

## RESEARCH ARTICLE

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

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Editor: Julie Olson

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## ABSTRACT

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

**Keywords:** Arthropod-plant-symbiont interaction; bacterial symbiont; fitness effects; host-plant use; mutualism; parasitism

## INTRODUCTION

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

Herbivores harbour a rich community of microorganisms, ranging from their gut microbiota and intracellular vertically transmitted endosymbionts to plant bacteria and viruses of which they serve as vectors, and there is growing evidence of the impact of such communities on herbivore performance on plants (Hosokawa *et al.* 2007; Clark, Karley and Hubbard 2010; Frago, Dicke and Godfray 2012; Hansen and Moran 2014; Oliver and Martinez 2014; Zhu, Poelman and Dicke 2014; Shikano *et al.* 2017). Obvious candidates to influence plant colonization by herbivorous arthropods are their heritable endosymbionts (Clark, Karley and Hubbard 2010; Feldhaar 2011; Ferrari and Vavre 2011;

Received: 21 May 2018; Accepted: 12 September 2018

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Frago, Dicke and Godfray 2012; Jaenike 2015). Due to their vertical mode of transmission, the fitness of such symbionts is tightly linked to that of their host and they are likely to benefit their host in order to increase their own transmission (Fine 1975). Indeed, endosymbionts have been shown to affect the host-plant range of herbivorous arthropods (Hosokawa et al. 2007; Tsuchida et al. 2011; Sugio et al. 2015; Wagner et al. 2015; Giron et al. 2017) or to increase performance on certain plant species (Wilkinson et al. 2001; Leonardo and Muiru 2003; Ferrari et al. 2004; Tsuchida, Koga and Fukatsu 2004; Ferrari, Scarborough and Godfray 2007; Hosokawa et al. 2007; Su et al. 2013; Su et al. 2015; Wagner et al. 2015), while decreasing performance on others (Chen, Montllor and Purcell 2000; Leonardo and Muiru 2003; Ferrari, Scarborough and Godfray 2007; Chandler, Wilkinson and Douglas 2008; McLean et al. 2011; Wagner et al. 2015). In some cases, increased host performance is due to endosymbionts acting as nutritional mutualists, directly supplying their arthropod hosts with nutrients or enzymes that are missing in their plant diet (reviewed by Chaves, Neto and Tenreiro 2009; Douglas 2009), or displaying compensatory effects during periods of nutritional deficiency (Su et al. 2014). Endosymbionts may also enable arthropods to manipulate phytohormonal profiles (Kaiser et al. 2010; Body et al. 2013), resource allocation (Hackett, Karley and Bennett 2013), and anti-herbivory defences (Barr et al. 2010; Su et al. 2015). Conversely, symbiont-mediated decreased host performance on particular plants might be due to the nutrient profile (e.g. specific amino acids and nitrogen content) of these plants, which promotes deleterious symbiont traits and disturbs the host control over bacterial abundance (Wilkinson, Koga and Fukatsu 2007; Chandler, Wilkinson and Douglas 2008). However, as these endosymbionts are vertically transmitted, their effects cannot easily be disentangled from that of the insect species or biotype/host race (e.g. Chen, Montllor and Purcell 2000; Leonardo and Muiru 2003; Simon et al. 2003; Ferrari et al. 2004; Chandler, Wilkinson and Douglas 2008). Artificial curing and/or transinfection should be performed to determine unambiguously the respective roles of symbionts and host genotypes, as well as their interactions (e.g. Leonardo 2004; Tsuchida, Koga and Fukatsu 2004; Ferrari, Scarborough and Godfray 2007; Hosokawa et al. 2007; McLean et al. 2011; Tsuchida et al. 2011; Su et al. 2013; Su et al. 2015; Wagner et al. 2015).

Such variable effects of endosymbionts on herbivore-plant interactions may contribute to variation in the abundance and distribution of herbivorous arthropods (Douglas 2009; Hansen and Moran 2014). Conversely, as symbiont-herbivore interactions may differ according to the host plant, and nutrition of the herbivore host can affect the within-host symbiont density (Wilkinson et al. 2001; Wilkinson, Koga and Fukatsu 2007; Chandler, Wilkinson and Douglas 2008; Zhang et al. 2016a), the host plant can also affect endosymbiont distribution in the field (Leonardo and Muiru 2003; Simon et al. 2003; Ferrari et al. 2004; Tsuchida, Koga and Fukatsu 2004; Chandler, Wilkinson and Douglas 2008; Ahmed et al. 2010; Brady and White 2013; Pan et al. 2013; Guidolin and Consoli 2017). However, most studies addressing these questions have been conducted on sap-feeding insects, and whether symbiont prevalence and their effects on their herbivorous host vary with the host plant remains unstudied in many other systems.

The two-spotted spider mite, *Tetranychus urticae*, a cosmopolitan agricultural and horticultural pest that feeds on cell content, is a highly polyphagous arthropod, feeding on more than 1100 plant species (Migeon and Dorkeld 2017). This generalist herbivore rapidly adapts to novel host plants (Fry

1990; Agrawal 2000; Magalhães et al. 2007a), sometimes forming host races (Magalhães et al. 2007b), and may harbour several endosymbiotic bacteria with variable prevalence among populations (Enigl and Schausberger 2007; Gotoh, Noda and Ito 2007a; Staudacher et al. 2017). Among them, *Wolbachia* is the most prevalent (Liu, Miao and Hong 2006; Gotoh et al. 2007b; Ros and Breeuwer 2009; Zhang et al. 2016b; Zélé et al. 2018a) and induces variable fitness effects in spider mites. For instance, it can decrease (Perrot-Minnot et al. 2002; Suh et al. 2015), not affect (Breeuwer 1997; Vala, Breeuwer and Sabelis 2000; Perrot-Minnot et al. 2002; Vala et al. 2002; Gotoh et al. 2007b), or increase (Vala et al. 2002; Gotoh et al. 2007b; Xie, Chen and Hong 2011) their fecundity. Given these variable effects, it is as yet unclear whether *Wolbachia* will facilitate or hamper host-plant colonization by spider mites.

Here, we measured the prevalence of the most prevalent endosymbionts of *T. urticae* on five different host plants in Portugal. The most common bacterial reproductive manipulators in arthropods are *Wolbachia*, *Cardinium*, *Rickettsia*, *Arsenophonus* and *Spiroplasma* (Duron et al. 2008; Weinert et al. 2015). Moreover, a previous study conducted on spider mites collected in the same geographical area and on similar host plants revealed that several mite populations carried *Wolbachia*, *Cardinium* and *Rickettsia*, but none carried *Arsenophonus* or *Spiroplasma* (Zélé et al. 2018a). Their presence is thus likely to be anecdotic and unlikely to vary according to the host plants. Hence, we focused on the former three endosymbionts in the current study. Subsequently, we explored whether the effect of *Wolbachia* on the performance of *T. urticae* hinges on the plant that is being colonized. Finally, we discuss the importance of possible mechanisms leading to our results as well as the potential adaptive significance of the presence of *Wolbachia* for plant colonization by *T. urticae*.

## MATERIALS AND METHODS

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

To determine whether the prevalence of *Wolbachia*, *Cardinium* and *Rickettsia* in natural *T. urticae* populations varied with the host plant, spider mites (red form only) were collected on bean (*Phaseolus vulgaris*, Fabaceae), eggplant (*Solanum melongena*, Solenaceae), purple morning glory (*Ipomoea purpurea*, Convolvulaceae), zucchini (*Cucurbita pepo*, Cucurbitaceae) and tomato (*Solanum lycopersicum*, Solenaceae) across 12 different locations in the region of Lisbon (Portugal) in June and July 2015 (Fig. 1, Table 1). These plants were selected because they are part of the natural host range of *T. urticae* and belong to different families. Sampling sites consisted of open fields, greenhouses or organic vegetable gardens, while being insecticide/pesticide-free to avoid this potential confounding effect. Infested leaves were detached and placed in closed plastic boxes that were brought to the laboratory. On the same day, 50 adult females were haphazardly picked from each population and their species determined at the individual level based on morphological characteristics under a stereoscope. These females were then placed on 2 cm<sup>2</sup> leaf discs of the same plant species on which they were found and allowed to lay eggs for four days. Subsequently, 20 of these females were randomly selected and individually tested for the presence of *Wolbachia*, *Cardinium* and *Rickettsia* on entire mites without DNA extraction by multiplex PCR using genus-specific primers as described in Zélé, Weill and Magalhães (2018b). Subsequently, for each population, the DNA of a pool consisting of one daughter from each of these females was

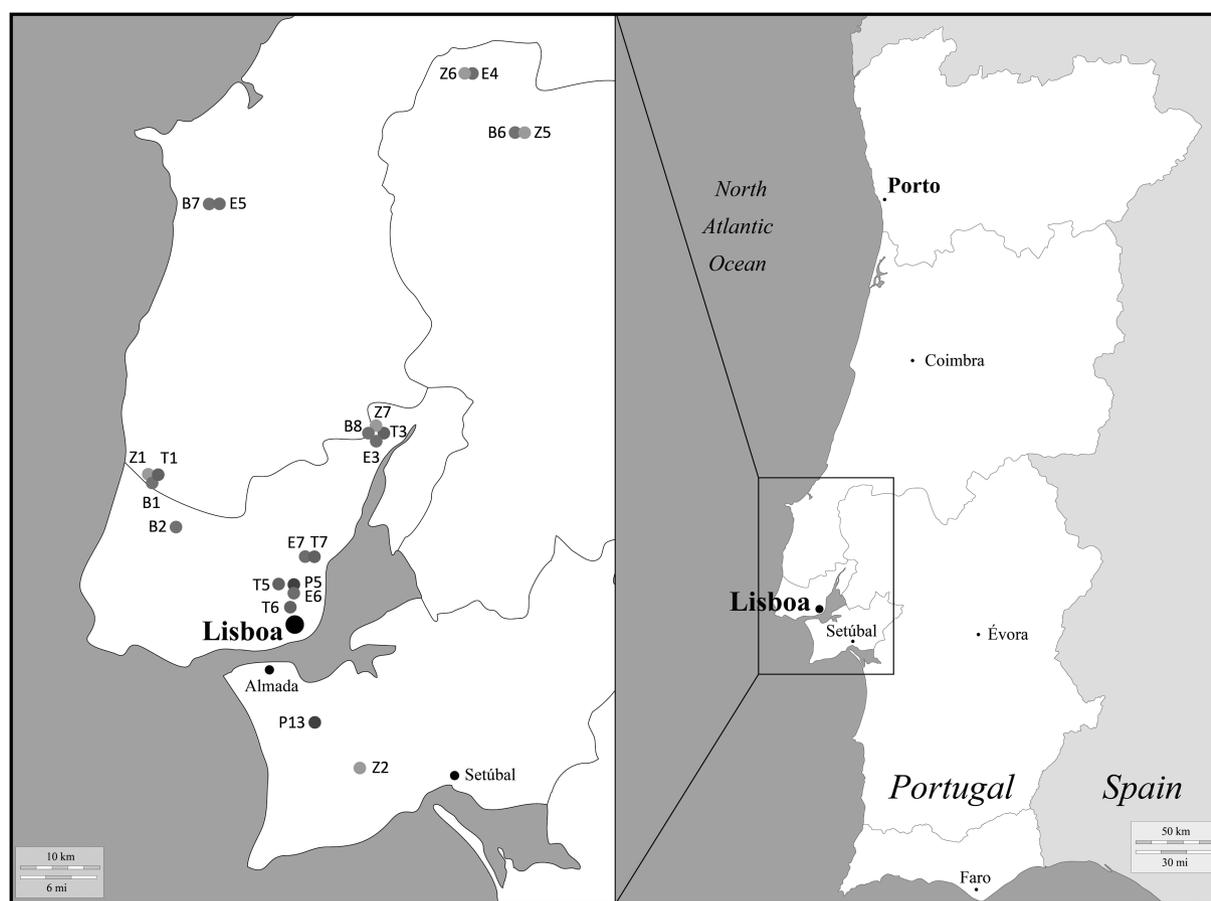


Figure 1. Map showing sampling sites (grey dots) where *T. urticae* spider mites were collected on five different host plants in the region of Lisbon in Portugal. Bean: B1, B2, B6, B7 and B8; eggplant: E3, E4, E5, E6 and E7; purple morning glory: P5 and P13; tomato: T1, T3, T5, T6 and T7; zucchini: Z1, Z2, Z5, Z6 and Z7.

Table 1. *Tetranychus urticae* populations collected on five different host plants across 12 different locations in the region of Lisbon in 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 morning glory ( <i>Ipomea 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

extracted, then a PCR-based method to identify the mite species was performed by multiplex PCR as described in Z  l  , Weill and Magalh  es (2018b). If a pool could not be assigned unambiguously to *T. urticae* (Table S1), all data concerning endosymbiont prevalence were discarded. This process was repeated until obtaining endosymbiont prevalence data for five populations per plant, except for morning glory, for which we could obtain only two populations of *T. urticae* due to the weak infestation rate of this plant by this spider-mite species, despite a large sampling effort (Table S1).

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

#### Spider-mite populations, tetracycline treatment and population rearing

The spider-mite population used belongs to the red form of *T. urticae* and was originally collected on *Datura* plants at Aldeia da Mata Pequena, Portugal, in November 2013 and kept in a mass-rearing environment (>5000 individuals) on bean plants (var. *Enana*), under controlled conditions ( $25 \pm 2^\circ\text{C}$ , 16/8 h L/D) since then. This population, hereafter called Wi, was found uninfected by *Rickettsia*, *Spiroplasma* or *Arsenophonus* but fully infected by *Wolbachia* in the field (Z  l   et al. 2018a). Although this population was also slightly infected by *Cardinium* (Z  l   et al. 2018a), this endosymbiont has been rapidly lost following laboratory rearing (unpublished data). To obtain a *Wolbachia*-uninfected (Wu) population with a similar genetic background, roughly three months after collection 30 adult females of the Wi population were placed in Petri dishes containing bean-leaf fragments placed on cotton with a tetracycline solution (0.1%, w/v). This treatment was applied continuously for three successive generations (Breeuwer 1997), then the population was maintained in a mass-rearing environment without antibiotics for c.a. 12 generations before the experiment to avoid (or limit) potential side-effects of the antibiotic treatment (e.g. O'Shea and Singh 2015) and allow mites to recover potential loss of gut microbiota. Before use, up to 20 individual females and pools of 100 females were checked by PCR to confirm the absence and presence of *Wolbachia* infection in Wu and Wi populations, respectively.

#### Performance of *Wolbachia*-infected and uninfected females on different host plants

To determine the effect of *Wolbachia* infection and of the host plant, as well as their possible interaction, on the performance of *T. urticae*, we measured life-history traits of individuals from Wi or Wu populations when placed on the same plant species as those from which mites were collected in the field study (bean: var. *Enana*, eggplant: var. *Larga Morada*, morning glory: var. *Vigorous*, zucchini: var. *Bellezza Negra*, and tomato: var. *Moneymaker*). To control for age, 100 females were allowed to lay eggs for three days on detached bean leaves placed on water-soaked cotton, and the adult females resulting from those eggs were used in the experiments. Fifty mated females (10–13 days old) were haphazardly picked from either Wi or Wu cohorts and placed individually on a 2 cm<sup>2</sup> leaf disc from one of the five different host plants. The replicates were distributed along five consecutive days (10 replicates per treatment per day). Females that were alive after three days were transferred to new leaf discs where they could lay eggs for another three days. Their survival (S) and the proportion of drowned females (PD) in the water-soaked cotton (i.e. accidental death of females trying to escape the leaf discs) were followed daily during six days. The fecundity of each female was measured

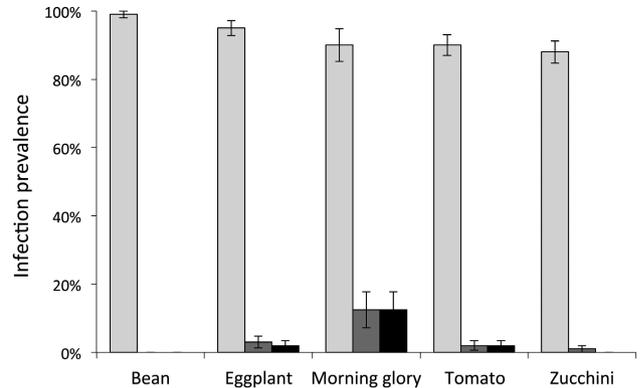


Figure 2. Endosymbiont prevalence in *T. urticae* females collected on different host plants. Bars represent the mean ( $\pm$  s.e.) infection frequencies by *Wolbachia* (light grey), *Cardinium* (dark grey), and *Rickettsia* (black) for several spider-mite populations collected on bean (n = 5), eggplant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

at days 3 and 6 and the average female daily fecundity was estimated taking into account their daily mortality ( $DF = \text{total number of eggs laid per female} / \text{number of days the female was alive}$ ). The number of unhatched eggs was counted five days later (i.e. days 8 and 11, respectively) to estimate the egg-hatching rate ( $HR = \text{hatched eggs} / \text{total number of eggs}$ ). Adult offspring ( $F_1$  females +  $F_1$  males) were counted after six additional days (i.e. days 14 and 17, respectively) and used to estimate juvenile mortality ( $JM = [\text{total number of eggs} - \text{number of unhatched eggs} - \text{number of } F_1 \text{ adults}] / \text{total number of eggs}$ ),  $F_1$  sex ratio ( $SR = \text{number of } F_1 \text{ males} / \text{number of } F_1 \text{ adults}$ ) and the number of viable offspring ( $VO = \text{total number of adult offspring per female per treatment observed at the end of the experiment on each plant}$ ). The entire experiment was repeated three months later (hereafter called blocks 1 and 2) except for replicates involving tomato plants. Indeed, given a very high PD ( $88 \pm 3.3\%$ ; data not shown) and because the surviving females laid on average less than one egg per day ( $0.32 \pm 0.05$ ; data not shown) on this plant, subsequent traits could not be measured and we decided to exclude it from this experiment. The entire experiment was conducted in a growth chamber under standard conditions ( $25 \pm 2^\circ\text{C}$ , 60% RH, 16/8 h L/D).

#### Statistical analyses

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

To analyse the effect of host plants on endosymbiont prevalence in field-collected mites, the prevalence of *Wolbachia* (model 1), *Cardinium* (model 2) and *Rickettsia* (model 3) were fit as binary response variables, the host plant on which mites were collected as fixed explanatory variable, and the location as random explanatory variable. Because of quasi-complete separation of some of our data, which usually causes problems with estimated regression coefficients, analyses were conducted using a mixed model *glmer* procedure (*blme* package) with a binomial error distribution (Pasch, Bolker and Phelps 2013). When the variable 'plant' was significant, a stepwise *a posteriori* procedure (Crawley 2007) to determine differences between plants was carried out by aggregating factor levels together and by testing the fit

of the simplified model using a likelihood ratio test (LRT), which is approximately distributed as a  $\chi^2$  distribution (Bolker 2008). Because none of the mites collected in this study were singly infected by *Cardinium* or *Rickettsia*, and the prevalence of each type of coinfection was very low (cf. Results), we did not have enough statistical power to study the effect of the host plants on the prevalence of coinfections.

To analyse the effect of *Wolbachia*, the host plant, and their interaction on the performance of spider mites, the infection status of females (i.e. Wi: infected or Wu: uninfected) and the host plants tested were fit as fixed explanatory variables, whereas block and day were fit as random explanatory variables (day nested within block). Survival data (S; model 4) were analysed using a Cox proportional hazards mixed-effect model (coxme, kinship package). Hazard ratios were obtained from this model as an estimate of the difference in mortality rate (Crawley 2007) between our control (Wi population on bean) and each of the other factor levels. PD, a binary response variable (drowned or not; model 5), was analysed using a generalised linear mixed model with a binomial distribution (glmer, lme4 package). DF, a continuous response variable (model 6) was analysed using linear mixed-effect models (lmer, nlme package). The other proportion variables, HR, SR and JM (models 7, 8 and 9, respectively), were computed using the function cbind (e.g. number of hatched eggs, males or dead juveniles vs. number of unhatched eggs, females or alive juveniles, respectively). However, due to the low DF of spider mites, these variables, as well as VO (model 10), were greatly overdispersed. One way of handling this overdispersion is by using quasi-binomial or negative binomial pseudo distributions (Crawley 2007), but, to our knowledge, this is not possible within the usual mixed model glmer procedure. Thus, we used instead a mixed model glmmadmb procedure (glmmADMB package) with zero-inflated binomial error distribution for HR, SR and JM, and zero-inflated negative binomial error distribution for VO. When a statistically significant interaction between the variables 'Wolbachia' (Wi or Wu) and 'plant' was found, the effect of *Wolbachia* was analysed for each plant separately. When only the variable 'plant' was significant, *a posteriori* contrasts between host plants were performed as before.

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

## RESULTS

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

The prevalence of *Wolbachia* was overall high ( $92.7 \pm 1.2\%$ ), while that of *Cardinium* ( $2.5 \pm 0.7\%$ ) and *Rickettsia* ( $2.0 \pm 0.7\%$ ) were low (Fig. 2). In addition, while  $89.3 \pm 1.5\%$  of the mites collected in this study were infected by *Wolbachia* only, none were infected by *Cardinium* or by *Rickettsia* only;  $1.4 \pm 0.6\%$  were coinfecting by *Wolbachia* and *Cardinium*,  $0.9 \pm 0.5\%$  were coinfecting by *Wolbachia* and *Rickettsia*, and  $1.14 \pm 0.5\%$  were infected by these three endosymbionts (see Fig. S1 for infection statuses at the individual level). The prevalence of *Wolbachia* and of *Rickettsia* were significantly affected by the plant on which *T. urticae* females

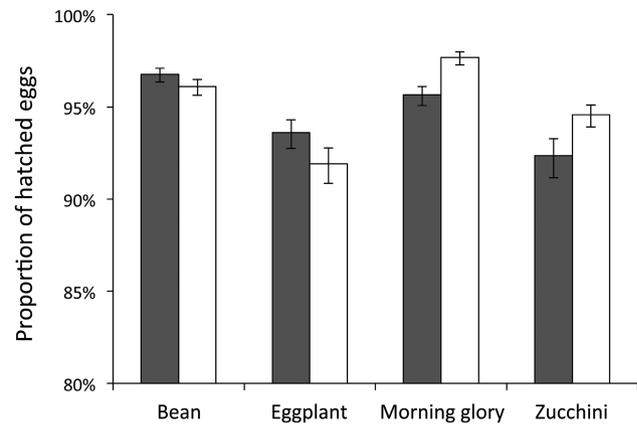


Figure 3. Effects of different host plants and of *Wolbachia* on the HR of *T. urticae*. Bars represent the mean ( $\pm$  s.e.) proportions of hatched eggs laid by *Wolbachia*-infected (Wi; grey bars) and uninfected (Wu; white bars) females on different host plants. Estimates were obtained from the GLMM 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 calculated from the upper and lower confidence intervals given by the model.

were collected ( $\chi^2_4 = 14.79$ ,  $P = 0.005$ ; model 1, and  $\chi^2_4 = 12.71$ ,  $P = 0.01$ ; model 3, respectively; Fig. 2). Contrast analyses revealed that the prevalence of *Wolbachia* was higher on bean and eggplant ( $97.0 \pm 1.7\%$ ; contrast bean vs. eggplant:  $\chi^2_1 = 0.51$ ,  $P = 0.47$ ) than on the three other plants ( $89.2 \pm 2.0\%$ ; contrast morning glory vs. tomato vs. zucchini:  $\chi^2_2 = 0.39$ ,  $P = 0.82$ ; contrast between the two groups of plants:  $\chi^2_1 = 14.34$ ,  $P = 0.0002$ ), and that of *Rickettsia* differed only on morning glory ( $12.5 \pm 5.3\%$ ) compared with all other plants ( $1.0 \pm 0.5\%$ ; contrast bean vs. eggplant vs. tomato vs. zucchini:  $\chi^2_3 = 2.95$ ,  $P = 0.40$ ; contrast between this group of plants and morning glory:  $\chi^2_1 = 9.76$ ,  $P = 0.002$ ). Finally, the prevalence of *Cardinium*, similarly to that of *Rickettsia*, tended to be higher on morning glory ( $12.5 \pm 5.3\%$ ) compared with the other plants ( $1.5 \pm 0.6\%$ ), but this effect was not statistically significant ( $\chi^2_4 = 1.61$ ,  $P = 0.81$ ; model 2).

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

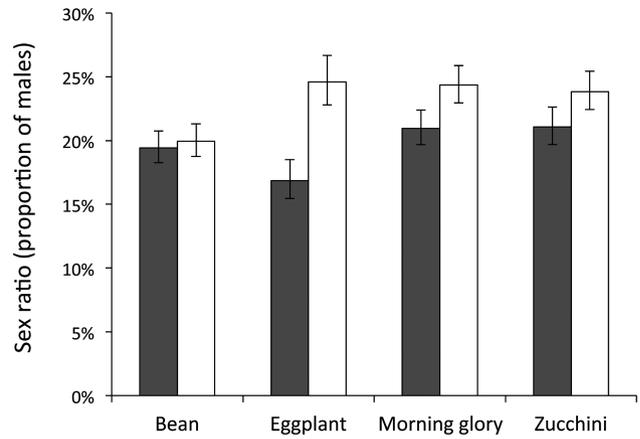
Overall, there was no significant effect of *Wolbachia* infection ( $\chi^2_1 = 0.73$ ,  $P = 0.39$ ), of host plants ( $\chi^2_3 = 6.84$ ,  $P = 0.07$ ), or of their interaction ( $\chi^2_3 = 3.34$ ,  $P = 0.34$ ; model 4; Table 1 and Fig. S2) on survival (S) over the first six days of the experiment.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

Daily fecundity (DF) was significantly affected by host plants ( $\chi^2_3 = 129.33$ ,  $P < 0.0001$ ), but not 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). Contrast analyses revealed that DF was similar on morning glory and zucchini ( $3.37 \pm 0.11$  eggs per day; contrast morning glory vs. zucchini:  $\chi^2_1 = 1.03$ ,  $P = 0.31$ ), but higher on bean ( $4.60 \pm 0.19$  eggs per day; contrast morning glory-zucchini vs. bean:  $\chi^2_1 = 40.14$ ,  $P < 0.0001$ ), and lower on eggplant ( $2.10 \pm 0.13$ ; Contrast eggplant vs. morning glory-zucchini:  $\chi^2_1 = 42.77$ ,  $P < 0.0001$ ).

The effect of *Wolbachia* on egg-hatching rate (HR) depended on the host plant tested (*Wolbachia*-plant interaction:  $F_{3,697} = 5.47$ ,  $P = 0.001$ ; model 7; Table 1 and Fig. 3). Indeed, *Wolbachia* reduced HR on morning glory ( $F_{1,172} = 10.05$ ,  $P = 0.002$ )

**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, morning glory, zucchini and eggplant) are represented for each one of the performance traits measured in this study. For HR, JM and SR, 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	Significance of explanatory variables and their interaction										
	Bean		Morning glory		Zucchini		Eggplant				
	Wi	Wu	Wi	Wu	Wi	Wu	Wi	Wu			
Log Hazard Ratio for survival (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	Plant * <i>Wolbachia</i> $\chi^2_3 = 3.34$ , P = 0.34	Plant $\chi^2_3 = 6.84$ , P = 0.08	<i>Wolbachia</i> $\chi^2_1 = 0.88$ , P = 0.35
Proportion of drowned mites (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
Egg-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	-	-
Number of 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 4.** Effects of different host plants and of *Wolbachia* on the offspring SR 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 calculated from the upper and lower confidence intervals given by the model.

and on zucchini ( $F_{1,177} = 19.74$ ,  $P < 0.0001$ ), but had no effect on bean and eggplant ( $F_{1,181} = 1.42$ ,  $P = 0.24$  and  $F_{1,158} = 1.56$ ,  $P = 0.21$ , respectively).

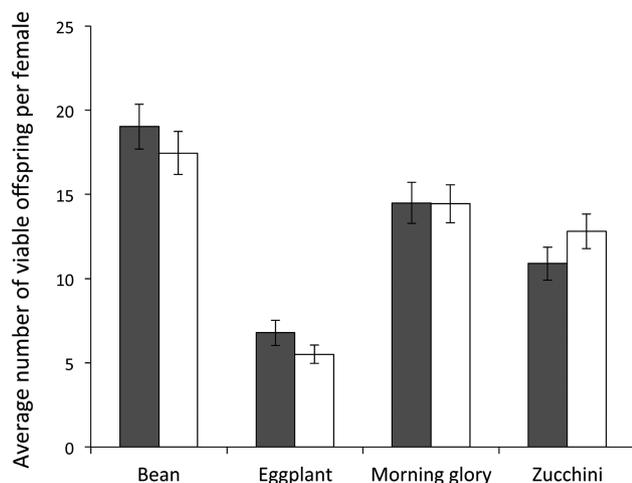
Juvenile mortality (JM) was not significantly affected by *Wolbachia* ( $F_{1,692} = 0.01$ ,  $P = 0.92$ ; model 8; Table 2), and this was consistent across all host plants (*Wolbachia*-plant interaction:  $F_{3,689} = 1.85$ ,  $P = 0.14$ ; model 8). However, host plant was a significant predictor of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

*Wolbachia* infection affected differently the sex ratio (SR) produced on the different plants (*Wolbachia*-plant interaction:  $F_{3,681} = 2.48$ ,  $P = 0.04$ ; model 9; Table 2 and Fig. 4). Indeed, *Wolbachia* decreased the proportion of males on morning glory ( $F_{1,168} = 5.51$ ,  $P = 0.02$ ) and on eggplant ( $F_{1,153} = 8.54$ ,  $P = 0.004$ ). On bean and zucchini, however, SR did not differ significantly between Wi and Wu mites ( $F_{1,179} = 5.51$ ,  $P = 0.54$  and  $F_{1,1726} = 2.28$ ,  $P = 0.13$ , respectively).

Although we found a significant *Wolbachia*-plant interaction on HR and SR, *Wolbachia* did not significantly influence the average number of viable offspring (VO;  $F_{1,789} = 0.78$ ,  $P = 0.38$ ), and this effect was independent of the host plant (*Wolbachia*-plant interaction:  $F_{3,786} = 0.70$ ,  $P = 0.55$ ; model 10; Table 2 and Fig. 5). Nonetheless, host plant significantly explained this trait ( $F_{3,790} = 48.72$ ,  $P < 0.0001$ ; model 10), with the highest values on bean, intermediate values on morning glory (contrast *morning glory vs. bean*:  $\chi^2_1 = 4.82$ ,  $P = 0.03$ ) and zucchini (contrast *zucchini vs. morning glory*:  $\chi^2_1 = 5.12$ ,  $P = 0.02$ ), and the lowest values on eggplant (contrast *eggplant vs. zucchini*:  $\chi^2_1 = 44$ ,  $P < 0.0001$ ).

## DISCUSSION

The prevalence of *Wolbachia* and *Rickettsia* in *T. urticae* females found in this study was relatively similar to that of an earlier



**Figure 5.** Effects of different host plants and of *Wolbachia* on the average VO 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.

study in the same geographical area (Zélé et al. 2018a). However, the prevalence of *Cardinium* was about five times lower in the current study than in the former one ( $2.5 \pm 0.7\%$  vs.  $13.6 \pm 2.9\%$ , respectively). As the populations were sampled on comparable host plants in this previous study (except for one population collected on *Datura stramonium*, the others were collected on bean, eggplant, tomato and zucchini), the discrepancy observed for the overall *Cardinium* prevalence between the two studies may be attributed to the time of collection. Indeed, mites were collected between September and December in the previous study and in June–July in the current one. Several studies have shown that the sampling period might affect endosymbiont prevalence and/or density in host populations (Toju and Fukatsu 2011; Dorfmeier et al. 2015; Martinez-Diaz, Latorre and Gil 2016; Sumi, Miura and Miyatake 2017), and the increase of *Cardinium* prevalence during summer might be compatible with the hypothesis of an accumulation of this symbiont throughout the season via horizontal transfers (Zélé et al. 2018a). In addition, the temperature strongly differed between the two sampling periods in the region of Lisbon, with, on average,  $21.6 \pm 0.3^\circ\text{C}$  in June–July 2015, and  $16.0 \pm 0.5^\circ\text{C}$  in September to December 2013 (data obtained from <https://www.tempo.pt/lisboa-sactual.htm>). Temperature variations may affect endosymbiont within-host densities, fitness components and maternal transmission depending on the symbiont species or strain, and many heritable bacteria have been shown to affect their host thermal tolerance, both to elevated temperature and to frost (reviewed by Corbin et al. 2017). As a result, several studies showed geographical variation in symbiont (including *Cardinium*) prevalence that may be attributable to temperature differences (e.g. Tsuchida et al. 2002; Toju and Fukatsu 2011; Morag et al. 2012; Kriesner et al. 2016).

We found that *Wolbachia* prevalence was overall high, but significantly higher on bean and eggplant than on the other plants. Whereas some earlier studies have shown that *Wolbachia* prevalence in herbivores varies according to the host plant (Ahmed et al. 2010; Toju and Fukatsu 2011; Guidolin and Consoli 2017), including a recent study conducted in the spider mite, *Tetranychus truncatus* (Zhu et al. 2018), others show no difference (Ji et al. 2015). Unfortunately, the scarcity of studies, along with the fact that they were mostly done in other systems, hampers a meaningful comparison among studies. In addition, it

is extremely difficult to sample spider-mite populations on all the plants tested within the same locality (Table S1). Consequently, this implies an important sampling effort to obtain only a very reduced number of populations that fit the criteria for such studies. For instance, despite a large sampling effort across 21 localities and 12 host plant species, Zhu et al. (2018) could only assess the effect of three common host plants (soybean, corn and tomato) from three different locations. Still, they did find a significant effect of the host plant on the prevalence of *Wolbachia*, which was about 30% higher on tomato than on corn. In our study, the amplitude of the observed effects is much lower, possibly due to a threshold effect, since the prevalence of *Wolbachia* that we observed in *T. urticae* is overall much higher than that observed in *T. truncatus* by Zhu et al. (2018). Clearly, differences in *Wolbachia* prevalence were not associated with plant phylogenetic distance, as it differed between the solanaceous plants used (eggplant and tomato). Moreover, the effect of an endosymbiont on arthropod–plant interactions may depend on both the genotype (or species) of symbiont (Leonardo and Muir 2003) and arthropod host (Chen, Montllor and Purcell 2000; Ferrari, Scarborough and Godfray 2007; McLean et al. 2011; Wagner et al. 2015), and/or their interaction (Ferrari, Scarborough and Godfray 2007). More studies on plant-dependent symbiont prevalence may thus shed light on the potential factors underlying the pattern observed and on the ecological meaning of such effects.

Here, we hypothesise that the variation in endosymbiont prevalence according to the host plant is, at least partially, due to plant-specific effects of these symbionts on spider-mite performance. Although we did find some variation of *Rickettsia* and *Cardinium* prevalences according to the host plant, they were very low, so we opted for addressing this issue using *Wolbachia* only. Overall, we found a strong effect of the host plant on spider-mite performance, with the highest values observed on bean. This is not surprising, given that bean was the rearing environment of the population used, and is generally a host plant of high quality for spider mites (e.g. Magalhães et al. 2011). Conversely, the lowest performances were found on Solanaceous plants (eggplant and tomato), being so low on tomato that we excluded these data from further analyses (cf. Material and Methods). In the other four plants, we found that some traits (S, PD, DF and JM) were not affected by *Wolbachia* whereas others (HR and SR) were affected in a plant-specific manner.

The plant-specific effects of *Wolbachia*, although of low amplitude, could be explained by several non-exclusive mechanisms. First, *Wolbachia* may impose a nutritional burden on its hosts, sequestering and using vital host nutrients for its own survival (Chandler, Wilkinson and Douglas 2008; Caragata et al. 2014; Ponton et al. 2015), and this may vary with the host plant. Indeed, the nutrient composition of plant material is often poor or unbalanced for herbivores (Schoonhoven et al. 2005; Karban and Baldwin 2007), and nutrient-deficient diet may increase the competition for resources between hosts and symbionts. In turn, this may lead to a decreased ability of infected spider mites to allocate enough nutrients to ensure egg viability on plants of low quality. Increased host–symbiont competition on such low-quality plants could also lead to a biased sex ratio towards males because females are produced from bigger eggs than males in *T. urticae* (Macke et al. 2011). In addition, the slight *Wolbachia*-induced female-biased SR observed on morning glory could be a consequence of the lower HR observed on this plant, as larger eggs are generally more likely to hatch (Macke et al. 2011). However, if this hypothesis would hold true, one would expect a

stronger cost of *Wolbachia* in spider mites on plants of lower quality for mites, which was not the case.

Second, *Wolbachia* may directly influence the metabolism of some plants, which in turn can affect the biology of its herbivorous hosts. For instance, *Wolbachia* possess a key gene involved in cytokinin biosynthesis in their genomes (Kaiser et al. 2010) and might be responsible for an increased level of cytokinins in infested plants (Kaiser et al. 2010; Body et al. 2013). As *Wolbachia* are present in high density in the gnathosoma of spider mites (Zhao et al. 2013), and cytokinins can be responsible for a sex ratio shift towards females in insects (Adam et al. 2017), further research could assess whether sex ratio shifts observed on morning glory and eggplant can be mediated by increased cytokinin levels induced by *Wolbachia*.

Third, *Wolbachia* may interfere with the mites' response towards plant defences. Indeed, endosymbionts found in herbivores, including *Wolbachia*, may directly manipulate the plant defences to benefit their host (Frago, Dicke and Godfray 2012; Hansen and Moran 2014; Zhu, Poelman and Dicke 2014; Sugio et al. 2015; Giron et al. 2017; Shikano et al. 2017). For instance, downregulation of several defence genes of maize by the western corn rootworm *Diabrotica virgifera* has been shown to be mediated by *Wolbachia* (Barr et al. 2010, but see Robert et al. 2013). Conversely, endosymbionts may have a detrimental effect on their host by increasing the level of induced plant defences (see Staudacher et al. 2017; although no causal link could be established between the changes in plant defences and mite performance in this study). Thus, whether the presence of *Wolbachia* in *T. urticae* can upregulate the defences of zucchini and morning glory, and whether this could explain the reduced egg hatchability observed here, remains to be tested.

From the *Wolbachia* perspective, a reduced egg hatchability is costly, as it will reduce its later transmission. Also, *Wolbachia* being maternally transmitted, it should always benefit from a more female-biased sex ratio (note that cytoplasmic incompatibility does not explain our results as this phenotype involves crosses between infected males and uninfected females; Gotoh et al. 2007b; Xie, Chen and Hong 2011; Suh et al. 2015). Despite the weak plant-specific effects of *Wolbachia* on mite performance, and that they do not affect VO, these effects may affect *Wolbachia* prevalence on different host plants. Here, *Wolbachia* decreases egg hatchability on zucchini, and occurs at low prevalence on this plant. Conversely, *Wolbachia* increases the proportion of females produced on eggplant, where it occurs at high prevalence. On morning glory, *Wolbachia* increases the proportion of daughters, decreases egg hatchability and occurs at intermediate prevalence. Finally, *Wolbachia* is not costly on bean, the plant where it occurs at very high prevalence. Hence, the observed balance of costs/benefits of *Wolbachia* on different host plants seems to be correlated to its prevalence.

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

among populations, and (iii) whether endosymbionts affect the host range of herbivores.

## SUPPLEMENTARY DATA

Supplementary data are available at [FEMSEC](https://femsec.oup.com/femsec/article-abstract/94/12/fy187/5097780) online.

## ACKNOWLEDGMENTS

We thank Murat Bakırdöven, Jacques Denoyelle, Leonor Rodrigues, and Inês Santos for their help in spider-mite collection. We also thank IS for the maintenance of the plants and mite populations, JD for designing the map, and Nelson Martins, Jordi Moya Laraño and Susana Verala for advice in statistical analysis.

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

## FUNDING

This work was funded by an FCT-ANR project (FCT-ANR//BIA-EVF/0013/2012) to SM and Isabelle Olivieri and by a FCT-Tubitak project (FCT-TUBITAK/0001/2014) to SM and Ibrahim Cakmak. FZ and DG were funded through FCT Post-Doc (SFRH/BPD/125 020/2016) and PhD (PD/BD/114 010/2015) fellowships, respectively. Funding agencies did not participate in the design or analysis of experiments.

**Conflicts of interest.** None declared.

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