

***Wolbachia* both aids and hampers the performance of spider mites on different host plants**

Flore Zélé^{1*}, Joaquim L. Santos¹, Diogo Prino Godinho¹ and Sara Magalhães¹

¹cE3c: centre for Ecology, Evolution and Environmental changes; Faculdade de Ciencias; Universidade de Lisboa, Edificio C2, Piso-3, Campo Grande, 1749-016 Lisbon, Portugal

* Corresponding author: email: fezele@fc.ul.pt

Keywords

Arthropod-plant-symbiont interaction, bacterial symbiont, fitness effects, host-plant use, mutualism, parasitism.

1 **Abstract**

2 In the last decades, many studies had revealed the potential role of arthropod bacterial
3 endosymbionts in shaping the host range of generalist herbivores and their performance on different
4 host plants, which, in turn, might affect endosymbiont distribution in herbivores populations. We
5 tested this by measuring the prevalence of endosymbionts in natural populations of the generalist
6 spider mite *Tetranychus urticae* on different host plants. Focusing on *Wolbachia*, we then analysed
7 how symbionts affected mite life-history traits on the same host-plants in the laboratory. Overall, the
8 prevalences of *Cardinium* and *Rickettsia* were low, whereas that of *Wolbachia* was high, with the
9 highest values on bean and eggplant and the lowest on purple, tomato and zucchini. Although most
10 mite life-history traits were affected by the plant species only, *Wolbachia* infection was detrimental
11 for egg hatching rate on purple and zucchini, and led to a more female-biased sex ratio on purple and
12 eggplant. These results suggest that endosymbionts may affect the host range of polyphagous
13 herbivores, both by aiding and hampering their performance, depending on the host plant and on
14 the life-history trait that affects performance the most. Conversely, endosymbiont spread may be
15 facilitated or hindered by the plants on which infected herbivores occur.

16 INTRODUCTION

17 Although generalist herbivores are able to colonize several host plants, their performance on
18 different host plants is variable. Whereas some studies suggest that the host range of herbivores is
19 mostly determined by geographical location (Calatayud *et al.*, 2016), others suggest that this range is
20 determined by host-plant nutritional quality (Schoonhoven *et al.*, 2005) or host-plant defences
21 (Becerra, 1997). Still, the proximate mechanisms allowing populations to colonize particular host
22 plants remain elusive.

23 Herbivores harbour a rich community of microorganisms, ranging from their gut microbiota
24 and intracellular vertically-transmitted endosymbionts to plant bacteria and viruses of which they
25 serve as vectors, and there is growing evidence of the impact of such communities on herbivore
26 performance on plants (Hosokawa *et al.*, 2007, Clark *et al.*, 2010, Frago *et al.*, 2012, Hansen & Moran,
27 2014, Oliver & Martinez, 2014, Zhu *et al.*, 2014, Shikano *et al.*, 2017). Obvious candidates to influence
28 plant colonization by herbivorous arthropods are their heritable endosymbionts (Clark *et al.*, 2010,
29 Feldhaar, 2011, Ferrari & Vavre, 2011, Frago *et al.*, 2012, Jaenike, 2015). Due to their vertical mode
30 of transmission, the fitness of such symbionts is tightly linked to that of their host and they are likely
31 to benefit their host in order to increase their own transmission (Fine, 1975). Indeed, endosymbionts
32 have been shown to affect the host-plant range of herbivorous arthropods (Hosokawa *et al.*, 2007,
33 Tsuchida *et al.*, 2011, Sugio *et al.*, 2015, Wagner *et al.*, 2015, Giron *et al.*, 2017) or to increase
34 performance on certain plant species (Wilkinson *et al.*, 2001, Leonardo & Muiru, 2003, Ferrari *et al.*,
35 2004, Tsuchida *et al.*, 2004, Ferrari *et al.*, 2007, Hosokawa *et al.*, 2007, Su *et al.*, 2013, Su *et al.*, 2015,
36 Wagner *et al.*, 2015), while decreasing performance on others (Chen *et al.*, 2000, Leonardo & Muiru,
37 2003, Ferrari *et al.*, 2007, Chandler *et al.*, 2008, McLean *et al.*, 2011, Wagner *et al.*, 2015). In some
38 cases, increased host performance is due to endosymbionts acting as nutritional mutualists, directly
39 supplying their arthropod hosts with nutrients or enzymes that are missing in their plant diet
40 (reviewed by Chaves *et al.*, 2009, Douglas, 2009), or displaying compensatory effects during periods
41 of nutritional deficiency (Su *et al.*, 2014). Finally, endosymbionts may also enable arthropods to
42 manipulate phytohormonal profiles (Kaiser *et al.*, 2010, Body *et al.*, 2013), resource allocation
43 (Hackett *et al.*, 2013), and anti-herbivory defences (Barr *et al.*, 2010, Su *et al.*, 2015). Conversely,
44 symbiont-mediated decreased host performance on particular plants might be due to the nutrient
45 profile (e.g., specific amino acids and nitrogen content) of these plants, which promotes deleterious
46 symbiont traits and disturbs the host control over bacterial abundance (Wilkinson *et al.*, 2007,
47 Chandler *et al.*, 2008).

48 Such variable effects of endosymbionts on herbivore plant use may contribute to variation in
49 the abundance and distribution of herbivorous arthropods (Douglas, 2009, Hansen & Moran, 2014).
50 Conversely, as symbiont-herbivore interactions may differ according to the host plant, and nutrition

51 of herbivore host can affect the within-host symbiont density (Wilkinson *et al.*, 2001, Wilkinson *et al.*,
52 2007, Chandler *et al.*, 2008, Zhang *et al.*, 2016), the host plant can also affect endosymbiont
53 distribution in the field (Leonardo & Muiru, 2003, Simon *et al.*, 2003, Ferrari *et al.*, 2004, Tsuchida *et*
54 *al.*, 2004, Chandler *et al.*, 2008, Ahmed *et al.*, 2010, Brady & White, 2013, Pan *et al.*, 2013, Guidolin &
55 Consoli, 2017). However, most studies addressing these questions have been conducted on sap-
56 feeding insects and whether symbiont prevalence and their effects on their herbivorous host vary
57 with the host plant remains unstudied in other systems.

58 The two-spotted spider mite *Tetranychus urticae*, a cosmopolitan agricultural and
59 horticultural pest that feeds on cell content, is a highly polyphagous arthropod, feeding on more than
60 1100 plant species (Migeon & Dorkeld, 2006-2017). This generalist herbivore rapidly adapts to novel
61 host plants (Fry, 1990, Agrawal, 2000, Magalhães *et al.*, 2007), sometimes forming host races
62 (Magalhães *et al.*, 2007), and may harbour several endosymbiotic bacteria with variable prevalence
63 among populations (Enigl & Schausberger, 2007, Gotoh *et al.*, 2007, Staudacher *et al.*, 2017). Among
64 them, *Wolbachia* is the most prevalent (Liu *et al.*, 2006, Gotoh *et al.*, 2007, Ros & Breeuwer, 2009,
65 Zhang *et al.*, 2016, Zélé *et al.*, 2018) and induces variable fitness effects in spider mites. For
66 instance, it can decrease (Perrot-Minnot *et al.*, 2002, Suh *et al.*, 2015), not affect (Breeuwer, 1997,
67 Vala *et al.*, 2000, Perrot-Minnot *et al.*, 2002, Vala *et al.*, 2002, Gotoh *et al.*, 2007), or increase (Vala *et*
68 *al.*, 2002, Gotoh *et al.*, 2007, Xie *et al.*, 2011) their fecundity. Given these variable effects, it is as yet
69 unclear whether *Wolbachia* will facilitate or hamper host-plant colonization by spider mites.

70 Here, we measured the prevalence of the three most prevalent endosymbionts of *T. urticae*,
71 namely *Wolbachia*, *Cardinium*, and *Rickettsia*, on five different host plants in Portugal. Subsequently,
72 we explored whether the effect of *Wolbachia* on the performance of *T. urticae* hinges on the plant
73 that is being colonized. Finally, we discuss the importance of possible mechanisms leading to our
74 results as well as the potential adaptive significance of the presence of *Wolbachia* for plant
75 colonization by *T. urticae*.

76

77

78 **MATERIALS AND METHODS**

79

80 **Effect of the host plant on endosymbiont prevalence in the field**

81 To determine whether the prevalence of *Wolbachia*, *Cardinium* and *Rickettsia* in natural *T. urticae*
82 populations varied with the host plant, spider mites were collected on bean (*Phaseolus vulgaris*,
83 Fabaceae), eggplant (*Solanum melongena*, Solenaceae), purple morning glory (*Ipomoea purpurea*,
84 Convolvulaceae, hereafter "purple"), zucchini (*Cucurbita pepo*, Cucurbitaceae), and tomato (*Solanum*
85 *lycopersicum*, Solenaceae) across 12 different locations (Table 1). These plants were selected because

86 they are part of the natural host range of *T. urticae* but belong to different families. Sampling sites
 87 consisted of open fields, greenhouses or organic vegetable gardens, while being insecticide/pesticide
 88 free to avoid this potential confounding effect. Infested leaves were detached and placed in closed
 89 plastic boxes that were brought to the laboratory. On the same day, 50 adult females were
 90 haphazardly picked from each population and their species determined at the individual level based
 91 on morphological characteristics under a binocular microscope. These females were then placed on 2
 92 cm² leaf discs of the same plant species on which they were found, and allowed to lay eggs for 4
 93 days. Subsequently, 20 of these females were randomly selected and individually tested for the
 94 presence of *Wolbachia*, *Cardinium* and *Rickettsia* on entire mites without DNA extraction by
 95 multiplex PCR using genus-specific primers as described in (Zélé *et al.*, 2018). Subsequently, for each
 96 population, the DNA of a pool consisting of one daughter from each of these females was extracted,
 97 then a PCR-based method to identify the mite species was performed by multiplex PCR as described
 98 in (Zélé *et al.*, 2018). If a pool could not be assigned unambiguously to *T. urticae* (see Table S1 in
 99 Additional file 1), all data concerning endosymbiont prevalence were discarded. This process was
 100 repeated until obtaining endosymbiont prevalence data for 5 populations per plant, except for
 101 purple, for which we could obtain only 2 populations of *T. urticae* due to the weak infestation rate of
 102 this plant by this spider-mite species, and despite a large sampling effort (Table S1).

103

104 **Table 1.** *Tetranychus urticae* populations collected on five different host plants across 12 different locations in
 105 June-July 2015 and used to study the plant effect on the prevalence of *Wolbachia*, *Cardinium* and *Rickettsia*.

Host plant	Name	Date	Location	Coordinates
Bean (<i>Phaseolus vulgaris</i>)	B1	08-06-2015	Hortas da Cortesia, São João das Lampas	38.865278, -9.384006
	B2	08-06-2015	Pêro Pinheiro	38.851900, -9.326903
	B6	10-06-2015	Correias	39.342914, -8.797936
	B7	10-06-2015	Biofrade, Lourinhã	39.258314, -9.294675
	B8	10-06-2015	Aromas do Outeiro, Carregado	39.026500, -8.982278
Eggplant (<i>Solanum melongena</i>)	E3	10-06-2015	Aromas do Outeiro, Carregado	39.026500, -8.982278
	E4	10-06-2015	Ribeira de Fráguas	39.366414, -8.851036
	E5	10-06-2015	Biofrade, Lourinhã	39.258314, -9.294675
	E6	15-06-2015	Alvalade, Lisbon	38.755283, -9.147203
	E7	16-06-2015	Quinta Pedagógica dos Olivais, Lisbon	38.762897, -9.112419
Purple (<i>Ipomoea purpurea</i>)	P5	14-06-2015	Alvalade, Lisbon	38.755283, -9.147203
	P13	08-07-2015	Fernão Ferro	38.580006, -9.102147
Tomato (<i>Solanum lycopersicum</i>)	T1	08-06-2015	Hortas da Cortesia, São João das Lampas	38.865278, -9.384006
	T3	10-06-2015	Aromas do Outeiro, Carregado	39.026500, -8.982278
	T5	13-06-2015	Campo Grande, Lisbon	38.755775, -9.156075
	T6	16-06-2015	Campo Pequeno, Lisbon	38.744336, -9.144289
	T7	16-06-2015	Quinta Pedagógica dos Olivais, Lisbon	38.762897, -9.112419
Zucchini (<i>Cucurbita pepo</i>)	Z1	08-06-2015	Hortas da Cortesia, São João das Lampas	38.865278, -9.384006
	Z2	09-06-2015	Quinta do Poial, Galeotas	38.536103, -9.000375
	Z5	10-06-2015	Correias	39.342914, -8.797936
	Z6	10-06-2015	Ribeira de Fráguas	39.366414, -8.851036
	Z7	10-06-2015	Aromas do Outeiro, Carregado	39.026500, -8.982278

106

107

108 **Effect of *Wolbachia*, the host plant, and their interaction on the performance of spider mites**

109 *Spider mite populations, tetracycline treatment and population rearing*

110 The spider-mite population used was originally collected on *Datura* plants at Aldeia da Mata
111 Pequena, Portugal, in November 2013 and kept in a mass-rearing environment (>5 000 individuals)
112 on bean plants (var. *Enana*), under controlled conditions (25°C, photoperiod of 16L:8D) since then.
113 This population, hereafter called Wi, was found uninfected by *Rickettsia*, *Spiroplasma* or
114 *Arsenophonus* but fully infected by *Wolbachia* in the field (Zélé *et al.*, 2018). Although this population
115 was also slightly infected by *Cardinium* (Zélé *et al.*, 2018), this endosymbiont has been rapidly lost
116 following laboratory rearing (unpublished data). To obtain a *Wolbachia*-uninfected (Wu) population
117 with a similar genetic background, roughly 3 months after collection 30 adult females of the Wi
118 population were placed in petri dishes containing bean leaf fragments placed on cotton with a
119 tetracycline solution (0.1 %, w/v). This treatment was applied continuously for three successive
120 generations (Breeuwer, 1997), then the population was maintained in a mass-rearing environment
121 without antibiotics for c.a. 12 generations before the experiment to avoid (or limit) potential side
122 effects of the antibiotic treatment (e.g. O'Shea & Singh, 2015) and allow mites to recover potential
123 loss of gut. Before use, up to 20 individual females and pools of 100 females were checked by PCR to
124 confirm the absence and presence of *Wolbachia* infection in Wu and Wi populations, respectively.

125

126 *Performance of *Wolbachia*-infected and uninfected females on different host plant*

127 To determine the effect of *Wolbachia* infection and of the host plant, as well as their possible
128 interaction, on the performance of *T. urticae*, we measured life history traits of individuals from Wi
129 or Wu populations when placed on the same plant species as those from which mites were collected
130 in the field study (bean: var. *Enana*, eggplant: var. *Larga Morada*, purple: var. *Vigorous*, zucchini: var.
131 *Bellezza Negra*, and tomato: var. *Money Maker*). To control for age, 100 females were allowed to lay
132 eggs for three days on detached bean leaves placed on water-soaked cotton, and the adult females
133 resulting from those eggs were used in the experiments. Fifty mated females (10-13 days old) were
134 haphazardly picked from either Wi or Wu cohorts and placed individually on a 2 cm² leaf disc from
135 one of the 5 different host plants. The replicates were distributed along 5 temporal blocks (10
136 replicates per treatment per day during 5 consecutive days). Females that were alive after 3 days
137 were transferred to new leaf discs where they could lay eggs for another 3 days. Their survival (S) and
138 the proportion of drowned females in the water-soaked cotton (i.e. accidental death of females
139 trying to escape the leaf discs; PD) were followed daily during six days. The fecundity of each female
140 was measured at days 3 and 6 and the average female daily fecundity was estimated taking into
141 account their daily mortality (DF = total number of eggs laid per female / number of days the female
142 was alive). The number of unhatched eggs was counted 5 days later (i.e. days 8 and 11, respectively)

143 to estimate the hatching rate (HR = hatched eggs / total number of eggs). Adult offspring (F₁ females
144 + F₁ males) was counted after 6 additional days (i.e. days 14 and 17, respectively) and used to
145 estimate juvenile mortality (JM = [total number of eggs - number of unhatched eggs - number of F₁
146 adults]/ total number of eggs), F₁ sex ratio (SR = number of F₁ males/number of F₁ adults) and the
147 number of viable offspring (VO = total number of adult offspring per female per treatment observed
148 at the end of the experiment on each plant). The entire experiment was repeated three months later
149 (hereafter called blocks 1 and 2) except for replicates involving tomato plants. Indeed, given a very
150 high proportion of drowned females (88 ± 3.3 %; data not shown) and because the surviving females
151 laid on average less than 1 egg per day (0.32 ± 0.05; data not shown) on this plant, subsequent traits
152 could not be measured and we decided to exclude it from this experiment.

153

154 **Statistical analyses**

155 Analyses were carried out using the R statistical package (v. 3.3.2). The different statistical models
156 built to analyse the effect of host-plant on endosymbiont prevalence in field-collected spider-mite
157 populations and the effects of *Wolbachia* on different host plants are described in the electronic
158 supplementary material (Additional file 1), Table S2.

159 To analyse the effect of host plants on endosymbiont prevalence in field-collected mites, the
160 prevalence of *Wolbachia* (model 1), *Cardinium* (model 2) and *Rickettsia* (model 3) were fit as binary
161 response variables, the host plant on which mites were collected as fixed explanatory variable, and
162 the location as random explanatory variable. Because of quasi-complete separation of some of our
163 data, which usually causes problems with estimated regression coefficients, analyses were conducted
164 using a mixed model *bgfmer* procedure (*blme* package) with a binomial error distribution (Pasch *et*
165 *al.*, 2013). When the variable “plant” was significant, a stepwise *a posteriori* procedure (Crawley,
166 2007) to determine differences between plants was carried out by aggregating factor levels together
167 and by testing the fit of the simplified model using a likelihood ratio test (LRT), which is
168 approximately distributed as a χ^2 distribution (Bolker, 2008). Because none of the mites collected in
169 this study were singly infected by *Cardinium* or *Rickettsia*, and the prevalence of each type of
170 coinfection was very low (cf. Results), we did not have enough statistical power to study the effect of
171 the host plants on the prevalence of coinfections.

172 To analyse the effect of *Wolbachia*, the host plant, and their interaction on the performance
173 of spider mites, the infection status of females (i.e. Wi: infected or Wu: uninfected) and the host
174 plants tested were fit as fixed explanatory variables, whereas block and day were fit as random
175 explanatory variables (day nested within block). Survival data (S; model 4) were analysed using a Cox
176 proportional hazards mixed-effect model (*coxme*, *kinship* package). Hazard ratios were obtained
177 from this model as an estimate of the difference in mortality rate (Crawley, 2007) between our

178 control (Wi population on bean) and each of the other factor levels. PD, a binary response variable
179 (drowned or not; model 5), was analysed using a generalized linear mixed model with a binomial
180 distribution (*glmer*, *lme4* package). DF, a continuous response variable (model 6) was analysed using
181 linear mixed-effect models (*lmer*, *nlme* package). The other proportion variables HR, SR and JM
182 (models 7, 8, and 9, respectively) were computed using the function *cbind* (e.g. number of hatched
183 eggs, males, or dead juveniles vs. number of unhatched eggs, females, or alive juveniles,
184 respectively). However, due to the low daily fecundity of spider mites, these variables, as well as VO
185 (model 10) were greatly over-dispersed. One way of handling this over-dispersion is by using
186 quasibinomial or negative binomial pseudo distributions (Crawley, 2007) but, to our knowledge, this
187 is not possible within the usual mixed model *glmer* procedure. Thus, we used instead a mixed model
188 *glmmadmb* procedure (*glmmADMB* package) with zero-inflated binomial error distribution for HR, SR
189 and JM, and zero-inflated negative binomial error distribution for VO. When a statistically significant
190 interaction between the variables “*Wolbachia*” (Wi or Wu) and “plant” was found, the effect of
191 *Wolbachia* was analysed for each plant separately. When only the variable “plant” was significant, *a*
192 *posteriori* contrasts between host plants were performed as before.

193 For all analyses, maximal models were simplified by sequentially eliminating non-significant
194 terms to establish a minimal model (Crawley, 2007), and the significance of the explanatory variables
195 was established using χ^2 -tests or *F*-tests to account for overdispersion (Bolker, 2008). The significant
196 values given in the text are for the minimal model, while non-significant values correspond to those
197 obtained before deletion of the variable from the model (Crawley, 2007). Full datasets are given in
198 Additional files 2 and 3.

199

200

201 RESULTS

202

203 Effect of the host plant on endosymbiont prevalence in the field

204 The prevalence of *Wolbachia* was overall high (92.7 ± 1.2 %), while that of *Cardinium* (2.5 ± 0.7 %)
205 and *Rickettsia* (2.0 ± 0.7 %) were low (Fig. 1). In addition, while 89.3 ± 1.5 % of the mites collected in
206 this study were infected by *Wolbachia* only, none were infected by *Cardinium* or by *Rickettsia* only.
207 1.4 ± 0.6 % were coinfecting by *Wolbachia* and *Cardinium*, 0.9 ± 0.5 % were coinfecting by *Wolbachia*
208 and *Rickettsia*, and 1.14 ± 0.5 % were infected by these three endosymbionts (see Fig. S1 in
209 Additional file 1 for infection statuses at the individual level). The prevalence of *Wolbachia* and of
210 *Rickettsia* were affected by the plant on which *T. urticae* females were collected ($\chi^2_4=14.79$, $p=0.005$;
211 model 1, and $\chi^2_4=12.71$, $p=0.01$; model 3, respectively; Fig. 1). Contrast analyses revealed that the
212 prevalence of *Wolbachia* was higher on bean and eggplant (97.0 ± 1.7 %; *contrast bean vs eggplant*:

213 $\chi^2_1=0.51$, $p=0.47$) than on the 3 other plants (89.2 ± 2.0 %; *Contrast purple vs tomato vs zucchini:*
214 $\chi^2_2=0.39$, $p=0.82$; *Contrast between the two groups of plants:* $\chi^2_1=14.34$, $p=0.0002$), and that of
215 *Rickettsia* differed only on purple (12.5 ± 5.3 %) compared to all other plants (1.0 ± 0.5 %; *contrast*
216 *bean vs eggplant vs tomato vs zucchini:* $\chi^2_3=2.95$, $p=0.40$; *Contrast between this group of plants and*
217 *purple:* $\chi^2_1=9.76$, $p=0.002$). Finally, the prevalence of *Cardinium*, similarly to that of *Rickettsia*, tended
218 to be higher on purple (12.5 ± 5.3 %) compared to the other plants (1.5 ± 0.6 %), but this effect was
219 not statistically significant ($\chi^2_4=1.61$, $p=0.81$; model 2).

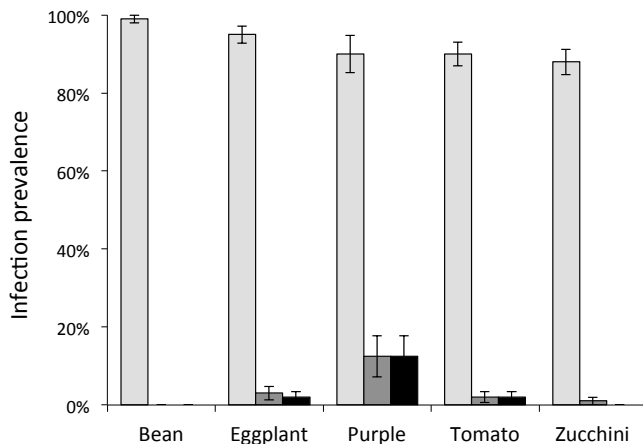


Figure 1. Endosymbiont prevalence in *T. urticae* females collected on different host plants. Bars represent the mean (\pm s.e.) infection frequencies by *Wolbachia* (light grey), *Cardinium* (dark grey), and *Rickettsia* (black) for several spider mite populations collected on bean (n=5), eggplant (n=5), purple (n=2), tomato (n=5), and zucchini (n=5).

230

231 **Effect of *Wolbachia*, the host plant, and their interaction on the performance of spider mites**

232 Overall, there was no significant effect of *Wolbachia* ($\chi^2_1=0.73$, $p=0.39$), of host plants ($\chi^2_3=6.84$,
233 $p=0.07$), or of their interaction ($\chi^2_3=3.34$, $p=0.34$; model 4; Table 1 and Fig. S2 in Additional file 1) on
234 survival (S) over the 6 first days of the experiment. However, host plants affected significantly the
235 proportion of drowned mites (PD; $\chi^2_3=23.14$, $p<0.0001$), regardless of *Wolbachia* infection
236 (*Wolbachia* effect: $\chi^2_1=1.35$, $p=0.25$; *Wolbachia*-plant interaction: $\chi^2_3=0.70$, $p=0.87$; model 5; Table
237 2).

238 Daily fecundity (DF) was significantly affected by host plants ($\chi^2_3=129.33$, $p<0.0001$), but not
239 by *Wolbachia* ($\chi^2_1=2.06$, $p=0.15$) or its interaction with the plant ($\chi^2_3=1.21$, $p=0.75$; model 6; table 2).
240 Contrast analyses revealed that DF was similar on purple and zucchini (3.37 ± 0.11 eggs per day;
241 *contrast purple vs zucchini:* $\chi^2_1=1.03$, $p=0.31$), but higher on bean (4.60 ± 0.19 eggs per day; *contrast*
242 *purple-zucchini vs bean:* $\chi^2_1=40.14$, $p<0.0001$), and lower on eggplant (2.10 ± 0.13 ; *Contrast eggplant*
243 *vs purple-zucchini:* $\chi^2_1=42.77$, $p<0.0001$).

244 The effect of *Wolbachia* on egg hatching rate (HR) depended on the host plant tested
245 (*Wolbachia*-plant interaction: $F_{3,697}=5.47$, $p=0.001$; model 7; Table 1 and Fig. 2). Indeed, *Wolbachia*
246 reduced HR on purple ($F_{1,172}=10.05$, $p=0.002$) and on zucchini ($F_{1,177}=19.74$, $p<0.0001$), but had no
247 effect on bean and eggplant ($F_{1,181}=1.42$, $p=0.24$ and $F_{1,158}=1.56$, $p=0.21$, respectively).

Table 2. Effect of *Wolbachia* and of host plants on the performance of spider mites. Mean (\pm s.e.) values of both *Wolbachia*-infected (Wi) and uninfected (Wu) *T. urticae* on the different plants studied (bean, purple, zucchini and eggplant) are represented for each one of the performance traits measured in this study. For hatching rate, juvenile mortality and sex ratio, estimates were obtained from the GLMM statistical models and take into account variation among females, as well as the correction for zero-inflation and day within block as random effect.

Variable of interest	Bean		Purple		Zucchini		Eggplant		Significance of explanatory variables and their interaction		
	Wi	Wu	Wi	Wu	Wi	Wu	Wi	Wu	Plant * <i>Wolbachia</i>	Plant	<i>Wolbachia</i>
Log Hazard Ratio (S)	-	0.15 \pm 0.21	-0.03 \pm 0.22	-0.21 \pm 0.31	-0.06 \pm 0.23	-0.59 \pm 0.32	0.18 \pm 0.22	-0.28 \pm 0.30	$\chi^2_3=3.34$, p=0.34	$\chi^2_3=6.84$, p=0.08	$\chi^2_1=0.88$, p=0.35
Proportion of drowned (PD)	0.16 \pm 0.04	0.13 \pm 0.03	0.26 \pm 0.04	0.22 \pm 0.04	0.34 \pm 0.05	0.26 \pm 0.04	0.34 \pm 0.05	0.34 \pm 0.05	$\chi^2_3=0.70$, p=0.87	$\chi^2_3=23.14$, p<0.0001	$\chi^2_1=1.35$, p=0.25
Daily fecundity (DF)	4.76 \pm 0.27	4.43 \pm 0.26	3.54 \pm 0.24	3.42 \pm 0.21	3.26 \pm 0.22	3.25 \pm 0.22	2.32 \pm 0.21	1.88 \pm 0.15	$\chi^2_3=1.21$, p=0.75	$\chi^2_3=129.33$, p<0.0001	$\chi^2_1=2.06$, p=0.15
Hatching rate (HR)	0.97 \pm 0.01	0.96 \pm 0.01	0.96 \pm 0.01	0.98 \pm 0.01	0.92 \pm 0.01	0.95 \pm 0.01	0.94 \pm 0.01	0.93 \pm 0.02	$F_{3,697}=5.47$, p=0.001	-	-
Juvenile mortality (JM)	0.18 \pm 0.03	0.20 \pm 0.01	0.12 \pm 0.01	0.11 \pm 0.01	0.19 \pm 0.02	0.16 \pm 0.02	0.32 \pm 0.02	0.27 \pm 0.03	$F_{3,689}=1.85$, p=0.14	$F_{3,693}=48.23$, p<0.0001	$F_{1,692}=0.01$, p=0.92
Sex ratio (SR)	0.19 \pm 0.01	0.20 \pm 0.01	0.21 \pm 0.01	0.24 \pm 0.02	0.21 \pm 0.02	0.23 \pm 0.02	0.17 \pm 0.02	0.24 \pm 0.03	$F_{3,681}=2.48$, p=0.04	-	-
Viable offspring (VO)	19.03 \pm 1.33	17.45 \pm 1.28	14.49 \pm 1.23	14.44 \pm 1.12	10.89 \pm 0.98	12.80 \pm 1.03	6.78 \pm 0.74	5.5 \pm 0.54	$F_{3,786}=0.70$, p=0.55	$F_{3,790}=48.72$, p<0.0001	$F_{1,789}=0.78$, p=0.38

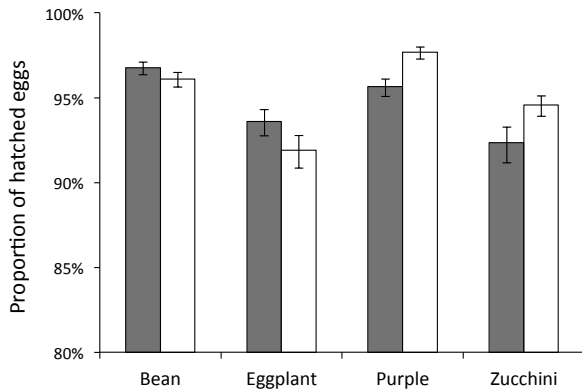


Figure 2. Effects of different host plants and of *Wolbachia* on the hatching rate of *T. urticae* eggs. Bars represent the mean (\pm s.e.) proportions of hatched eggs laid by *Wolbachia*-infected (Wi; grey bars) and uninfected (Wu; white bars) females on different host plants. Estimates were obtained from the GLMM statistical model that takes into account variation of fecundity among females, day within block as random effect, and corrects for zero-inflation. Standard errors were obtained from the upper and lower confidence intervals given by the model.

11

12 Juvenile mortality (JM) was not significantly affected by *Wolbachia* ($F_{1,692}=0.01$, $p=0.92$;
13 model 8; Table 2), and this was consistent across all host plants (*Wolbachia*-plant interaction:
14 $F_{3,689}=1.85$, $p=0.14$; model 8). However, host plant was a significant predictor of JM ($F_{3,693}=48.23$,
15 $p<0.0001$; model 8). Bean and zucchini did not differ significantly from each other (contrast bean vs
16 zucchini: $\chi^2_1=0.72$, $p=0.40$) and led to intermediate JM of $16.8 \pm 0.9\%$, while purple decreased it by
17 $5.2 \pm 1.5\%$ (contrast purple vs bean-zucchini: $\chi^2_1=53.82$, $p<0.0001$), and eggplant increased it by
18 $11.3 \pm 2.1\%$ (contrast bean-zucchini vs eggplant: $\chi^2_1=109.36$, $p<0.0001$).

19 *Wolbachia* infection affected differently the sex ratio (SR) produced on the different plants
20 (*Wolbachia*-plant interaction: $F_{3,681}=2.48$, $p=0.04$; model 9; Table 2 and Fig. 3). Indeed, *Wolbachia*
21 decreased the proportion of males on purple ($F_{1,168}=5.51$, $p=0.02$) and on eggplant ($F_{1,153}=8.54$,
22 $p=0.004$). On bean and zucchini, however, SR did not differ significantly between Wi and Wu mites
23 ($F_{1,179}=5.51$, $p=0.54$ and $F_{1,1726}=2.28$, $p=0.13$, respectively).

24

25

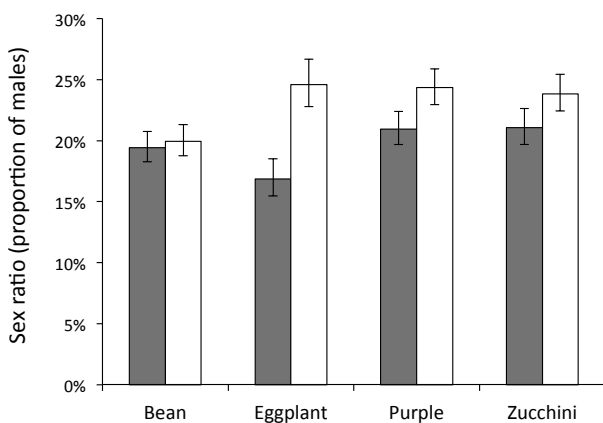


Figure 3. Effects of different host plants and of *Wolbachia* on the offspring sex ratio produced by *T. urticae* females. Bars represent the mean (\pm s.e.) proportions of male offspring produced by *Wolbachia*-infected (Wi; grey bars) and uninfected (Wu; white bars) females on different host plants. Estimates were obtained from the GLMM statistical model that takes into account variation of fecundity among females, day within block as random effect, and corrects for zero-inflation. Standard errors were obtained from the upper and lower confidence intervals given by the model.

37

38 Although we found a significant *Wolbachia*-plant interaction on HR and SR, *Wolbachia* did
39 not significantly influence the average number of viable offspring (VO; $F_{1,789}=0.78$, $p=0.38$), and this
40 effect was independent of the host plant (*Wolbachia*-plant interaction: $F_{3,786}=0.70$, $p=0.55$; model 10;
41 Table 2 and Fig. 4). Nonetheless, host plant significantly explained this trait ($F_{3,790}=48.72$, $p<0.0001$;
42 model 10), with the highest values on bean, intermediate values on purple (*contrast purple vs bean*:
43 $\chi^2_1=4.82$, $p=0.03$) and zucchini (*contrast zucchini vs purple*: $\chi^2_1=5.12$, $p=0.02$), and the lowest values
44 on eggplant (*contrast eggplant vs zucchini*: $\chi^2_1=44$, $p<0.0001$).
45

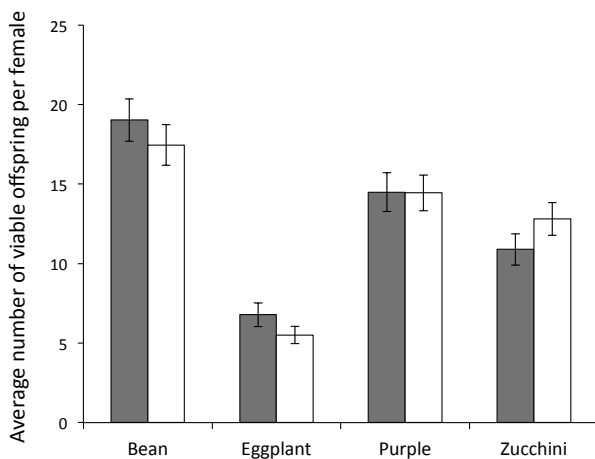


Figure 4. Effects of different host plants and of *Wolbachia* on the average number of viable offspring per female. Bars represent the mean (\pm s.e.) numbers of offspring (grey: sons; white: daughters) produced by *Wolbachia*-infected (Wi; grey bars) and uninfected (Wu; white bars) females on different host plants.

57
58
59 **DISCUSSION**

60
61 In this study, we confirmed that *Wolbachia* is highly prevalent in *T. urticae* in Portugal, while
62 *Cardinium* and *Rickettsia* were found at low prevalences (Z  l   et al., 2018). Moreover, this study
63 suggests that endosymbiont prevalence varied with the host plant, *Cardinium* and *Rickettsia* being
64 more prevalent on purple (although non-significantly for *Cardinium*) than on the other plants, and
65 *Wolbachia* being more prevalent on bean and eggplant than on tomato, purple and zucchini. In the
66 laboratory, *Wolbachia*-infected eggs had a lower hatching rate than uninfected ones on purple and
67 zucchini, while this was not the case on bean and eggplant.

68 The prevalence of *Wolbachia* and *Rickettsia* in *T. urticae* females found in this study was
69 relatively similar to that of an earlier study in the same geographical area (Z  l   et al., 2018).
70 However, the prevalence of *Cardinium* was about five times lower in the current study than in the
71 former one ($2.5 \pm 0.7\%$ vs $13.6 \pm 2.9\%$, respectively). As the populations were sampled on
72 comparable host plants in this previous study (except for one population collected on *Datura*

73 *stramonium*, the others were collected on bean, eggplant, tomato and zucchini), the discrepancy
74 observed for the overall *Cardinium* prevalence between the two studies may be attributed to the
75 time of collection. Indeed, mites were collected between September and December in the previous
76 study and in June-July in the current one. Several studies have shown that the sampling period might
77 affect endosymbiont prevalence and/or density in host populations (Toju & Fukatsu, 2011, Dorfmeier
78 *et al.*, 2015, Martinez-Diaz *et al.*, 2016, Sumi *et al.*, 2017). This increase of *Cardinium* prevalence
79 during summer is compatible with the hypothesis of an accumulation of this symbiont throughout
80 the season via horizontal transfers (Zélé *et al.*, 2018).

81 We found that *Wolbachia* prevalence was overall high, but significantly higher on bean and
82 eggplant than on the other plants. Whereas some earlier studies have shown that *Wolbachia*
83 prevalence in herbivores varies according to the host plant (Ahmed *et al.*, 2010, Toju & Fukatsu,
84 2011, Guidolin & Consoli, 2017), including a recent study conducted in the spider mite *Tetranychus*
85 *truncatus* (Zhu *et al.*, 2018), others show no difference (Ji *et al.*, 2015). Unfortunately, the scarcity of
86 studies, along with the fact that they were mostly done in other systems, hampers a meaningful
87 comparison among studies. In addition, it is extremely difficult to sample spider-mite populations on
88 all the plants tested within the same locality (see Table S1 in Additional file 1). Consequently, this
89 implies an important sampling effort to obtain only a very reduced number of populations that fit the
90 criteria for such studies. For instance, despite a large sampling effort across 21 localities and 12 host
91 plant species, Zhu *et al.* (2018) could assess the effect of three common host plants (soybean, corn,
92 and tomato) from three different locations only. Still, they did find that the prevalence of *Wolbachia*
93 was significantly affected by the host plant (about 30% higher in tomato than in corn). In our study,
94 the amplitude of the observed effects is much lower, possibly due to a threshold effect since the
95 prevalence of *Wolbachia* that we observed in *T. urticae* is overall much higher than that observed in
96 *T. truncatus* by Zhu *et al.* (2018). Clearly, differences in *Wolbachia* prevalence were not associated
97 with plant phylogenetic distance, as it differed between the solanaceous plants used (eggplant and
98 tomato). Moreover, the effect of an endosymbiont on arthropod-plant interactions may depend on
99 both the genotype (or species) of symbiont (Leonardo & Muir, 2003) and arthropod host (Chen *et*
100 *al.*, 2000, Ferrari *et al.*, 2007, McLean *et al.*, 2011, Wagner *et al.*, 2015), and/or their interaction
101 (Ferrari *et al.*, 2007). More studies on plant-dependent symbiont prevalence may thus shed light on
102 the potential factors underlying the pattern observed and on the ecological meaning of such effects.

103 Here, we hypothesize that the variation in endosymbiont prevalence according to the host
104 plant is, at least partially, due to plant-specific effects of these symbionts on spider-mite
105 performance. Although we did find some variation of *Rickettsia* and *Cardinium* prevalence according
106 to the host plant, their prevalence was very low, so we opted for addressing this issue using
107 *Wolbachia* only. Overall, we found a strong effect of the host plant on spider-mite performance, with

108 the highest values observed on bean. This is not surprising, given that bean was the rearing
109 environment of the population used, and is generally a host plant of high quality for spider mites (e.g.
110 Magalhães *et al.*, 2011). Conversely, the lowest performances were found on Solanaceous plants
111 (eggplant and tomato), being so low on tomato (cf. Material and Methods) that we excluded these
112 data from further analyses. In the other four plants, we found that some traits (proportion escaping,
113 female fecundity, and juvenile survival) were not affected by *Wolbachia* whereas others (egg
114 hatching rate and sex ratio) were affected in a plant-specific manner.

115 The plant-specific effects of *Wolbachia*, although of low amplitude, could be explained by
116 several non-exclusive mechanisms. First, *Wolbachia* may impose a nutritional burden to its hosts,
117 sequestering and using vital host nutrients for its own survival (Chandler *et al.*, 2008, Caragata *et al.*,
118 2014, Ponton *et al.*, 2015), and this may vary with the host plant. Indeed, the nutrient composition of
119 plant material is often poor or unbalanced for herbivores (Schoonhoven *et al.*, 2005, Karban &
120 Baldwin, 2007), and nutrient deficient diet may increase the competition for resources between
121 hosts and symbionts. In turn, this may lead to a decreased ability of infected spider mites to allocate
122 enough nutrients to ensure egg viability on plants of low quality. Increased host-symbiont
123 competition on such low-quality plants could also lead to a biased sex ratio towards males because
124 females are produced from bigger eggs than males in *T. urticae* (Macke *et al.*, 2011). In addition, the
125 slight *Wolbachia*-induced female-biased sex ratio observed on purple could be a consequence of the
126 lower hatching rate observed on this plant, as larger eggs are generally more likely to hatch (Macke
127 *et al.*, 2011). However, if this hypothesis would hold true, one would expect a stronger cost of
128 *Wolbachia* in spider mites on plants of lower quality for mites, and we did not find such pattern.

129 Second, *Wolbachia* may directly influence the metabolism of some plants, which in turn can
130 affect the biology of its herbivorous hosts. For instance, *Wolbachia* infecting the leaf-mining moth
131 *Phyllonorycter blancardella* might be responsible for an increased level of cytokinins (plant hormones
132 mainly involved in nutrient mobilisation and inhibition of senescence) in infested apple trees, *Malus*
133 *domestica*. In this system, *Wolbachia* thus helps its host to develop in photosynthetically active green
134 patches in otherwise senescent leaves (Kaiser *et al.*, 2010, Body *et al.*, 2013). Interestingly, cytokinins
135 have also been shown to be responsible for sex-ratio shift towards females in the sap-feeding insect
136 *Tupiocoris notatus* (although this effect was not mediated by *Wolbachia*; Adam *et al.* 2017). As
137 *Wolbachia* possess a key gene involved in cytokinin biosynthesis in their genomes (Kaiser *et al.*,
138 2010), frequently infect the salivary glands of its hosts (Dobson *et al.*, 1999) and are present in high
139 density in the gnathosoma of spider mites (Zhao *et al.*, 2013), one could speculate that the sex-ratio
140 shift towards females observed in *Wolbachia*-infected mites on purple and eggplant in our study is
141 mediated by increased cytokinin levels induced by *Wolbachia* in these two plants. Further research is
142 thus needed to test this hypothesis. In particular, whether the *Wolbachia* present in spider mites also

143 possess genes involved in cytokinin biosynthesis in their genomes is still unknown and the full
144 genome of *Wolbachia* isolated from spider-mite hosts has, to our knowledge, not yet been
145 sequenced.

146 Third, *Wolbachia* may interfere with the mites' response toward plant defences. Indeed,
147 endosymbionts found in herbivores, including *Wolbachia*, may directly manipulate the plant
148 defenses to benefit their host (Frago *et al.*, 2012, Hansen & Moran, 2014, Zhu *et al.*, 2014, Sugio *et*
149 *al.*, 2015, Giron *et al.*, 2017, Shikano *et al.*, 2017), or have a detrimental effect on their host by
150 increasing the level of induced plant defences. For instance, down-regulation of several defense
151 genes of maize by the western corn rootworm *Diabrotica virgifera* has been shown to be mediated
152 by *Wolbachia* (Barr *et al.*, 2010, but see Robert *et al.*, 2013). Moreover, in a recent study, Staudacher
153 *et al.* (2017) found that feeding by mites coinfecting with *Spiroplasma* and *Wolbachia* increased the
154 accumulation of 12-oxo-phytodienoic acid (a precursor of jasmonic acid) in tomato plants, compared
155 to *Spiroplasma*-infected or non-infected mites. However, the concentration of jasmonic, salicylic and
156 abscisic acids were not affected and no causal link could be established between the changes in plant
157 defenses and mite performance (although only fecundity and longevity have been studied). Whether
158 the presence of *Wolbachia* in *T. urticae* can upregulate the defences of zucchini and purple, and
159 whether this could explain the reduced egg hatchability observed here, thus remains to be tested.

160 Despite the weak plant-specific effects of *Wolbachia* on mite performance, and that they do
161 not affect the total number of viable offspring, they seem to be correlated with *Wolbachia*
162 prevalence on field populations of *T. urticae* collected on different host plants. Indeed, given that
163 *Wolbachia* is costly on egg hatchability on zucchini, we would expect a lower prevalence of this
164 symbiont on this plant. Conversely, as *Wolbachia* increases the proportion of females produced on
165 eggplant, we could expect a higher prevalence on this plant. Indeed, *Wolbachia* being maternally
166 transmitted, it should always benefit from a more female-biased sex ratio. Note that, although
167 *Wolbachia* may induce cytoplasmic incompatibility in *T. urticae* (Gotoh *et al.*, 2007, Xie *et al.*, 2011,
168 Suh *et al.*, 2015), the effects observed in this study on spider-mite sex ratio cannot be attributed to
169 this phenotype as it involves a cross between infected males and uninfected females, which was not
170 performed here. On purple, we could expect the prevalence of *Wolbachia* to be intermediate, as the
171 infection decreases egg hatchability but increases female proportion. Finally, bean being the plant on
172 which spider mites have, overall, the best performance and that *Wolbachia* is not costly on this plant,
173 we could expect its prevalence to be very high. Hence, by affecting the balance costs/benefits of
174 *Wolbachia* on its spider-mite hosts, plants may affect *Wolbachia* prevalence. From the host
175 perspective, however, although increased egg hatchability would probably benefit the spread of
176 spider mites, it is not clear whether a female-biased sex ratio would benefit mites, as this is expected
177 to depend on population structure (Hamilton, 1967, Macke *et al.*, 2011). More studies are thus

178 needed to shed light on the potential role of *Wolbachia* on the host plant range of spider mites, as
179 done in other systems (Hansen & Moran, 2014, Sugio *et al.*, 2015, Giron *et al.*, 2017).

180 In conclusion, our results show plant-dependent effects of *Wolbachia* on spider mites egg
181 hatchability and offspring sex ratio, two crucial traits for both spider-mite population dynamics and
182 *Wolbachia* spread among host populations. Although the amplitude of these effects is relatively low,
183 they may, at least partially, explain the prevalence of this symbiont in spider mite populations
184 collected on these different host plants. Moreover, our study highlights the importance of studying
185 different host plants and life history traits when addressing the effects of endosymbionts on the
186 performance of their herbivorous arthropods. These results also raised important questions, such as:
187 (i) whether the pattern observed in this study varies between host and/or symbiont genotype, (ii)
188 whether host plants affect the maintenance and/or spread of endosymbionts within and among
189 populations, and (iii) whether endosymbionts affect the host range of herbivores.

190

191 **SUPPLEMENTARY DATA**

192 Supplementary data are available at FEMSEC online.

193

194 **ACKNOWLEDGMENTS**

195 We thank M. Bakırdöven, J. Denoyelle, L. Rodrigues, and Inês Santos for their help in spider-mite
196 collection. We also thank IS for the maintenance of the plants and mite populations, Miguel Cruz,
197 Nelson Martins, Jordi Moya Laraño and Susana Verala for advices in statistical analysis.

198

199 **AUTHOR'S CONTRIBUTIONS**

200 Experimental conception and design: FZ, SM; field collections: JS, DG; acquisition of data: JS;
201 statistical analyses: FZ, JS; paper writing: FZ, SM, with input from all authors. All authors have read
202 and approved the final version of the manuscript.

203

204 **FUNDING**

205 This work was funded by an FCT-ANR project (FCT-ANR//BIA-EVF/0013/2012) to SM and Isabelle
206 Olivieri and by a FCT-Tubitak project (FCT-TUBITAK/0001/2014) to SM and Ibrahim Cakmak. FZ and
207 DG were funded through FCT Post-Doc (SFRH/BPD/125020/2016) and PhD (PD/BD/114010/2015)
208 fellowships, respectively. Funding agencies did not participate in the design or analysis of
209 experiments.

210 **Conflict of interest.** None declared.

211 **REFERENCES**

- 212 Adam N, Erler T, Kallenbach M, Kaltenpoth M, Kunert G, Baldwin IT & Schuman MC (2017) Sex ratio
213 of mirid populations shifts in response to hostplant co-infestation or altered cytokinin signaling.
214 *J Integr Plant Biol* **59**: 44-59.
- 215 Agrawal AA (2000) Host-range evolution: Adaptation and trade-offs in fitness of mites on alternative
216 hosts. *Ecology* **81**: 500-508.
- 217 Ahmed MZ, Ren SX, Mandour NS, Greeff JM & Qiu BL (2010) Prevalence of *Wolbachia* supergroups A
218 and B in *Bemisia tabaci* (Hemiptera: Aleyrodidae) and some of its natural enemies. *J Econ*
219 *Entomol* **103**: 1848-1859.
- 220 Barr KL, Hearne LB, Briesacher S, Clark TL & Davis GE (2010) Microbial symbionts in insects influence
221 down-regulation of defense genes in maize. *PLoS One* **5**.
- 222 Becerra JX (1997) Insects on plants: Macroevolutionary chemical trends in host use. *Science* **276**: 253-
223 256.
- 224 Body M, Kaiser W, Dubreuil G, Casas J & Giron D (2013) Leaf-miners co-opt microorganisms to
225 enhance their nutritional environment. *J Chem Ecol* **39**: 969-977.
- 226 Bolker BM (2008) *Ecological models and data in R* Princeton University Press, New Jersey.
- 227 Brady CM & White JA (2013) Cowpea aphid (*Aphis craccivora*) associated with different host plants
228 has different facultative endosymbionts. *Ecol Entomol* **38**: 433-437.
- 229 Breeuwer JAJ (1997) *Wolbachia* and cytoplasmic incompatibility in the spider mites *Tetranychus*
230 *urticae* and *T. turkestanii*. *Heredity* **79**: 41-47.
- 231 Calatayud J, Horreo JL, Madrigal-Gonzalez J, Migeon A, Rodriguez MA, Magalhães S & Hortal J (2016)
232 Geography and major host evolutionary transitions shape the resource use of plant parasites.
233 *Proceedings of the National Academy of Sciences of the United States of America* **113**: 9840-
234 9845.
- 235 Caragata EP, Rances E, O'Neill SL & McGraw EA (2014) Competition for amino acids between
236 *Wolbachia* and the mosquito host, *Aedes aegypti*. *Microb Ecol* **67**: 205-218.
- 237 Chandler SM, Wilkinson TL & Douglas AE (2008) Impact of plant nutrients on the relationship
238 between a herbivorous insect and its symbiotic bacteria. *Proc R Soc B* **275**: 565-570.
- 239 Chaves S, Neto M & Tenreiro R (2009) Insect - symbiont systems: From complex relationships to
240 biotechnological applications. *Biotechnology journal* **4**: 1753-1765.
- 241 Chen DQ, Montllor CB & Purcell AH (2000) Fitness effects of two facultative endosymbiotic bacteria
242 on the pea aphid, *Acyrtosiphon pisum*, and the blue alfalfa aphid, *A. kondoi*. *Entomol Exp Appl*
243 **95**: 315-323.
- 244 Clark EL, Karley AJ & Hubbard SF (2010) Insect endosymbionts: manipulators of insect herbivore
245 trophic interactions? *Protoplasma* **244**: 25-51.

- 246 Crawley MJ (2007) *The R Book* John Wiley & Sons, Ltd, Chichester, England.
- 247 Dobson SL, Bourtzis K, Braig HR, Jones BF, Zhou WG, Rousset F & O'Neill SL (1999) *Wolbachia*
248 infections are distributed throughout insect somatic and germ line tissues. *Insect Biochem Mol*
249 *Biol* **29**: 153-160.
- 250 Dorfmeier EM, Vadopalas B, Frelier P & Friedman CS (2015) Temporal and spatial variability of native
251 geoduck (*Panopea generosa*) endosymbionts in the pacific northwest. *J Shellfish Res* **34**: 81-90.
- 252 Douglas AE (2009) The microbial dimension in insect nutritional ecology. *Functional Ecology* **23**: 38-
253 47.
- 254 Enigl M & Schausberger P (2007) Incidence of the endosymbionts *Wolbachia*, *Cardinium* and
255 *Spiroplasma* in phytoseiid mites and associated prey. *Exp Appl Acarol* **42**: 75-85.
- 256 Feldhaar H (2011) Bacterial symbionts as mediators of ecologically important traits of insect hosts.
257 *Ecol Entomol* **36**.
- 258 Ferrari J & Vavre F (2011) Bacterial symbionts in insects or the story of communities affecting
259 communities. *Philosophical Transactions of the Royal Society B-Biological Sciences* **366**: 1389-
260 1400.
- 261 Ferrari J, Scarborough CL & Godfray HCJ (2007) Genetic variation in the effect of a facultative
262 symbiont on host-plant use by pea aphids. *Oecologia* **153**: 323-329.
- 263 Ferrari J, Darby AC, Daniell TJ, Godfray HCJ & Douglas AE (2004) Linking the bacterial community in
264 pea aphids with host-plant use and natural enemy resistance. *Ecol Entomol* **29**: 60-65.
- 265 Fine PEM (1975) Vectors and vertical transmission - epidemiologic perspective. *Annals of the New*
266 *York Academy of Sciences* **266**: 173-194.
- 267 Frago E, Dicke M & Godfray HCJ (2012) Insect symbionts as hidden players in insect-plant
268 interactions. *Trends in Ecology & Evolution* **27**: 705-711.
- 269 Fry JD (1990) Trade-offs in fitness on different hosts - Evidence from a selection experiment with a
270 phytophagous mite. *Am Nat* **136**: 569-580.
- 271 Giron D, Dedeine F, Dubreuil G, Huguet E, Mouton L, Outreman Y, Vavre F & Simon JC (2017)
272 Influence of microbial symbionts on plant-insect interactions. *Insect-Plant Interactions in a Crop*
273 *Protection Perspective*, Vol. 81 (Sauvion N, Thierry D & Calatayud PA, eds.), p.^pp. 225-257.
- 274 Gotoh T, Noda H & Ito S (2007) *Cardinium* symbionts cause cytoplasmic incompatibility in spider
275 mites. *Heredity* **98**: 13-20.
- 276 Gotoh T, Sugawara J, Noda H & Kitashima Y (2007) *Wolbachia*-induced cytoplasmic incompatibility in
277 Japanese populations of *Tetranychus urticae* (Acari : Tetranychidae). *Exp Appl Acarol* **42**: 1-16.
- 278 Guidolin AS & Consoli FL (2017) Symbiont diversity of *Aphis* (Toxoptera) *citricidus* (Hemiptera:
279 Aphididae) as Influenced by host plants. *Microb Ecol* **73**: 201-210.

- 280 Hackett SC, Karley AJ & Bennett AE (2013) Unpredicted impacts of insect endosymbionts on
281 interactions between soil organisms, plants and aphids. *Proc R Soc B* **280**: 7.
- 282 Hamilton WD (1967) Extraordinary sex ratios. *Science* **156**: 477-488.
- 283 Hansen AK & Moran NA (2014) The impact of microbial symbionts on host plant utilization by
284 herbivorous insects. *Molecular Ecology* **23**: 1473-1496.
- 285 Hosokawa T, Kikuchi Y, Shimada M & Fukatsu T (2007) Obligate symbiont involved in pest status of
286 host insect. *Proc R Soc B* **274**: 1979-1984.
- 287 Jaenike J (2015) Heritable symbionts contribute to host plant adaptation. *Functional Ecology* **29**:
288 1371-1372.
- 289 Ji HL, Qi LD, Hong XY, Xie HF & Li YX (2015) Effects of host sex, plant species, and putative host
290 species on the prevalence of *Wolbachia* in natural populations of *Bemisia tabaci* (Hemiptera:
291 Aleyrodidae): A modified nested PCR study. *J Econ Entomol* **108**: 210-218.
- 292 Kaiser W, Huguet E, Casas J, Commin C & Giron D (2010) Plant green-island phenotype induced by
293 leaf-miners is mediated by bacterial symbionts. *Proc R Soc B* **277**: 2311-2319.
- 294 Karban R & Baldwin IT (2007) *Induced responses to herbivory*. University of Chicago Press, Chicago, IL,
295 USA.
- 296 Leonardo TE & Muir GT (2003) Facultative symbionts are associated with host plant specialization in
297 pea aphid populations. *Proc R Soc B* **270**: S209-S212.
- 298 Liu Y, Miao H & Hong XY (2006) Distribution of the endosymbiotic bacterium *Cardinium* in Chinese
299 populations of the carmine spider mite *Tetranychus cinnabarinus* (Acari : Tetranychidae). *J Appl*
300 *Entomol* **130**: 523-529.
- 301 Macke E, Magalhães S, Bach F & Olivieri I (2011) Experimental evolution of reduced sex ratio
302 adjustment under local mate competition. *Science* **334**: 1127-1129.
- 303 Macke E, Magalhães S, Khan HD-T, Luciano A, Frantz A, Facon B & Olivieri I (2011) Sex allocation in
304 haplodiploids is mediated by egg size: evidence in the spider mite *Tetranychus urticae* Koch. *Proc*
305 *R Soc B* **278**: 1054-1063.
- 306 Magalhães S, Blanchet E, Egas M & Olivieri I (2011) Environmental effects on the detection of
307 adaptation. *J Evol Biol* **24**: 2653-2662.
- 308 Magalhães S, Fayard J, Janssen A, Carbonell D & Olivieri I (2007) Adaptation in a spider mite
309 population after long-term evolution on a single host plant. *J Evol Biol* **20**: 2016-2027.
- 310 Magalhães S, Forbes MR, Skoracka A, Osakabe M, Chevillon C & McCoy KD (2007) Host race
311 formation in the Acari. *Exp Appl Acarol* **42**: 225-238.
- 312 Martinez-Diaz V, Latorre A & Gil R (2016) Seasonal changes in the endosymbiotic consortia of aphids
313 from the genus *Cinara*. *Microbes Environ* **31**: 137-144.

- 314 McLean AHC, van Asch M, Ferrari J & Godfray HCJ (2011) Effects of bacterial secondary symbionts on
315 host plant use in pea aphids. *Proc Roy Soc B-Biol Sci* **278**: 760-766.
- 316 Migeon A & Dorkeld F (2006-2017) Spider Mites Web: a comprehensive database for the
317 Tetranychidae. p.^pp.
- 318 O'Shea KL & Singh ND (2015) Tetracycline-exposed *Drosophila melanogaster* males produce fewer
319 offspring but a relative excess of sons. *Ecol Evol* **5**: 3130-3139.
- 320 Oliver KM & Martinez AJ (2014) How resident microbes modulate ecologically-important traits of
321 insects. *Current Opinion in Insect Science* **4**: 1-7.
- 322 Pan HP, Chu D, Liu BM, Xie W, Wang SL, Wu QJ, Xu BY & Zhang YJ (2013) Relative amount of
323 symbionts in insect hosts changes with host-plant adaptation and insecticide resistance. *Environ*
324 *Entomol* **42**: 74-78.
- 325 Pasch B, Bolker BM & Phelps SM (2013) Interspecific dominance via vocal interactions mediates
326 altitudinal zonation in Neotropical singing mice. *The American Naturalist* **182**: E161-E173.
- 327 Perrot-Minnot MJ, Cheval B, Migeon A & Navajas M (2002) Contrasting effects of *Wolbachia* on
328 cytoplasmic incompatibility and fecundity in the haplodiploid mite *Tetranychus urticae*. *J Evol*
329 *Biol* **15**: 808-817.
- 330 Ponton F, Wilson K, Holmes A, Raubenheimer D, Robinson KL & Simpson SJ (2015) Macronutrients
331 mediate the functional relationship between *Drosophila* and *Wolbachia*. *Proc R Soc B* **282**: 9.
- 332 Robert CAM, Frank DL, Leach KA, Turlings TCJ, Hibbard BE & Erb M (2013) Direct and indirect plant
333 defenses are not suppressed by endosymbionts of a specialist root herbivore. *J Chem Ecol* **39**:
334 507-515.
- 335 Ros VID & Breeuwer JAJ (2009) The effects of, and interactions between, *Cardinium* and *Wolbachia* in
336 the doubly infected spider mite *Bryobia sarothamni*. *Heredity* **102**: 413-422.
- 337 Schoonhoven LM, Van Loon JJ & Dicke M (2005) *Insect-plant biology*. Oxford University Press, Oxford,
338 UK.
- 339 Shikano I, Rosa C, Tan CW & Felton GW (2017) Tritrophic interactions: Microbe-mediated plant
340 effects on insect herbivores. *Annual Review of Phytopathology, Vol 55*, Vol. 55 (Leach JE &
341 Lindow SE, eds.), p.^pp. 313-331.
- 342 Simon JC, Carre S, Boutin M, Prunier-Leterme N, Sabater-Munoz B, Latorre A & Bournoville R (2003)
343 Host-based divergence in populations of the pea aphid: insights from nuclear markers and the
344 prevalence of facultative symbionts. *Proc R Soc B* **270**: 1703-1712.
- 345 Staudacher H, Schimmel BCJ, Lamers MM, Wybouw N, Groot AT & Kant MR (2017) Independent
346 effects of a herbivore's bacterial symbionts on its performance and induced plant defences. *Int J*
347 *Mol Sci* **18**: 182.

- 348 Su Q, Oliver KM, Xie W, Wu QJ, Wang SL & Zhang YJ (2015) The whitefly-associated facultative
349 symbiont *Hamiltonella defensa* suppresses induced plant defences in tomato. *Functional Ecology*
350 **29**: 1007-1018.
- 351 Su Q, Xie W, Wang SL, Wu QJ, Liu BM, Fang Y, Xu BY & Zhang YJ (2014) The endosymbiont
352 *Hamiltonella* increases the growth rate of its host *Bemisia tabaci* during periods of nutritional
353 stress. *PLoS One* **9**: 6.
- 354 Su Q, Oliver KM, Pan HP, *et al.* (2013) Facultative symbiont *Hamiltonella* confers benefits to *Bemisia*
355 *tabaci* (Hemiptera: Aleyrodidae), an invasive agricultural pest worldwide. *Environ Entomol* **42**:
356 1265-1271.
- 357 Sugio A, Dubreuil G, Giron D & Simon JC (2015) Plant-insect interactions under bacterial influence:
358 ecological implications and underlying mechanisms. *Journal of Experimental Botany* **66**: 467-478.
- 359 Suh E, Sim C, Park J-J & Cho K (2015) Inter-population variation for *Wolbachia* induced reproductive
360 incompatibility in the haplodiploid mite *Tetranychus urticae*. *Exp Appl Acarol* **65**: 55-71.
- 361 Sumi T, Miura K & Miyatake T (2017) *Wolbachia* density changes seasonally amongst populations of
362 the pale grass blue butterfly, *Zizeeria maha* (Lepidoptera: Lycaenidae). *PLoS One* **12**: 10.
- 363 Toju H & Fukatsu T (2011) Diversity and infection prevalence of endosymbionts in natural
364 populations of the chestnut weevil: relevance of local climate and host plants. *Mol Ecol* **20**: 853-
365 868.
- 366 Tsuchida T, Koga R & Fukatsu T (2004) Host plant specialization governed by facultative symbiont.
367 *Science* **303**: 1989-1989.
- 368 Tsuchida T, Koga R, Matsumoto S & Fukatsu T (2011) Interspecific symbiont transfection confers a
369 novel ecological trait to the recipient insect. *Biology Letters* **7**: 245-248.
- 370 Vala F, Breeuwer JAJ & Sabelis MW (2000) *Wolbachia*-induced 'hybrid breakdown' in the two-spotted
371 spider mite *Tetranychus urticae* Koch. *Proc R Soc B* **267**: 1931-1937.
- 372 Vala F, Weeks A, Claessen D, Breeuwer JAJ & Sabelis MW (2002) Within- and between-population
373 variation for *Wolbachia*-induced reproductive incompatibility in a haplodiploid mite. *Evolution*
374 **56**: 1331-1339.
- 375 Wagner SM, Martinez AJ, Ruan YM, Kim KL, Lenhart PA, Dehnel AC, Oliver KM & White JA (2015)
376 Facultative endosymbionts mediate dietary breadth in a polyphagous herbivore. *Functional*
377 *Ecology* **29**: 1402-1410.
- 378 Wilkinson TL, Koga R & Fukatsu T (2007) Role of host nutrition in symbiont regulation: Impact of
379 dietary nitrogen on proliferation of obligate and facultative bacterial endosymbionts of the pea
380 aphid *Acyrtosiphon pisum*. *Applied and Environmental Microbiology* **73**: 1362-1366.
- 381 Wilkinson TL, Adams D, Minto LB & Douglas AE (2001) The impact of host plant on the abundance
382 and function of symbiotic bacteria in an aphid. *Journal of Experimental Biology* **204**: 3027-3038.

- 383 Xie RR, Chen XL & Hong XY (2011) Variable fitness and reproductive effects of *Wolbachia* infection in
384 populations of the two-spotted spider mite *Tetranychus urticae* Koch in China. *Appl Entomol*
385 *Zool* **46**: 95-102.
- 386 Zélé F, Weill M & Magalhães S (2018) Identification of spider-mite species and their endosymbionts
387 using multiplex PCR. *Exp Appl Acarol* **74**: 123-138.
- 388 Zélé F, Santos I, Olivieri I, Weill M, Duron O & Magalhães S (2018) Endosymbiont diversity and
389 prevalence in herbivorous spider mite populations in South-Western Europe. *FEMS Microbiology*
390 *Ecology* **94**: fiy015.
- 391 Zhang YC, Cao WJ, Zhong LR, Godfray HCJ & Liu XD (2016) Host plant determines the population size
392 of an obligate symbiont (*Buchnera aphidicola*) in aphids. *Appl Environ Microbiol* **82**: 2336-2346.
- 393 Zhang YK, Chen YT, Yang K, Qiao GX & Hong XY (2016) Screening of spider mites (Acari:
394 Tetranychidae) for reproductive endosymbionts reveals links between co-infection and
395 evolutionary history. *Sci Rep* **6**: 27900.
- 396 Zhao DX, Zhang XF, Chen DS, Zhang YK & Hong XY (2013) *Wolbachia*-host Interactions: Host mating
397 patterns affect *Wolbachia* density dynamics. *PLoS One* **8**: e66373.
- 398 Zhu F, Poelman EH & Dicke M (2014) Insect herbivore- associated organisms affect plant responses to
399 herbivory. *New Phytologist* **204**: 315-321.
- 400 Zhu Y-X, Song Y-L, Zhang Y-K, Hoffmann AA, Zhou J-C, Sun J-T & Hong X-Y (2018) Incidence of
401 facultative bacterial endosymbionts in spider mites associated with local environment and host
402 plant. *Appl Environ Microbiol* **84**: e02546-02517.