaked with 10 ml of water (Service *et al*., 1985). The same procedure was followed by Harcombe and Hoffmann (2004) to check starvation tolerance between infection status without control. Five replicates were tested at two temperatures (22 ± 1 ºC and 29 ± 1 °C) for WF and WI lines. Mortality was assessed twice a day.

Statistical analysis

All experimental data showed a normal distribution and variability. Univariate analysis using General Linear Model (SPSS 10.1) was performed to determine the influence of temperature on fecundity, hatch rate, and viability for both WF and WI lines. Two methods were used in order to obtain more accurate results. Survival data were analyzed with Kaplan-Meier (KM) log rank tests, to check the mortality rates for each temperature of both lines on the basis of sex. Three way ANOVA was also used to compare the survival at both temperature on the basis of days and sex for both lines. Nutritional and starvation stress was checked by three-way ANOVA with relation to temperature stress on developmental time (days) and survival (hours) for both lines. For the multiple comparisons of tests, means were separated by Tukey-Kramer (HSD) test at 5% significance level (Sokal and Rohlf, 1995).

Results

Wolbachia was proven to consistently infect our field collected *D. suzukii* population. PCR assays showed that *Wolbachia* was originally present in maintained culture with 93.8% average infection rate, 87.5% in males and 100% in females. No *Wolbachia* was detected in treated WF specimens.

Fecundity

Significant variation in fecundity among crosses ($F_{3, 480} = 50.82$, $P < 0.01$), temperatures ($F_{1, 480} = 1595.89$, $P < 0.01$, and time ($F_{11, 480} = 38.9$, $P < 0.01$) was found (figure 1). Cross 3 ($WI_{♂} \times WF_{♀}$) showed a significant decrease in fecundity after *Wolbachia* removal whereas cross 4 ($WF_{♂} \times WF_{♀}$) was not significantly different from crosses 1 ($WI_{♂} \times WI_{♀}$) and 2 ($WF_{♂} \times WI_{♀}$). Fewer eggs were laid at 29 °C than at 22 °C; moreover, during 12 days of egg laying, fecundity increased day by day.

The interactions temperature × crosses and temperature × days were significant ($F_{3, 480} = 4.22$, $P < 0.01$; $F_{11, 480} = 20.23$, $P < 0.01$, respectively), while the interactions crosses × days ($F_{33, 480} = 0.30$, $P = 1.00$) and temperature × days × crosses ($F_{33, 480} = 0.34$, $P = 1.00$) were not significant.

In short, the higher temperature drastically reduced the ability of both WF and WI lines to lay eggs than at 22 °C, although crosses 3 ($WI_{♂} \times WF_{♀}$) and 4 ($WF_{♂} \times WF_{♀}$) produced fewer eggs than crosses 1 ($WI_{♂} \times WI_{♀}$) and 2 ($WF_{♂} \times WI_{♀}$) at 29 °C.

Hatch rate and viability

The combined effect of temperature and antibiotic treatment affected the hatch rate and viability. All crosses (1-4) did not significantly differ at 22 °C ($F_{3, 240} = 0.10$, $P = 0.95$) whereas they did at 29 °C ($F_{3, 240} = 16.98$, $P < 0.01$). A reduction in hatch rate was found in crosses 3 ($WI_{♂} \times WF_{♀}$) and 4 ($WF_{♂} \times WF_{♀}$) at 29 °C (figure 2).

No major changes were recorded in viability of adults at 22 °C ($F_{3, 240} = 1.02$, $P = 0.38$), while at 29 °C fewer adults were obtained from the crosses having WF females compared to WI ones with significant differences ($F_{3, 240} = 13.16$, $P < 0.01$) (figure 3).

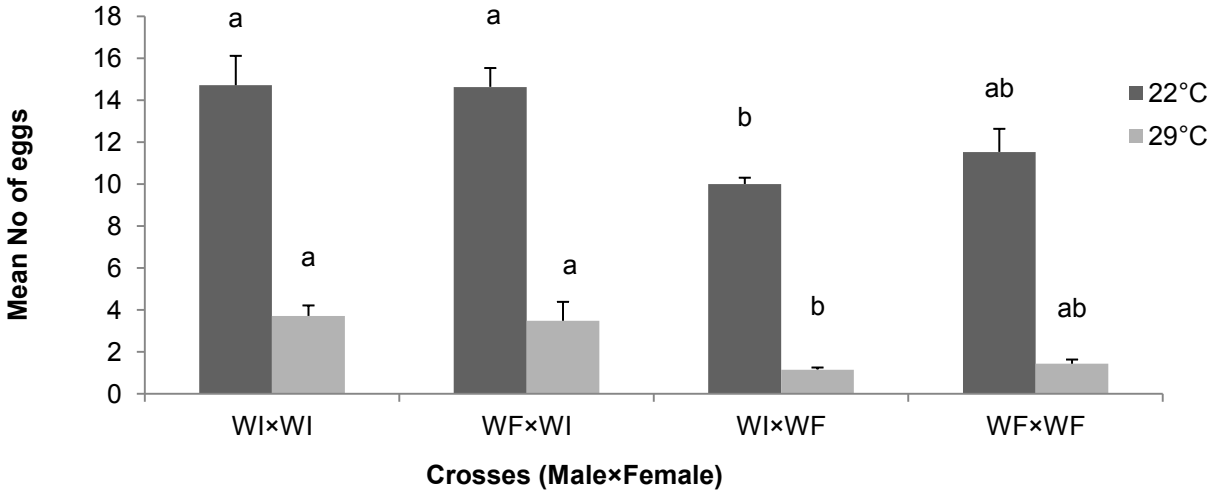

Figure 1. Mean number (± SE) of eggs of *D. suzukii* females during 1-12 days in the four crosses (1) $WI_{♂} \times WI_{♀}$, (2) $WF_{♂} \times WI_{♀}$, (3) $WI_{♂} \times WF_{♀}$, (4) $WF_{♂} \times WF_{♀}$ under two temperatures (22 and 29 °C). Different letters above histogram bars indicate significant differences (P < 0 .05) in pairwise comparisons within the same temperature.

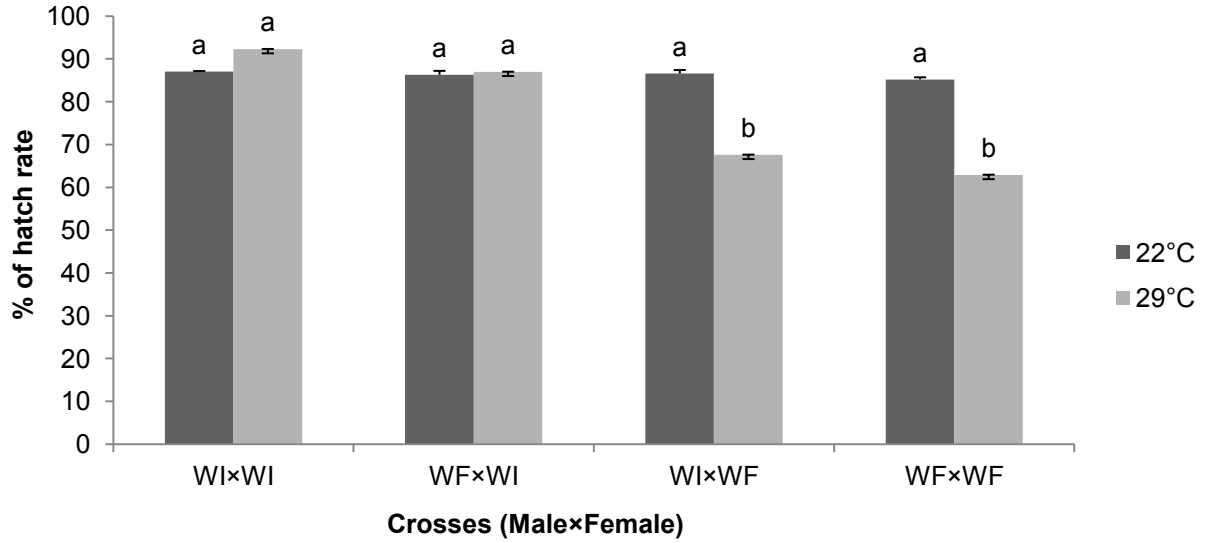

Figure 2. Mean (± SE) percentage of eggs hatching from each of four crosses under two temperatures (22 and 29 °C). Different letters above histogram bars indicate significant differences (P < 0.05) in pairwise comparisons within the same temperature.

Survival

As main effect, temperature significantly affected the longevity of both male and female flies (F = 319.58, df = 1, P < 0.01). At 22 °C *D. suzukii* adults survived longer (20-30 days) than at 29 °C (figure 4A, 4B). Regarding the influence of bacterial infection at 22 °C, infection status did not display any difference in survival (F = 0.48, df = 1, 408, P = 0.47) while it was noticed at 29 °C (F = 4.93, df = 1, 408, P = 0.02) (figure 4A, 4B and 5). There was a significant interaction between temperature × infection status (F = 4.8, df = 1, 816, P = 0.02).

According to Kaplan Meier analyses, temperature significantly affected survival (χ^2 = 96.45, df = 1, P < 0.01) At 22 °C, infection status did not impact survival (χ^2 = 0.70, df = 1, P = 0.78) while it did at 29 °C (χ^2 = 3.35, df = 1, P = 0.05) (figure 5). There was significant influence between temperatures for the survival of both WI and WF populations (χ^2 = 62.68, df = 1, P < 0.01 and χ^2 = 35.89, df = 1, P < 0.01, respectively). No difference was observed for the survival of both males and females in WF and WI lines at 22 °C (χ^2 = 0.49, df = 1, P = 0.48 and χ^2 = 0.38, df = 1, P = 0.53, respectively) (figure 4A) and 29 °C (χ^2 = 1.89, df = 1, P = 0.16 and χ^2 = 1.45, df = 1, P = 0.22, respectively) (figure 4B).

Nutritional and starvation stress

Nutritional effects assays showed that, food media ($F_{1, 40}$ = 0.10, P = 0.74 and infection status ($F_{1, 40}$ = 0.43,

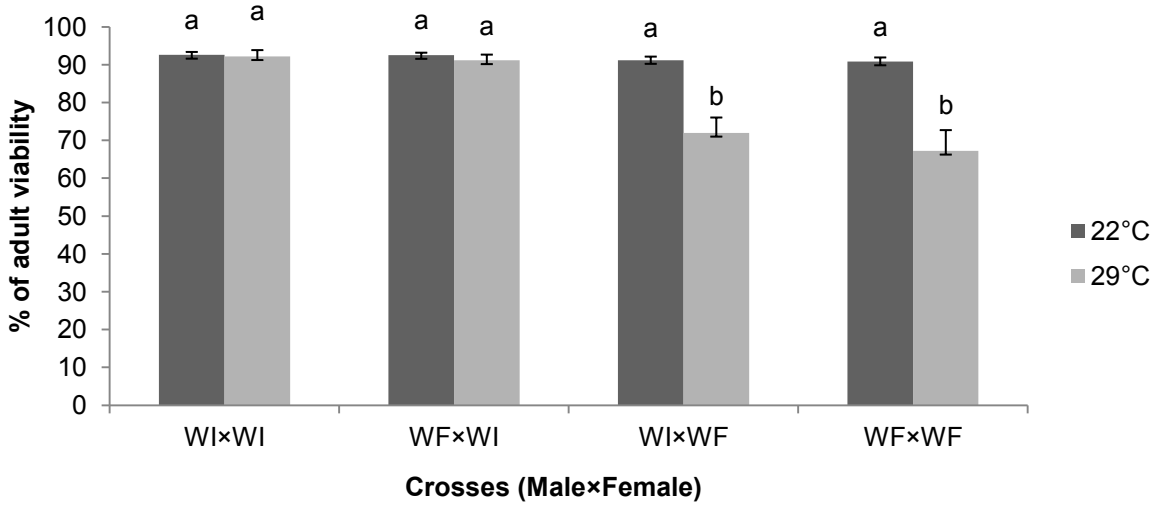

Figure 3. Mean (± SE) percentage of adult *D. suzukii* emergence from each cross under two temperature regimes (22 and 29 °C). Different letters above histogram bars indicate significant differences ($P < 0.05$) in pairwise comparison within the same temperature.

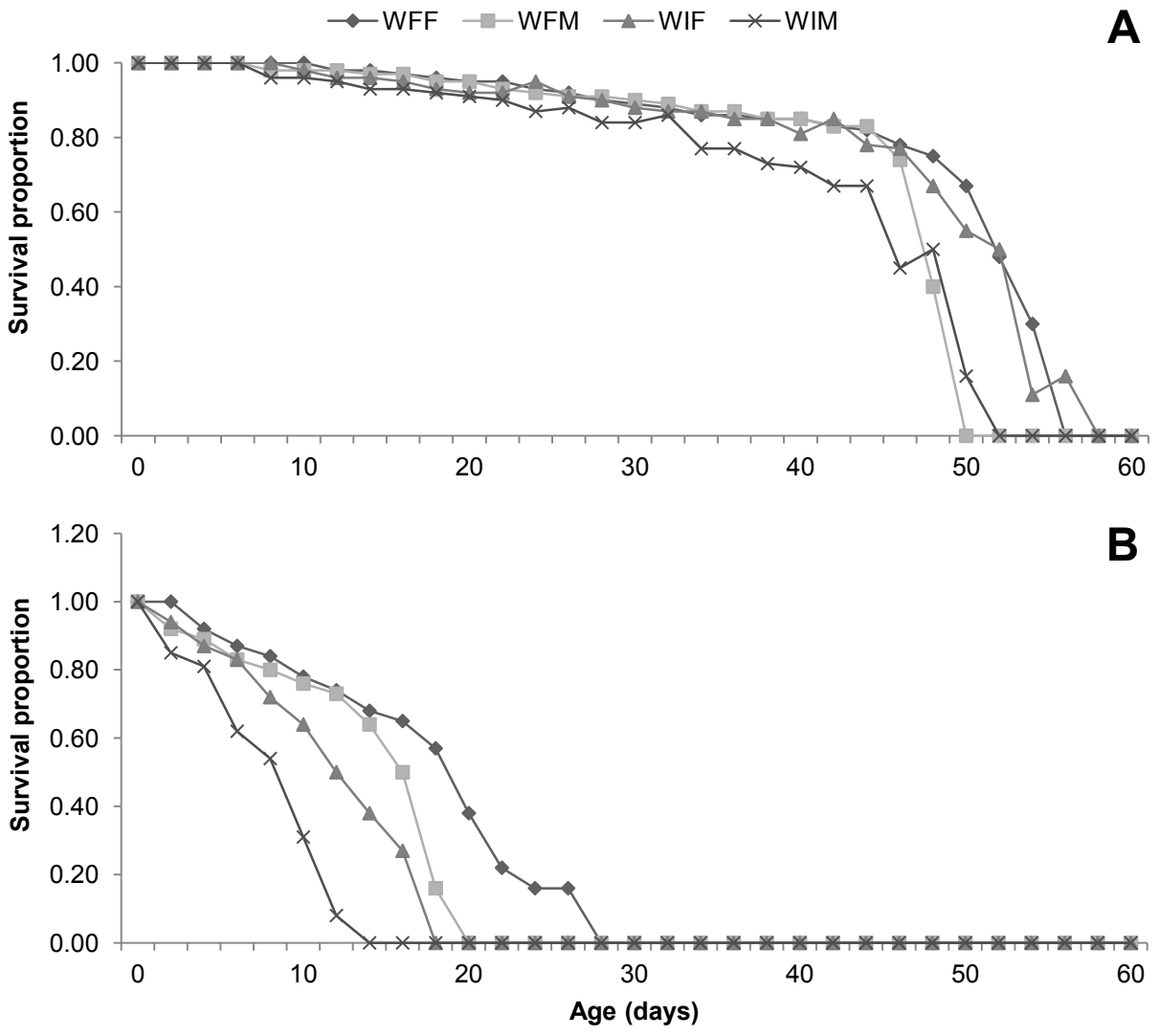

Figure 4. Proportion of survival for both WF and WI lines of *D. suzukii* males (M) and females (F) at 22 (A) and 29 °C (B).

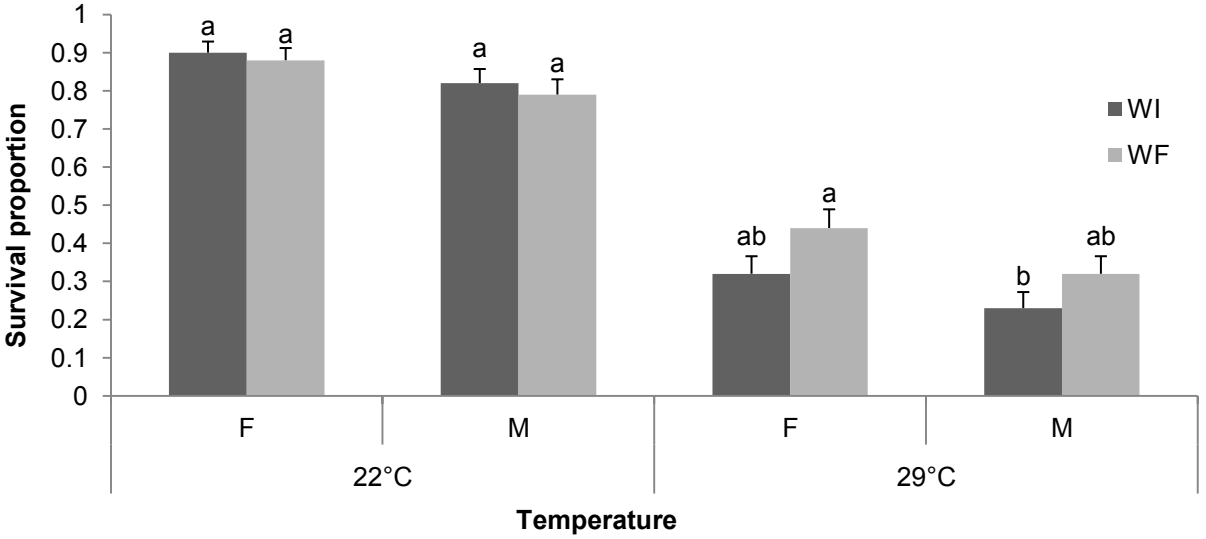

Figure 5. Average of survival proportion for both WF and WI lines of *D. suzukii* males (M) and females (F) at 22 and 29°C. Different letters above histogram bars indicate significant differences ($P < 0.05$) within each temperature among sex and infection status.

$P = 0.52$) did not influence the adult developmental time, which was 1.4 times longer at 22 than at 29 °C ($F_{1, 40} = 62.27$, $P < 0.01$). No significant interaction was found between media × infection status ($F_{1, 40} = 0.00$, $P = 1.00$), media × temperature ($F_{1, 40} = 0.00$, $P = 1.00$), infection status × temperature ($F_{1, 40} = 0.10$, $P = 0.74$) and media × infection status × temperature ($F_{1, 40} = 0.10$, $P = 0.74$).

Infection status did not impact the starvation resistance either ($F_{1, 40} = 3.13$, $P = 0.09$) but temperature did ($F_{1, 40} = 696.15$, $P < 0.01$), as both WF and WI flies lived 4 times longer at 22 than at 29 °C. Sex had an impact on starvation ($F_{1, 40} = 7.25$, $P = 0.01$), as females lived slightly longer than males. There was no interaction between infection status × sex ($F_{1, 40} = 0.29$, $P = 0.59$), infection status × temperature ($F_{1, 40} = 3.41$, $P = 0.09$), sex × temperature ($F_{1, 40} = 1.0$, $P = 0.33$) or infection status × sex × temperature ($F_{1, 40} = 0.15$, $P = 0.70$).

Discussion

The findings of this study elucidate the combined impact of temperature and *Wolbachia* infection on the fitness of *D. suzukii*. We found that *Wolbachia* had a positive effect on egg hatch rates and viability at 29 °C, while at the same temperature there was a negative effect on adult survival. No significant effect was detected due to nutritional and starvation stress, irrespective of temperature on WF and WI flies.

Our experiments showed that fecundity dropped in crosses with WF females. High temperature also decreased the number of eggs but the pattern of egg increase and decrease was the same in all crosses at both temperatures. Tochen *et al.* (2014) also obtained fewer eggs at 30 °C in *D. suzukii* infected with *Wolbachia*. Evans *et al*. (2017) suggested that *D. suzukii* prefers microclimate with mild temperatures and high humidity for reproduction and does not perform well when exposed to direct sunlight with extreme heat. Our data thus suggest that female fecundity was beneficially influenced by *Wolbachia*. Mutualistic links where *Wolbachia* had positive impacts on fecundity are known for many insects (Dobson *et al*., 2002; Fry *et al*., 2004; Mazzetto *et al*., 2015) and in filarial nematodes (Bandi *et al*., 1999). This phenotype may be one of the reasons for *Wolbachia* persistence in the wild (Fenton *et al*., 2011), as well as in *D. suzukii* populations from North America and Europe (Cattel *et al*., 2016).

In addition, our results indicate that the change in fertility may result from the combined effect of temperature and antibiotic treatment. No difference was recorded in egg hatch rate at 22 °C in all crosses having WF and WI lines whereas it was lower at 29 °C in WF females. The reason for this is that heat shock and the consequent endogenous rise in heat shock proteins could play a role (Snook *et al*., 2000), although our results do not indicate occurrence of CI, as already observed in this species (Mazzetto *et al.*, 2015). However, a *Wolbachia* decrease was also reported in hosts fed at a temperature above 30 °C (Van Opijnen and Breeuwer, 1999; Zhukova *et al*., 2008) and below 13 °C (Pintureau *et al*., 2003). These two elements (temperature and antibiotic) help in understanding the species performance in a fluctuating environment, as explained by Araripe *et al*. (2004). As in hatch rate, we did not observe any indication of viability difference between WF and WI flies at 22 °C as found at 29 °C. Similar to our outcome, Fry *et al*. (2004) proved that the viability of some strains of *D. melanogaster* caused harmful modifications in fitness.

An ectothermic animal's survival greatly relies on temperature conditions (Cossins and Bowler, 1987). In our findings, when the temperature rose from 22 ± 1 °C to 29 ± 1 °C, WF females survived longer (28 days) than WI females (19 days), as previously detected by Tochen *et al*. (2014). This result confirms that obtained

by Min and Benzer (1997) with the strain *w*MelPop of *D. melanogaster*, who found that all WI flies kept at 29 °C died within 14 days and WF flies within 28-30 days. However, when survival was evaluated at 22 ± 1 °C, WF and WI flies did not maintain the differences in life-span. These findings show that the virulence of *Wolbachia* depends on temperature (Mint and Benzer, 1997; Carrington *et al*., 2009; Rohrscheib *et al*., 2016; Chrostek and Teixeira, 2017), which could play a vital role in host-*Wolbachia* interactions (Mouton *et al*., 2006; Kusmintarsih, 2012). Our study extended these findings to *D. suzukii*. The effects were also observed in strains of *D. melanogaster*, although the influence on fitness associated with the removal of *Wolbachia* varied according to the strain (Fry and Rand, 2002; Fry *et al*., 2004). Two main causes were suggested by Strunov (2013) to explain the early death of WI flies: first, the multiple obliteration of brain cells and the evacuation of replicating bacteria were seen at high temperature (also in Rasgon *et al*., 2006), second, the increase in proportion of clusters of bacteria may be the main cause of steady deterioration.

Energy and protein resources are imperative in adult *Drosophila* as they hunt for food (Harcombe and Hoffmann, 2004; Brownile, 2009; Hoffmann *et al*., 2011). The prevalence of nutrition supplementation in other endosymbiotic systems suggested that *Wolbachia* might also influence nutrition (Harcombe and Hoffman, 2004). In this study, both nutrition and starvation assays did not detect any changes between infected and cured flies. This suggests that *Wolbachia* does not offer *D. suzukii* any fitness benefit linked to nutritional tolerance.

We conclude that the effect of temperature is a key element influencing *D. suzukii*-*Wolbachia* strains. So, testing the association of *Wolbachia* with *D. suzukii* under different fluctuating temperature regimes may lead to a better understanding of the varying performance of the pest when exposed to seasonal temperature variation.

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