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► **To cite this version:**

Gwenaëlle Deconninck, Jordy Larges, Hélène Henri, Laureen Beaugeard, Vincent Foray, et al.. Wolbachia improves the performance of an invasive fly after a diet shift. *Journal of Pest Science*, 2024, 97, pp.2087-2099. 10.1007/s10340-023-01739-w . hal-04589869

HAL Id: hal-04589869

<https://hal.science/hal-04589869v1>

Submitted on 9 Oct 2024

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1 ***Wolbachia* improves the performance of an invasive fly after a diet shift**

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17

18 **ABSTRACT**

19

20 Polyphagy is an adaptative strategy allowing species to develop and survive on multiple diets. Phytophagous
21 insects can be exposed to sub-optimal resources, as host plants vary in nutritional quality. Some insects may
22 rely on symbiotic bacteria to better utilize less favorable substrates. The invasive fruit fly *Drosophila suzukii*
23 is a highly successful pest worldwide. The use of ripening and ripe fruits, a niche neglected by other
24 drosophilids, and its polyphagy may have contributed to its global spread. The role of *D. suzukii*'s *Wolbachia*
25 strain (*wSuz*) remains unconfirmed, although a mutualistic role has been hypothesized *via* virus protection or
26 dealing with abiotic stress. In some insect species, *Wolbachia* acts as a nutritional mutualist. This study
27 explored the role of *wSuz* in *D. suzukii* adaptation to fluctuating diet regimes. To simulate a diet shift, we used
28 *Wolbachia*-infected and uninfected flies, maintained on a corn-based (low-starch) or a grape-based (high-
29 starch) diet for at least 10 generations. Then, we placed individuals from each line on one or the other diet for
30 30 days. The effects of *Wolbachia* and the diet treatments were assessed by quantifying female fecundity, egg-
31 to-adult development time and survival, and offspring mass. The presence of *Wolbachia* positively affected
32 female fecundity and offspring mass after a diet shift. *Wolbachia* also increased survival during larval
33 development regardless of the diet, supporting its mutualistic role. Our results underline the role of *wSuz* on
34 *D. suzukii* diet tolerance and performance. A better understanding of the role of symbionts in invasive species
35 could help to improve management strategies.

36

37 **Keywords:** *Drosophila suzukii*, endosymbiont, fecundity, mutualism, nutrition, polyphagy

38

39 **KEY MESSAGE**

40

- 41 • Generalist phytophagous insects may face diets varying in nutritional quality.
- 42 • Endosymbionts may contribute to better tolerance of diet shifts.
- 43 • The role of *Drosophila suzukii*'s *Wolbachia* strain (*wSuz*) is currently unconfirmed.
- 44 • We demonstrate a mutualistic role of *wSuz*, increasing survival and offspring mass after a diet shift.
- 45 • Understanding the role of symbionts in invasive species could help to improve management strategies.

46

47 **INTRODUCTION**

48

49 The ability to develop and reproduce on several diets – known as polyphagy – is an adaptive strategy that
50 is often successful in heterogeneous environments (Singer 2008). However, polyphagy among phytophagous
51 insects is the exception rather than the rule as most hypotheses on niche breadth relate on host specialization
52 (reviewed by Jousset and Elias 2019). While specialists can only develop and survive on a very limited
53 number of plant species, polyphagous insects can complete their cycle on a large spectrum of plants with
54 varying quality. Encountering both low- and high-quality resources, the generalist strategy can be costly for
55 individuals as offspring performance (e.g., developmental rate, survival) may vary between host plants (Janz
56 2003; Poyet et al. 2015). This cost could be offset by a female laying eggs on numerous host plants, ensuring
57 that at least some offspring survive or achieve optimal development (Jaenike 1990; Singer 2008) and leading
58 to a trade-off between host quality and availability (Singer 2008). Many successful pest insects are
59 polyphagous (Singer 2008). This is the case for the fruit flies (Diptera: Tephritidae), which include some
60 species with extreme polyphagy, for which selection pressure acts towards the ability of individuals to use any
61 fruit that are readily available (Wilson et al. 2012).

62 Extreme polyphagy may act in synergy with other strategies to help improve performance when
63 encountering a poor-quality host plant. A particularly important factor affecting insect host range is their
64 associated microbiome (Frago et al. 2012; Sugio et al. 2015; Giron et al. 2018). For example, facultative
65 secondary endosymbionts broaden the range of suitable host plants for the whitefly *Bemisia tabaci* (Benhamou
66 et al. 2021). Associations between microbes and insect hosts can be beneficial or deleterious, facultative or
67 obligatory (Sugio et al. 2015; Gurung et al. 2019). Amongst those with beneficial interactions, bacteria are the
68 most studied organisms. They have a role in insect immunity, protection against parasitoids and parasites,
69 helping insects coping with abiotic stress, as well as with nutritional stress (Dillon and Dillon 2004; Oliver et
70 al. 2010; Eleftherianos et al. 2013; Sannino et al. 2018; Gurung et al. 2019). Bacteria can play multiple
71 nutritional roles in insects, including improving their ability to live on suboptimal diets, increasing the
72 efficiency of digestion, providing digestive enzymes, detoxifying defense compounds and provisioning
73 vitamins (Dillon and Dillon 2004; Ashra and Nair 2022). For example, *Candidatus Erwinia dacicola*, an
74 obligate symbiont of the tephritid olive fruit fly, *Bactrocera olea* (Rossi), provides adult flies with essential
75 amino acids that are absent in unripe olive fruits (Ben-Yosef et al. 2014). In *Drosophila melanogaster* larvae,

76 *Acetobacter pomorum* and *Lactobacillus plantarum* allow them to better cope with changes in nutrient
77 availability, optimizing host fitness (Consuegra et al. 2020).

78 Among bacterial endosymbionts, *Wolbachia* is widely acknowledged to be the most abundant and prevalent
79 in insect species (Zug and Hammerstein 2015). Initially known for their reproductive parasitism that allows
80 them to spread, research has increasingly shed light on *Wolbachia*'s complex effects on hosts, ranging from
81 parasitism to mutualism (Brownlie et al. 2009; Zug and Hammerstein 2015; Newton and Rice 2020; Pimentel
82 et al. 2021). As mutualists, *Wolbachia* can protect the host against viruses (Chrostek et al. 2013; Cattel et al.
83 2016b; Pimentel et al. 2021), increase thermal tolerance (Saeed et al. 2018) and provide nutritional benefits
84 (Brownlie et al. 2009; Hosokawa et al. 2010; Zug and Hammerstein 2015; Newton and Rice 2020; Lindsey et
85 al. 2023). Nucleotides and vitamins have been suggested to be supplemented or modulated by *Wolbachia*
86 (Newton and Rice 2020; Lindsey et al. 2023). In the bed bug *Cimex lectularius*, the *Wolbachia* strain *wCle*
87 provides B vitamins to its host that are essential for growth and reproduction (Hosokawa et al. 2010; Nikoh et
88 al. 2014). In *D. melanogaster*, *Wolbachia* plays a role in iron metabolism (Brownlie et al. 2009) and was
89 recently found to be highly beneficial under nutrient limited conditions (Lindsey et al. 2023). The nutritional
90 role of *Wolbachia* could explain its prevalence in some populations even when no reproductive manipulation
91 is found (Newton et al. 2020). Overall, however, the role of *Wolbachia* as a potential promotor of invasion
92 and/or pest success has received little attention (but see Lu et al. 2016). In this study, we explored its role in
93 an important invasive fruit fly.

94 Originating from Asia, *Drosophila suzukii* Matsumara spread in the late 2000s into Europe, America and,
95 more recently, Africa (Asplen et al. 2015; Kwadha et al. 2021). Unlike other *Drosophila* species that oviposit
96 on rotting fruit, *D. suzukii* lays eggs on still ripening and ripe fruits using a serrated ovipositor (Atallah et al.
97 2014; Clemente et al. 2018). This ability, in addition with multiple generations per year, makes *D. suzukii* an
98 important pest of soft and thin-skinned crops, resulting in significant economic losses for fruit growers (Walsh
99 et al. 2011). Being highly polyphagous, the fly exploits a wide variety of cultivated (e.g., red fruits, cherry,
100 plum, peach, grape; Walsh et al. 2011) and wild fruits (e.g., elderberry, mistletoe, wild black cherry; Poyet et
101 al. 2015; Kenis et al. 2016; Ulmer et al. 2022), ensuring its persistence year-round (Poyet et al. 2015). However,
102 this polyphagy exposes the fly to diets of differing quality, which results in various effects on its development
103 (Poyet et al. 2015). The *Wolbachia* strain *wSuz* has been reported in *D. suzukii* (Cattel et al. 2016b). In nature,
104 this strain has an imperfect maternal transmission (Hamm et al. 2014), while in laboratory the maternal
105 transmission is perfect (Cattel et al. 2016a). It does not induce reproductive manipulation in American
106 populations (Hamm et al. 2014; Cattel et al. 2016b), yet it induces moderate cytoplasmic incompatibility in
107 European populations (Cattel et al. 2016b, 2018). Its prevalence in natural populations is higher than expected
108 by chance, indicating that *wSuz* must provide benefits to its host (Cattel et al. 2016b), but costs (see below)
109 and drift (Oliver et al. 2014) certainly can play against the maintenance of *Wolbachia* within populations.
110 Studies have attempted to identify these beneficial effects, but the available information remains inconclusive.
111 Some studies have reported an increase in fecundity (Mazzetto et al. 2015) and others a decrease (Hamm et al.
112 2014) associated with *Wolbachia* presence. Hamm et al. (2014) found no effect of *wSuz* on starvation tolerance
113 or desiccation resistance while Saeed et al. (2018) reported an increase in thermal tolerance. These results must

114 be taken with caution as different host genetic backgrounds or the presence/absence of antimicrobial agents in
115 the diet may have led to confounding effects (Cattel et al. 2016b). Cattel et al. (2016b) revealed protection
116 against two viruses, Drosophila C virus and Flock House virus. However, no studies have yet addressed the
117 potential effects of *wSuz* on nutritional provisioning or diet tolerance. Taking into account the high level of
118 polyphagy displayed by *D. suzukii*, the variable resource availability across time and space, and the difference
119 in *wSuz* prevalence across geographical ranges, we hypothesized that *wSuz* benefits *D. suzukii* by improving
120 the fly's ability to deal with the diet shifts it experiences.

121 This study therefore investigated the role of the *D. suzukii* *Wolbachia* strain *wSuz* in dealing with nutritional
122 shifts. We used two fly lines sharing the same genetic background but differing in the presence or absence of
123 *Wolbachia*. Two diets were tested: a corn diet and a grape diet, which differed mainly in their starch content.
124 The numbers of eggs produced, developmental time, offspring survival to adulthood and offspring mass were
125 assessed following different combinations of nutritional diet shifts. We predicted differences in fecundity and
126 developmental parameters between the two diets, regardless of the *Wolbachia* infection status of the flies.
127 Also, we hypothesized that the presence of *Wolbachia* would increase fly survival after a diet shift, especially
128 if *Wolbachia* plays a nutritional mutualistic role in the context of polyphagy, as described above.

129
130

131 MATERIALS AND METHODS

132

133 Rearing of *D. suzukii* stocks

134

135 *Drosophila suzukii* flies originated from a field collection made on infested raspberries in Rennes, France
136 (48°7'2.158"N, 1°40'40.053"W), in October 2020, by the ECOBIO Laboratory (Rennes). Five hundred fruits
137 from multiple plants were individually placed in vials for *D. suzukii* emergence. One hundred and ninety fly
138 pairs were obtained and maintained separately on artificial diet (banana-based). In November 2020, nine lines
139 infected by *Wolbachia* (*wSuz*) were detected using specific PCR assays with *Wolbachia*-specific primers
140 targeting the *wsp* gene (81F/691R), following Braig et al. (1998). These infected lines were mixed in cages,
141 and the population amplified. To obtain separate lines differing by the presence (W+) or absence (W-) of
142 *Wolbachia*, half of the population was reared on a diet supplemented with tetracycline for four generations, as
143 previously described by Hague et al. (2020). Then, the two lines were reared in 100 ml plastic bottles
144 containing ~30 ml of standard cornmeal diet and incubated at 20°C, LD 12:12 h and 75% RH (STRADER,
145 EV1300, Angers, France). At least 30 bottles of 50–200 flies per line were used for continuous maintenance.
146 Before and after experiments, *Wolbachia* presence in the two lines was assessed by diagnostic PCR following
147 the abovementioned method. To evaluate the impact of the tetracycline treatment on the other microbiota of
148 the flies, we restored the microbiota of W- flies that have been raised for about 20 generation in the laboratory
149 using W+ flies' faeces for three generations, as described by Strunov et al. (2022). Then we compared W-
150 flies with or without microbiota restoration by performing a metabarcoding analysis with primers targeting the

151 variable region V3-V4 of the 16S rRNA gene to characterize Bacteria diversity and ITS2 to assess the diversity
152 of fungi (see Supplementary Material).

153

154 *Experimental diets*

155

156 We used two diets to analyze the influence of different nutritional substrates on *D. suzukii* development, a
157 standard cornmeal diet (Corn) and an industrial diet made from grape (Grape). These two media were
158 contrasted in their composition, both were artificial but the Grape diet included grape powder, a fruit that *D.*
159 *suzukii* can use for its development (e.g., Shu et al. 2022). The flies were divided into two populations reared
160 on each diet and maintained for several generations (~10, six months) before starting the experiment. A detailed
161 analysis of macronutrients (carbohydrates, lipids and proteins) in each diet was performed as described by
162 Foray et al. (2012). For analyses of amino acid, B vitamins and iron content, samples were sent to Eurofins®.

163

164 **Table 1** Treatment codes corresponding to diet combinations for *Wolbachia*-infected and uninfected flies. N_i
165 = initial sample size; N_f = final sample size after removing the data from females that died before the end of
166 the experiment.

| Code | Treatment | N_i | N_f |
|--------|---|-------|-------|
| W+ C/C | <i>Wolbachia</i> -infected flies from corn culture placed on Corn | 25 | 21 |
| W+ G/C | <i>Wolbachia</i> -infected flies from grape culture placed on Corn | 25 | 21 |
| W+ G/G | <i>Wolbachia</i> -infected flies from grape culture placed on Grape | 25 | 22 |
| W+ C/G | <i>Wolbachia</i> -infected flies from corn culture placed on Grape | 25 | 23 |
| W- C/C | <i>Wolbachia</i> -uninfected flies from corn culture placed on Corn | 25 | 19 |
| W- G/C | <i>Wolbachia</i> -uninfected flies from grape culture placed on Corn | 25 | 14 |
| W- G/G | <i>Wolbachia</i> -uninfected flies from grape culture placed on Grape | 25 | 22 |
| W- C/G | <i>Wolbachia</i> -uninfected flies from corn culture placed on Grape | 25 | 22 |

167

168 *Effect of diet on D. suzukii development*

169

170 Egg laying boxes were designed for the experiment: a hole was drilled in 60×45×50 mm crystal polystyrene
171 boxes to receive a 6×30 mm 3D print egg laying cylinder containing 5 ml of diet. One female and three males
172 aged 24 h and collected randomly from the culture vials were placed in each laying box. There were four diet
173 combinations for *Wolbachia*-infected and uninfected flies, resulting in eight treatments (Table 1). Each
174 treatment had 25 replicates (i.e., 25 females). C/C and G/G treatments were used as control as they correspond
175 to the routine rearing. A pinch of active yeast was dusted on each laying cylinder to stimulate egg laying
176 (Plantamp et al. 2017). The laying boxes were maintained under standard culture conditions (see above). Every
177 day over 30 days, eggs were counted on each laying cylinder under a stereomicroscope. The laying cylinder
178 was replaced every 48 h and the one removed was placed on a Petri dish containing ~30 ml of diet to continue
179 larval development. Developmental conditions were the same as for the rearing (see above). For each Petri
180 dish, the numbers of developed pupae and the numbers of emerged flies were recorded daily. Egg-to-pupa,
181 pupa-to-adult and egg-to-adult survival and development time were assessed. The emerging adults were

182 counted and frozen at -20°C for later weighing. From each female and treatment, 120 emerging individuals
183 were weighed on a laboratory precision balance (0.01 mg; QUINTIX125D-1S; Sartorius, Göttingen,
184 Germany).

185

186 *Statistical analyses*

187

188 All data were analyzed in R version 4.1.2 (R Core Team 2022). Differences in macronutrient composition
189 between diets (i.e., glucose, starch, proteins and lipids) were assessed using the Wilcoxon-Mann-Whitney test.
190 Data from females that died before the end of the experiment were removed from the analyses (Table 1). The
191 total numbers of eggs laid and numbers of offspring were compared among treatments with generalized linear
192 models using a Poisson distribution. The chosen explanatory variables were: the *Wolbachia* infectious status,
193 the laying diet and the presence of a diet shift between the diet from which the female originated and the one
194 it was made to oviposit on. Egg-to-pupa, pupa-to-adult and egg-to-adult survival, mean development time and
195 offspring mass were compared using analysis of variance (ANOVA) after square root transformation to
196 achieve normality and homoscedasticity of error variance. Offspring sex-ratio was also analysed with
197 ANOVA. Where ANOVA was significant, significant differences between means were then identified using
198 Tukey's HSD tests or the emmeans() function from the emmeans package.

199

200

201 **RESULTS**

202

203 *Diet analyses*

204

205 Macronutrient analysis indicated that the Grape diet was enriched in carbohydrates (more starch) compared
206 to the Corn diet, but that both diets provided a similar amount of proteins and lipids (Table S1). Therefore, the
207 Grape diet provided more energy than the Corn. Analyses of amino acid, B vitamins and iron contents revealed
208 that both diets were comparable in these three compound groups (Table S2). Therefore we concluded that any
209 differences found in fly performance between the diet treatments must be interpreted as a consequence of
210 varying starch levels, as the protein:total carbohydrate ratios were similar between the two diets (P:C Corn =
211 0.011 ± 0.006 ; P:C Grape = 0.010 ± 0.005 ; $p = 0.84$).

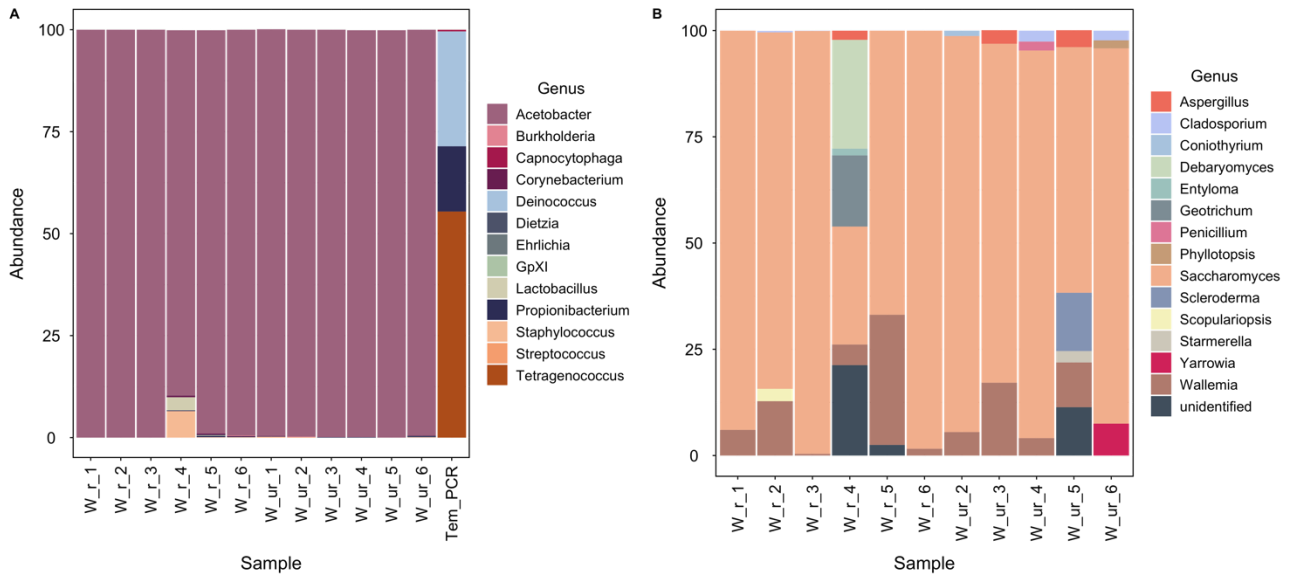
212

213 *Microbiota analysis*

214

215 The microbiota consisted almost exclusively of *Acetobacter* for bacteria and *Saccharomyces* and *Wallemia*
216 for fungi (Fig. 1, see Supplementary Material for more details). No significant difference was found between
217 W- flies with or without microbiota restoration. Because this assessment is indirect, we still cannot totally
218 exclude the confounding effect of microbiota. However, the absence of difference in the microbiota of W-
219 flies (many generations after exposure to antibiotic) before and after exposure to W+ faeces strongly suggests

220 that our W⁺ and W⁻ lines shared the same microbiota. Moreover, the protocol to restore the microbiota of W⁻
 221 flies was used in previous studies and we are therefore confident that it provides adequate outputs. Therefore,
 222 we considered that the two lines W⁺ and W⁻ share similar microbiota and that the results reported hereafter
 223 reflect the presence/absence of *Wolbachia*.
 224



225
 226 **Fig.1** Metabarcoding analysis to characterize Bacteria (A) and fungi (B) diversity in *Drosophila suzukii*. W_r
 227 samples correspond to W⁻ flies with microbiota restoration using W⁺ flies' faeces for three generations as
 228 described by Strunov et al. (2022) and W_{ur} to W⁻ flies without microbiota restoration (see Supplementary
 229 Material for more details). Each column corresponds to one individual.

230
 231 *Effect of diet treatment and Wolbachia status on female fecundity*

232
 233 Overall, there was a large variation between females in the total numbers of eggs laid, from 18 to 568 eggs
 234 during the 30 days of the experiment. The total number of eggs laid by a female was significantly influenced
 235 by the laying diet, the diet shift, *Wolbachia* presence and the interaction terms (Table 2, Fig. 2A). Females laid
 236 significantly more eggs on Grape (mean ± sd: 214 ± 134 eggs) than on Corn (157 ± 96 eggs). When confronted
 237 to a diet shift, females from the Corn line laid more eggs on Grape than they did on Corn (263 ± 143 vs. 201
 238 ± 111 eggs; $z = -32.4, p < 0.0001$) and females from the Grape line laid fewer eggs on Corn than they did on
 239 Grape (107 ± 38 vs. 163 ± 105 eggs; $z = 31.3, p < 0.0001$). In addition, W⁺ females laid fewer eggs than W⁻,
 240 although the difference was not significant for females from C/C treatment.

241
 242 *Effect of diet treatment and Wolbachia status on offspring survival*

243
 244 *Wolbachia* presence was the only variable to impact significantly the egg-to-adult survival (Table 2, Fig.
 245 2B). Mean egg-to-adult survival was about 66 ± 14% and did not differ significantly between diet treatments.
 246 *Wolbachia* presence increased survival across diet conditions ($F = 39.2, p < 0.0001$), although this was not

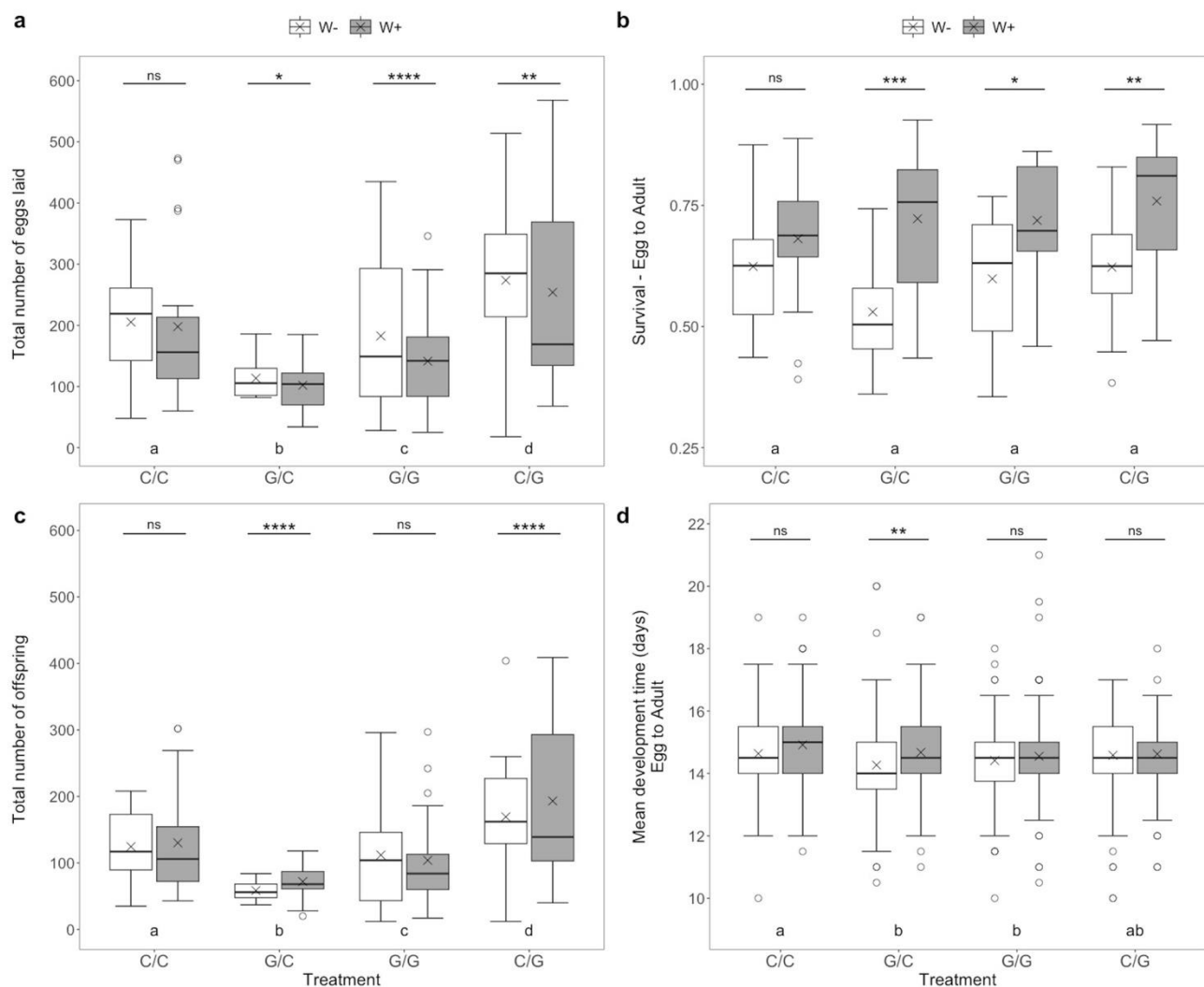


Fig. 2 Influence of diet treatment and *Wolbachia* infectious status (W+ or W-) on *Drosophila suzukii* reproductive parameters: (A) Total number of eggs laid in 30 d, (B) Egg to adult survival, (C) Total number of offspring per female (emerged adult flies), and (D) Mean development time from egg to adult emergence in days. The diet treatments were: flies from corn culture placed on corn diet (C/C), flies from grape culture placed on corn diet (G/C), flies from grape culture placed on grape diet (G/G), and flies from corn culture placed on grape diet (C/G). Significance of differences between diet treatments and *Wolbachia* infection status are represented by different letters (a, b, c, d; bottom of each panel) or by asterisks, respectively (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$, ns: non-significant; top of each panel)

247 significant for the C/C treatment, with a mean increase of 17%. *Wolbachia* presence primarily acted on larval
 248 survival (egg-to-pupa) and did not have a significant effect on pupal survival (Table S3, Fig. S1).

249 Overall, individual females produced between 12 and 409 surviving adult offspring over the experiment,
 250 with means of 145 ± 98 and 99 ± 60 offspring when laying on Grape and Corn respectively. The effect of diet
 251 treatment on the total number of offspring per female was the same as on the total number of eggs laid (Table
 252 2, Fig. 2C). A mean of 67 ± 23 offspring was produced in the G/C treatment, almost a third of those produced
 253 in the C/G treatment (182 ± 106 offspring). The numbers of offspring produced in treatments not involving a
 254 diet shift were 108 ± 75 and 127 ± 67 for G/G and C/C, respectively. *Wolbachia* had a significant effect only

255 in the treatments involving a diet shift (C/G and G/C), where the numbers of offspring were increased by
 256 14.1% and 23.1%, respectively.

257

258 *Effect of diet treatment and Wolbachia status on developmental time*

259

260 Across all treatments, the development time from egg to adult was 14.6 ± 1.1 days, with a minimum of 10
 261 days and a maximum of 21 days. The laying diet, *Wolbachia* status and occurrence of a diet shift had an
 262 interactive effect on the development time (Table 2). The longest development time was observed on Corn
 263 when no diet shift occurred while the shortest times were observed on the G/C and G/G treatments (Fig. 2D).
 264 *Wolbachia* presence had a significant effect only on the G/C treatment, increasing the development time by
 265 almost half a day (W-: 14.3 ± 1.4 days, W+: 14.7 ± 1.2 days; $F = 14.7674$, $p = 0.008$). *Wolbachia* presence
 266 had no effect on egg-to-pupa development time (Table S4). The larval phase lasted about 8.2 ± 1.0 days and
 267 was significantly shorter in the G/C and G/G treatments, where larvae pupated from 3 to 7 hours earlier than
 268 in the other treatments (Fig. S2A). The pupal phase lasted 6.4 ± 0.8 days and even if *Wolbachia* presence had
 269 an overall effect on pupa-to-adult development time, the effect was not significant anymore within the diet
 270 treatment (Table S4, Fig. S2B). Diet treatment did not have a significant influence on pupa-to-adult
 271 development time (Table S4, Fig. S2B).

272

273 *Effect of diet treatment and Wolbachia status on offspring mass and sex-ratio*

274

275 Overall, female offspring were almost twice as heavy as males, with a mean mass of 1.28 ± 0.40 mg vs.
 276 0.75 ± 0.24 mg. The diet treatments had comparable effects on female and male mass, with the heaviest

Table 2 Summary of results for generalized linear models for total number of eggs laid, egg-to-adult survival, total number of offspring, and egg-to-adult development time. Df = degree of freedom; Dev. = deviance; $P > |\chi^2|$ = P-value

| Effect | Total number of eggs laid | | | Egg-to-adult survival | | | Total number of offspring | | | Egg-to-adult development time | | |
|------------------------|---------------------------|---------|----------------|-----------------------|-------|----------------|---------------------------|---------|----------------|-------------------------------|------|----------------|
| | Df | Dev. | $P > \chi^2 $ | Df | Dev. | $P > \chi^2 $ | Df | Dev. | $P > \chi^2 $ | Df | Dev. | $P > \chi^2 $ |
| <i>Wolbachia</i> (Wol) | 1 | 136.71 | <0.0001 | 1 | 0.605 | <0.0001 | 1 | 9.05 | 0.0026 | 1 | 19.1 | 0.0001 |
| Laying diet (LD) | 1 | 670.46 | <0.0001 | 1 | 0.041 | 0.105 | 1 | 723.91 | <0.0001 | 1 | 5.5 | 0.039 |
| Diet shift (S) | 1 | 32.33 | <0.0001 | 1 | 0.003 | 0.650 | 1 | 43.89 | <0.0001 | 1 | 2.1 | 0.201 |
| Wol × LD | 1 | 0.20 | 0.651 | 1 | 0.001 | 0.763 | 1 | 1.70 | 0.192 | 1 | 6.2 | 0.029 |
| Wol × S | 1 | 0.07 | 0.791 | 1 | 0.045 | 0.090 | 1 | 13.92 | 0.0002 | 1 | 0.0 | 0.864 |
| LD × S | 1 | 2112.90 | <0.0001 | 1 | 0.029 | 0.174 | 1 | 1526.86 | <0.0001 | 1 | 21.5 | <0.0001 |
| Wol × LD × S | 1 | 24.75 | <0.0001 | 1 | 0.035 | 0.134 | 1 | 0.46 | 0.498 | 1 | 1.5 | 0.282 |

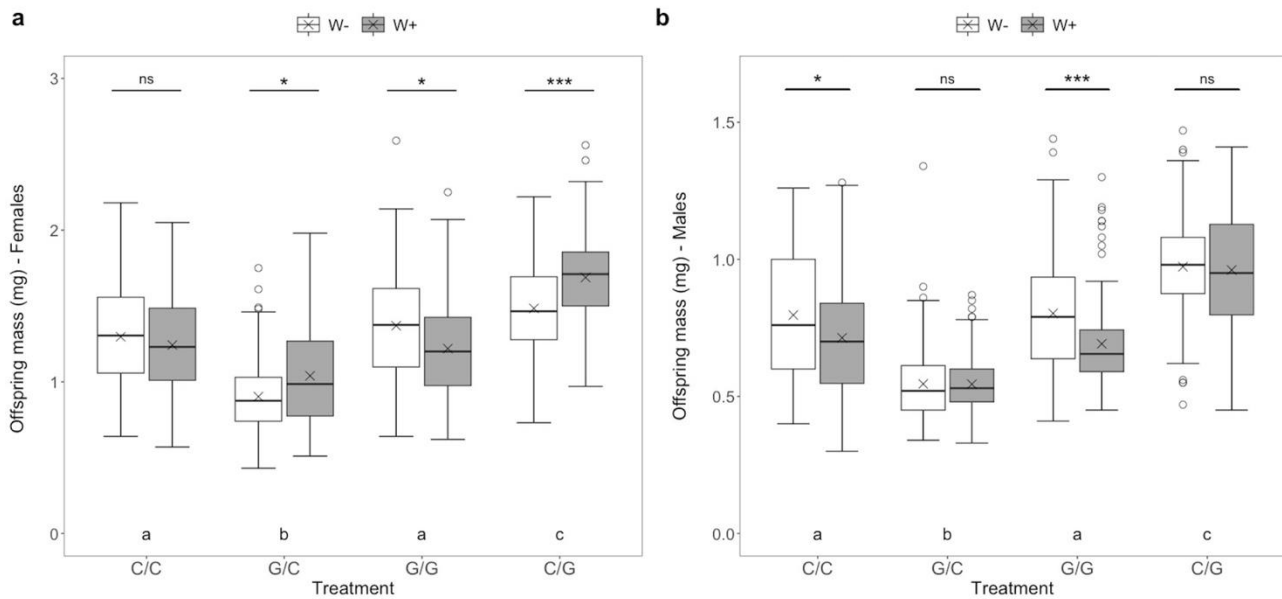


Fig. 3 Influence of diet treatment and *Wolbachia* presence (W+ or W-) on *Drosophila suzukii* offspring mass in mg with (A) females and (B) males. The treatments are the same as in Fig. 2. N = 120. Analysis of variance (ANOVA) was performed after square root transformation. Statistically significant differences are represented either by different letters (a, b, c, d; bottom of each panel) or by asterisks (*: $p < 0.05$, ***: $p < 0.001$; ns: non-significant; top of each panel)

277 individuals obtained in the C/G treatment and the lightest ones from the G/C treatment. When there was no
 278 diet shift (C/C and G/G treatments), the adult masses were intermediate (Table 3, Fig. 3). *Wolbachia* presence
 279 had an effect on the mass of both sexes produced, but only when a diet shift occurred (Table 4). When there
 280 was no diet shift, the mass of W+ offspring was significantly lower than those of W- for both males and
 281 females, although this was not significant in females from the C/C treatment (Fig. 3). In contrast, under diet
 282 shifts, the offspring mass did not differ in relation to infection status for males (Fig. 3B), and the W+ offspring
 283 were significantly heavier than W- offspring for females (Fig. 3A).

284 The mean offspring sex-ratio (females/males) was 0.51 ± 0.06 . *Wolbachia* had no effect on sex ratio
 285 (ANOVA, $F = 1.099$, $p = 0.2961$). The sex-ratio of offspring from G/C treatment was significantly higher
 286 (more females) than the sex-ratio of offspring from the G/G treatment (G/C: 0.53 ± 0.07 , G/G: 0.49 ± 0.06 , p
 287 $= 0.0258$). The sex-ratio did not differ between other treatments.

288
 289

290 DISCUSSION

291

292 Across their lifetime, polyphagous insects are exposed to various diets that may change across generations
 293 and phenology. Polyphagy at the population level is generally interpreted as an individual developing on a
 294 single plant species, yet being able to develop on several hosts (Singer 2008), so that the female has a range of
 295 host plants on which to oviposit. The larvae produced can thereby be exposed to diets varying in quality.
 296 Endosymbiotic partners may contribute to improve the performance of insects faced with such diet shifts, and

Table 3 Summary of results from generalized linear models for female and male offspring mass. For details, see legend of Table 3

| Effect | Offspring mass Female | | | Offspring mass Male | | |
|------------------------|--------------------------|-------|-------------------|------------------------|-------|-------------------|
| | Df | Dev. | $P > \chi^2 $ | Df | Dev. | $P > \chi^2 $ |
| <i>Wolbachia</i> (Wol) | 1 | 0.048 | 0.132 | 1 | 0.93 | 0.0001 |
| Laying diet (LD) | 1 | 5.006 | <0.0001 | 1 | 19.61 | <0.0001 |
| Diet shift (S) | 1 | 0.015 | 0.409 | 1 | 0.09 | 0.238 |
| Wol × LD | 1 | 0.007 | 0.555 | 1 | 0.06 | 0.342 |
| Wol × S | 1 | 0.840 | <0.0001 | 1 | 0.77 | 0.0004 |
| LD × S | 1 | 4.310 | <0.0001 | 1 | 19.91 | <0.0001 |
| Wol × LD × S | 1 | 0.046 | 0.142 | 1 | 0.00 | 0.896 |

297 could be especially critical for invasive species. Here, we show that the invasive fly *D. suzukii* performs better
 298 in terms of survival when it hosts the *Wolbachia* strain *wSuz*, in particular if it is faced with diet shifts. Hosting
 299 this endosymbiont may, however, also result in costs, since a negative effect was observed on other life history
 300 traits, such as fecundity or offspring mass. As the fly is an invasive species, and because it is present year-
 301 round in temperate regions, the influence of *Wolbachia* presence on its survival likely provides an overall
 302 advantage to *D. suzukii*. This effect may provide an important element explaining the maintenance of *wSuz* in
 303 natural populations and the success of this pest fly species worldwide. Caution is needed however when
 304 interpreting our findings, as they are specific to a single *Wolbachia* genotype and one *D. suzukii* population.
 305 To ensure broader applicability, it is essential to evaluate our results with various *D. suzukii* genotypes and
 306 *Wolbachia* strains, given that numerous studies has revealed that the influence of *Wolbachia* is contingent on
 307 both its own genotype and that of its host (Fry et al. 2004; Capobianco et al. 2018; Serga et al. 2021; Strunov
 308 et al. 2022).

309
 310 *Wolbachia* presence and the tradeoff between fecundity and offspring survival

311
 312 In all treatments, *Wolbachia*-infected females laid fewer eggs than non-infected ones, although this
 313 tendency was not significant for flies on Corn without diet shift. This apparent fitness cost was counterbalanced
 314 by the large increase seen in offspring survival, specifically larval survival, across all treatments, which
 315 resulted in almost perfect compensation in the number of offspring when the diet was stable, and even
 316 overcompensation (i.e., more surviving offspring in total) under diet shift treatments. This effect of *Wolbachia*
 317 on overall offspring survival contrasts with some previous studies. Hamm et al. (2014) reported a negative
 318 effect of *Wolbachia* presence on progeny production while Mazzetto et al. (2015) reported an increase of 30-
 319 50% in progeny production by *Wolbachia*-infected flies. However, the diet history of the flies was not taken
 320 into account in these studies. Mazzetto et al. (2015) reared flies on various organic fruits, then transferring

321 them to a standard diet, while Hamm et al. (2014) collected flies from an organic raspberry field and then
322 reared them on blueberry. For both, no antibiotic was used on the fruits they used as diet and they only had a
323 few populations, which may have led to genetic confounding effects. In our study, larval survival included
324 both hatch rate and direct larval survival, which means *Wolbachia* could have acted either on one parameter
325 or the other or both. *Wolbachia* is already known to act on hatch rate but its effects are inconclusive. Saeed et
326 al. (2018) showed that *Wolbachia* maintained a high egg hatch rate in *D. suzukii* even at increased temperature
327 while in Cattel et al. (2018) infected flies had a lower hatch rate than uninfected flies.

328 Saeed et al. (2018) concluded that *Wolbachia* presence does not offer any fitness benefit linked to nutritional
329 tolerance. However, their study mainly focused on survival of eggs and subsequent development time on a
330 yeast-deprived diet, which may have been insufficient to detect any *Wolbachia*-associated benefit. Our results
331 suggest that the influence of *Wolbachia* presence may differ depending on the diet history (i.e., the maintenance
332 of a stable diet or occurrence of a diet shift). The increases we observed in offspring survival also provide
333 support for the hypothesis that *Wolbachia* plays a mutualistic role for *D. suzukii*. Increasing host survival
334 certainly is beneficial for the bacteria since this effect likely contributes to its higher prevalence in the
335 population (Newton and Rice 2020).

336 The mechanisms at play in this nutritional mutualism were not addressed directly in this study. As in other
337 insect-*Wolbachia* nutritional mutualisms (Nikoh et al. 2014), *Wolbachia* may produce and supply nutrients
338 that contribute to *D. suzukii* fitness (Newton and Rice 2020). Indeed, studies of other insect species have
339 confirmed that *Wolbachia* genomes can possess pathways for B vitamins, either complete (e.g., riboflavin B2)
340 or partial (e.g., thiamine B1, pyridoxine B6, biotin B7, and folate B9) (Nikoh et al. 2014), as well as pathways
341 for nucleotide and heme syntheses (Newton and Rice 2020). For example, *Wolbachia* supplements biotin and
342 riboflavin to bedbugs (Moriyama et al. 2015; Balvín et al. 2018) and planthoppers (Ju et al. 2020). The bacteria
343 also influence iron metabolism in the parasitoid wasp *Asobara tabida*, the mosquito *Aedes aegypti* and the flies
344 *Drosophila simulans* (Kremer et al. 2009) and *D. melanogaster* (Brownlie et al. 2009). Supplementation seems
345 of particular importance for insect species that feed on single diets (e.g., blood, plant-sap, and grain), as some
346 important nutrients (mostly amino acids and vitamins) are missing from these diets (Ju et al. 2020). Similar
347 effects may also be useful for polyphagous insects developing using diets of varying quality. In our diets,
348 riboflavin and tyrosine differed quantitatively, even if we cannot assess statistical significance. If *wSuz* have
349 a role in riboflavin and tyrosine metabolism, we would have expected *W*-flies to struggle in Grape diet,
350 resulting in a lower survival or mass. It was not the case (Fig. 2, 3), either because *wSuz* does not play a role
351 in riboflavin or tyrosine metabolism, or because our diet was not lacking these nutrients and just had a reduced
352 amount, limiting the potential beneficial impact of *Wolbachia*. The diets were suboptimal in their P:C ratio for
353 *D. suzukii* (Silva-Soares et al. 2017; Young et al. 2018) and differed mostly in starch content (Table S1). The
354 role of starch reserves, which likely determine the energetic status of flies, in this nutritional mutualism
355 requires further investigation to identify the mechanisms involved.

356 The possible contributions of other bacteria, such as *Acetobacter*, to the observed differences also cannot
357 at present be completely excluded. Acetic acid bacteria are important components of the microbiota associated
358 with *Drosophila* (Winans et al. 2017; McMullen et al. 2021), and a role in *D. suzukii* gut response to diet

359 modification has been suggested (Vacchini et al. 2016). Also, *Wolbachia* presence can itself modulate the
360 relative titers of these bacteria, although the mechanisms involved are not known (Simhadri et al. 2017). We
361 compared the microbiota of W⁻ flies and W⁻ flies with restored microbiota after being in contact with faeces
362 of W⁺ flies, but some bacteria species may not be transferred by this mechanism and future research should
363 explore these aspects. It is also known that cultivating insects for numerous generations on the same diet
364 stabilizes the gut-microbiome (Sugio et al. 2015; Bing et al. 2018). Overall, we assume that the differences
365 observed in this study are most likely directly linked with *Wolbachia* presence rather than other bacteria such
366 as *Acetobacter*.

367 Finally, our data indicate that experiencing stressful conditions can increase the beneficial effects of
368 *Wolbachia* presence. In the current study, the stress was induced by the diet shift, through exposure to a new
369 nutritional environment. In other studies on the effect of *Wolbachia* in a nutritional context, benefits are seen
370 in nutrient-deprived situations. For example, *Wolbachia* presence increased *D. melanogaster* fecundity under
371 iron-restricted diets (Brownlie et al. 2009). Similarly, *wC1e* conferred fitness advantages to the host *Cimex*
372 *lectularius* when fed on B-vitamin-deprived food (Nikoh et al. 2014). Considering other microbes, Bing et al.
373 (2018) showed that the general microbiota of *D. suzukii* is advantageous in the context of a nutrient-poor diet,
374 while having no influence or even deleterious effects when the fly was cultivated on a nutrient-rich diet.
375 Thermal stress is also known to have differential effects on *Wolbachia* and the host insect (Strunov et al. 2013;
376 Saeed et al. 2018). As *Wolbachia* can be both mutualistic and parasitic with the same host (Zug and
377 Hammerstein 2015), studies of its effects should include stressful conditions that can impact the entire
378 holobiont.

379

380 *Diet affects reproductive traits*

381

382 As expected, fecundity was affected by the laying diet treatment applied. However, the direction of the
383 effect observed was unexpected. When the diet was stable, the maximum number of laid eggs and surviving
384 offspring were observed on Corn rather than Grape, with the latter containing grape powder, a fruit that *D.*
385 *suzukii* can use for its development (e.g., Shu et al. 2022). *Drosophila suzukii* is known to favor a low protein
386 diet on which to oviposit (Silva-Soares et al. 2017; Young et al. 2018). This is related to the particular behavior
387 of *D. suzukii* of ovipositing in ripening and ripe fruit, stages at which the fruit reach their maximum sugar
388 content (Burrack et al. 2013) and lowest protein content (Silva-Soares et al. 2017). Sugars subsequently
389 decrease and proteins increase as the fruit starts decaying (Silva-Soares et al. 2017). The overall protein content
390 of our two diets did not differ. Both diets had anti-microbial agent (Table S1), which could have led to different
391 microbial proliferation and divergence in microbiota between Corn and Grape, but it did not seem to influence
392 our results. However, as with proteins, the sugar content influences the number of eggs laid (Burrack et al.
393 2013). Therefore, females may have relied on sugar concentrations to drive their reproductive behavior in our
394 experiments, thereby preferring to oviposit on the Corn diet, which had lower sugars content even if it had the
395 same P:C ratio of 1:10.

396 Another factor which may have influenced oviposition is the penetration force required by the diet. Burrack
397 et al. (2013) found that the optimal force required by *D. suzukii* is around 30 cN, which corresponds to the
398 Grape diet. Our direct observations of oviposition suggest that the penetration force required on the Corn diet
399 may have been inferior and therefore suboptimal, even though the females laid more eggs on Corn. Conversely,
400 Silva-Soaeres et al. (2017) reported no difference in the proportions of eggs laid by females on diet patches
401 differing in agar concentration. These observations might explain why, in our experiment, the difference
402 observed between Corn and Grape diets was small, about 40 eggs and 19 offspring. In their study of *D. suzukii*,
403 Olazcuaga et al. (2021) described positive fitness changes across generations when a population was exposed
404 to a new diet after 12 generations and more. As our populations were reared on Grape for about ten generations,
405 it is therefore possible that the differences we observed between Grape and Corn could disappear completely
406 over a longer period.

407 Notably, diet shift had a strong directional effect on fecundity. Females originating from Grape that were
408 forced to lay eggs on Corn laid the minimum amount of eggs while, in the inverse sequence, females originating
409 from Corn and forced to lay eggs on Grape laid the maximum amount of eggs. In the short term, the Grape
410 diet possible allowed better performance of *D. suzukii* than the Corn diet. In the current study we only tested
411 two artificial diets, but Olazcuaga et al. (2021) used eight fruit and obtained similar results: depending on the
412 diet on which the population were reared, fitness would increase or decrease depending on the new diet, but
413 this effect would disappear after >12 generations on the same diet. In the natural environment, this feature has
414 clear relevance to the multitrophic context in which *D. suzukii* develops. Depending on the context (i.e.,
415 availability of different food sources), the species' high polyphagy could either be an advantage or a
416 disadvantage. For instance, after several generations laying eggs on the same fruit (e.g., during a fruit harvest
417 season), females would have to find new fruit to oviposit on, either increasing or decreasing their abundance.
418 This is a general pattern in polyphagous insect pests that depend on dynamic cropping systems (Kennedy and
419 Storer 2000). It would also be of importance in temperate climates when fruit resources are scarce during
420 winter and alternative resources provide nutritional support (Stockton et al. 2019).

421 Offspring survival was not affected by any diet treatment. The slight differences in diet composition may
422 not have been sufficient to induce a difference in larval mortality (see Young et al. 2018). However, the diet
423 treatment did affect surviving offspring mass and development time. When the diet was stable, offspring mass
424 did not differ between Corn and Grape, though the development time in Corn was longer than in Grape.
425 However, the differences in development time were small and may not have been sufficient to induce
426 differences in offspring mass. These findings are in agreement with Silva-Soares et al. (2017), who found a
427 faster development and larger offspring when *D. suzukii* was raised in intermediate protein concentrations, and
428 also with Jaramillo et al. (2015), who reported faster development on a low sugar diet (blueberry) than on a
429 sugar-rich diet (corn-molasses based) and no difference on phenotypic traits (e.g., wing length). Our results
430 differ however from those of Young et al. (2018) who observed longer egg-to-adult development time and/or
431 smaller adult mass when flies developed in high sugar environments. One possible explanation relies on egg
432 density, which can lead to overcrowding. Overcrowding generally induces longer development time and
433 smaller adult size (Da Silva et al. 2019) and can promote cannibalism in some insects (Richardson et al. 2010),

434 including *D. suzukii* (Da Silva et al. 2019). We did not control for egg density, but the replacement of the diet
435 every two days contributed to avoid reaching densities that can be detrimental. As seen in real fruit (Da Silva
436 et al. 2019), when larval density is higher, thanks to the large quantity of resource in the experimental Petri
437 dishes larvae pupated around the Petri dish edges in order to avoid competition.

438 When a diet shift was enforced the development time was shorter on Corn and offspring were lighter, while
439 on Grape the development time was longer and offspring were heavier. Fellous and Xuéreb (2017) found the
440 same pattern when comparing the larval performance of *D. suzukii* on diets differing in their protein / sugar
441 concentrations, with the shortest larval development time on high protein, low sugar diets. On the other hand,
442 Shu et al. (2022) found larval development time to be shorter and adult mass to be greater on high protein, low
443 sugar diets. However, the latter study took place on actual fruits, which might have impacted larval
444 development differently to artificial diets, especially because of the absence of antimicrobial agents (Young et
445 al. 2018). Generally, it might be expected that, the longer the development time, the heavier the offspring will
446 be as they can benefit from the diet over a longer period. However, this is not always the case as insects are
447 also able to increase their foraging rate, becoming heavier in a shorter time (Da Silva et al. 2019). Faster
448 development without effects on body size can also indicate a higher quality diet (Edgar 2006). In this study,
449 the diet shift induced modifications in both development time and in offspring mass and it is plausible that the
450 diet shift affected both the foraging rate of the larvae and their nutrient intake. Even if both Corn and Grape
451 diets had the same P:C ratio and mainly differed in starch content, the slight differences in nutrients between
452 them may have induced the same consequences observed in studies with diet differing in their P:C ratio. The
453 role and even importance of starch in the performance of *Drosophila* flies remains undetermined and future
454 studies should investigate its role in modulating fitness during diet changes.

455

456 CONCLUSIONS

457

458 In our study, *Wolbachia* presence improved the survival of *D. suzukii* in the earlier stages of development.
459 The beneficial effects on offspring performance were particularly clear when females experienced a diet shift.
460 Our results unveil an unappreciated role of *Wolbachia* in polyphagous insects: the bacteria support survival of
461 the fly and this effect is especially important during a diet shift. These findings suggest that *Wolbachia* could
462 supplement the diet of its host when it is developing on a low-quality resource, but this hypothesis requires
463 further investigation. Such a role could also explain the maintenance of the *wSuz* strain in *D. suzukii* globally.
464 Depending on specific host fruit availability and quality, *Wolbachia* presence could be more or less
465 advantageous to *D. suzukii*. *Wolbachia* presence is also associated with cost that can offset the benefits, such
466 as costs related to the energy needed to host the endosymbiont (Clavé et al. 2022). Further studies are required
467 to investigate how the cost-benefice balance is modulated in a multitrophic context, depending on the host
468 plants. Abiotic variables (e.g., temperature, humidity) also need to be taken into account as it has been shown
469 that the effect of *Wolbachia* can vary with temperature (Saeed et al. 2018). An integrative approach is needed
470 to properly understand the role *Wolbachia* plays in polyphagous insects. If *Wolbachia* has a mutualistic role,
471 its presence would benefit the fly during invasion, helping with establishment and maintenance of populations

472 in heterogeneous environments. A better understanding of these dynamics is crucial to identify and apply
473 efficient management strategies to limit the incidence of this invasive species on crops.

474

475 **ACKNOWLEDGEMENTS**

476

477 We are grateful to Hervé Colinet and Romuald Cloteau for their great efforts in raising the *Drosophila*
478 *suzukii* lines used in this study, and to Laurence Mouton for identifying the lines hosting *Wolbachia*. We thank
479 Aurélien Vigneron who inspired us for this study, Nina Joffard who helped with the fly weighing and Olivier
480 Chabrerie for his comments on a previous version. Finally, we are thankful to Peter Convey who proofread the
481 manuscript, and to two anonymous reviewers for their insightful comments. This work was supported by the
482 grant ANR DroThermal (ANR-20-CE02-0011).

483

484 **STATEMENTS & DECLARATIONS**

485

486 This work was supported by the grant ANR DroThermal (ANR-20-CE02-0011). The authors have no
487 relevant financial or non-financial interests to disclose.

488

489 **DATA AVAILABILITY**

490

491 The datasets generated during and/or analyzed during the current study are available from the corresponding
492 author on reasonable request.

493

494 **AUTHOR CONTRIBUTION STATEMENT**

495

496 GD, SP, and VF conceived and designed research. GD and JL conducted experiments. HH did the
497 microbiota analysis. LB helped with the rearing. GD analyzed the data. The first draft of the manuscript was
498 written by GD and all authors commented on previous versions of the manuscript. All authors read and
499 approved the final manuscript.

500

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