

# Population-specific effect of *Wolbachia* on the cost of fungal infection in spider mites

Flore Zélé<sup>1</sup>, Mustafa Altıntaş<sup>2</sup>, Inês Santos<sup>1</sup>, Ibrahim Cakmak<sup>2</sup>, Sara Magalhães<sup>1</sup>

# Corresponding author: fezele@fc.ul.pt

<sup>1</sup> Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências da Universidade de Lisboa, Edifício C2, 3º Piso Campo Grande, 1749-016 Lisboa, Portugal

<sup>2</sup> Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, 09010 Aydin, Turkey

## ABSTRACT

Many studies have revealed the ability of the endosymbiotic bacteria *Wolbachia* to protect its arthropod hosts against diverse pathogens. However, as *Wolbachia* may also increase the susceptibility of its host to infection, predicting the outcome of a particular *Wolbachia*-host-pathogen interaction remains elusive. Yet, understanding such interactions is crucial for disease and pest control strategies. *Tetranychus urticae* spider mites are herbivorous crop pests, causing severe damage on numerous economically important crops. Due to the rapid evolution of pesticide resistance, biological control strategies using generalist entomopathogenic are being developed. However, although spider mites are infected with various *Wolbachia* strains worldwide, whether this endosymbiont protects them from fungi is as yet unknown. Here, we compared the survival of two populations, treated with antibiotics or harbouring different *Wolbachia* strains, after exposure to the fungal biocontrol agents *Metarhizium brunneum* and *Beauveria bassiana*. In one population, *Wolbachia* affected survival in absence of fungi but not in their presence, whereas in the other population *Wolbachia* increased the mortality induced by *B. bassiana*. To control for potential effects of the bacterial community of spider mites, we also compared the susceptibility of two populations naturally uninfected by *Wolbachia*, treated with antibiotics or not. The antibiotic treatment increased the susceptibility of spider mites to *M. brunneum* in one naturally *Wolbachia*-uninfected population, but it had no effect in the other treatments. These results highlight the complexity of within-host pathogens interactions, and the importance of considering the whole bacterial community of arthropods when assessing the effect of *Wolbachia* in a particular system.

## KEY-WORDS

*Tetranychus urticae*; entomopathogenic fungi; parasite-induced mortality; endosymbionts; bacterial community; antibiotic treatment.

## INTRODUCTION

The maternally-inherited bacterium *Wolbachia*, first identified in *Culex pipiens* mosquitoes (Hertig and Wolbach 1924), is to date the best studied and probably the most common endosymbiont of arthropods. Indeed, it is estimated to infect up to 52% of arthropod species (Weinert et al. 2015), a success mainly attributed to its ability to induce various types of reproductive manipulation in hosts to increase the reproductive success of infected females, thereby increasing its own transmission (Werren et al. 2008). Such manipulations include induction of parthenogenetic reproduction of females, feminization of males (i.e. genotypic males develop as phenotypic females), male killing (i.e. infected males die during embryogenesis or late larval instars to the advantage of surviving infected female siblings) and cytoplasmic incompatibility (CI; i.e. modification of male sperm so that females cannot produce offspring unless they are infected with the same strain of *Wolbachia* as their mates). Such ability of *Wolbachia* to rapidly spread within and among host populations (Engelstadter and Hurst 2009), has thus raised growing interests in using it in biocontrol programmes (Bourtzis 2008; Bourtzis et al. 2014; Islam 2007).

Possible *Wolbachia*-based biocontrol strategies include the use of *Wolbachia* as microbial biocontrol agent, for instance to enhance productivity of natural predators and parasites such as parasitoids (e.g. Grenier et al. 1998; Stouthamer 1993; Stouthamer et al. 1999); as a potential gene-drive vehicle for population replacement strategies through cytoplasmic drive (i.e. provides a mechanism for the autonomous spread of desired genes into targeted populations; (Dobson 2003; Dobson et al. 2002; Durvasula et al. 1997; Sinkins and Godfray 2004; Turelli and Hoffmann 1999); or for sterile insect techniques (SIT) to suppress target pest populations by repeated sweeps with infected individuals (Calvitti et al. 2015; Laven 1967; Zhang et al. 2015a; Zhong and Li 2014). Subsequently, the recent discovery of the ability of *Wolbachia* to protect its hosts against a wide array of pathogens, including viruses, protozoan parasites, fungi, or pathogenic bacteria (reviewed in Cook and McGraw 2010) has provided new avenues for the control of vector-borne diseases (reviewed by Iturbe-Ormaetxe et al. 2011; Vavre and Charlat 2012). For instance, deliberate introductions of *Wolbachia* into *Aedes aegypti* mosquito populations are currently being successfully undertaken in several regions worldwide to control dengue virus (e.g. Hoffmann et al. 2014; 2011; Nguyen et al. 2015). However, such ability of *Wolbachia* to interfere with diverse host pathogens may have undesirable effects on biocontrol strategies if, for instance, the bacterium interferes with parasitic biocontrol agents, a possibility that has been largely overlooked. Conversely, natural *Wolbachia* infections in several host species may also increase host susceptibility to parasite infection (e.g. Fytrou et al. 2006; Graham et al. 2012; reviewed in Hughes et al. 2014), raising the possibility that *Wolbachia* could actually facilitate the action of other biocontrol agents. Hence, assessing the effect of *Wolbachia* infection on the efficiency of different strains and/or species of parasitic biocontrol agents should be a prerequisite for the development of efficient and long-lasting control strategies

(Zindel et al. 2011).

Spider mite of the genus *Tetranychus* (Acari: Tetranychidae) are ubiquitous major crop pests of c.a. 1100 plant species belonging to more than 140 different plant families (Migeon and Dorkeld 2006-2017). Due to their short generation time and high fecundity, spider mites rapidly develop resistance to pesticides (Van Leeuwen et al. 2010), which led to the development of alternative control strategies such as the use of essential oils or natural enemies (e.g. predators, entomopathogenic bacteria and fungi; (Attia et al. 2013). Among them, entomopathogenic fungi have been successfully used in Integrated Pest Management (IPM) programs, and commercial formulations are currently available to farmers in most parts of the world (Skinner et al. 2014). In particular, fungi such as *Beauveria bassiana*, *Metarhizium* spp., *Isaria* spp. and *Lecanicillium* spp., have been identified as good candidates for efficient spider mite control (e.g. Bugeme et al. 2008; Chandler et al. 2005; Maniania et al. 2008; Shi et al. 2008; Shin et al. 2017), and their compatibility with other control methods, such as predatory mites (e.g. Dogan et al. 2017; Seiedy 2014; Seiedy et al. 2012; Seiedy et al. 2013; Ullah and Lim 2017; Vergel et al. 2011; Wu et al. 2016) or pesticides (e.g. Irigaray et al. 2003; Klingen and Westrum 2007; Shi et al. 2005) is widely studied. Curiously, however, the interaction between entomopathogenic fungi and bacterial endosymbionts of spider mites has, to our knowledge, never been investigated. This is at odd with the fact that, on the one hand, natural populations of spider mites often carry several maternally-inherited endosymbiotic bacteria with variable prevalence, *Wolbachia* being the most prevalent (Gotoh et al. 2007; Liu et al. 2006; Zélé et al. 2018b; Zhang et al. 2016); and, on the other hand, that *Wolbachia* has been shown to protect *Drosophila melanogaster* hosts against the mortality induced by *B. bassiana* (Panteleev et al. 2007, although no such effect has been found in *D. simulans*; Fytrou et al. 2006).

To examine the effect of the interaction between *Wolbachia* and fungal infection on spider mite survival, we carried out a fully factorial experiment using two naturally *Wolbachia*-infected and two naturally *Wolbachia*-uninfected spider mite populations belonging to the green and the red form of *T. urticae*, and treated or not with antibiotics. We used two generalist entomopathogenic fungi species, *Beauveria bassiana* and *Metarhizium brunneum*, as *Beauveria* and *Metarhizium* spp. are among the most used fungi in commercial production (Vega et al. 2009), with wide geographical and host ranges (Greif and Currah 2007; Gurlek et al. 2018; Meyling and Eilenberg 2007; Rehner 2005; Roberts and Leger 2004). The specific aims of this work were to determine: (i) whether infection with a natural *Wolbachia* strain protects spider mites against fungus-induced mortality, (ii) whether this effect depends on the *Wolbachia* strain and/or other bacteria present in spider mites, and (iii) whether it depends on the fungus species. We then discuss possible mechanisms leading to our results, the importance of considering the whole bacterial community of arthropods when assessing the effect of *Wolbachia* in a particular system, as well as the potential consequences of the presence of *Wolbachia* for the success of spider mite control strategies using entomopathogenic fungi.

## MATERIALS AND METHODS

### *Spider mite populations and rearing*

Four populations of *Tetranychus urticae* were used in this study, two (AlRo and AMP) belonging to the red form (also referred to as *T. cinnabarinus* by some authors; e.g. Li et al. 2009; Shi et al. 2005; Shi and Feng 2004), and two (DEF and TOM) belonging to the green form. These populations have been collected in the Iberian Peninsula from 2010 to 2017, on different plant species. All the information concerning these populations is summarized in Table S1. After collection, these populations were reared in the laboratory under standard conditions (24±2°C, 60% RH, 16/8h L/D) at high numbers (c.a. 500-1000 females per population) in insect-proof cages containing bean plants (*Phaseolus vulgaris*, cv. Contender seedlings obtained from Germisem, Oliveira do Hospital, Portugal).

### *Endosymbiont infection*

Upon collection from the field, the populations AMP (red form) and TOM (green form) were naturally infected by two different strains of *Wolbachia*, although these strains are very closely related, having 1 SNP difference on the sequences of both the *fbpA* and *coxA* genes in the multilocus sequence typing system developed by Baldo et al. (2006) for *Wolbachia*. The population AMP is infected by the *Wolbachia* strain ST481 (isolate 'Turt\_B\_wUrtAmp'; id: 1858 in the PubMLST *Wolbachia* MLST database available at <http://www.pubmlst.org/wolbachia/>); and the population TOM is infected by the *Wolbachia* strain ST280 (isolate 'Turt\_B\_wUrtTom'; id: 1857). The two other populations, AlRo (red form) and DEF (green form), were naturally uninfected by *Wolbachia* and none of the populations used in this study carried other maternally-inherited bacterial endosymbionts (i.e. *Cardinium*, *Rickettsia*, *Spiroplasma*, *Arsenophonus*) at the time of the experiment, as confirmed by PCR using the methods described in Zélé et al. (2018b; 2018c).

### *Antibiotic treatments*

Roughly 2 months (i.e. 4 spider mite generations) before the onset of the experiment, a rifampicin solution (0.05%, w/v) was used to treat mites (n=70 adult females initially) from each population for one generation (Gotoh et al. 2005). This allowed to obtain *Wolbachia*-uninfected AMP and TOM populations as well as controls for the antibiotic treatment for the naturally uninfected populations AlRo and DEF. During the treatment, mites were maintained in petri dishes containing bean leaf fragments placed on cotton with the antibiotic solution. After one generation, 100 adult mated daughters from each treated population were transferred in insect-proof cages containing bean plants, in the same laboratory conditions than the untreated populations, and these new populations were allowed to grow for 3 successive generations to avoid potential side effects of

antibiotic treatment (Ballard and Melvin 2007; O'Shea and Singh 2015; Zeh et al. 2012). One generation before the onset of the experiment pools of 100 females were taken from each treated population and checked by PCR to confirm that they were uninfected by *Wolbachia* as described in Zélé et al. (2018c).

#### *Entomopathogenic fungi strains and preparation of inoculum*

We used the strains V275 (= Met52, F52, BIPESCO 5) of *Metarhizium brunneum* and UPH-1103 of *Beauveria bassiana*, obtained from Swansea University (UK) and from Siedlce University (Poland), respectively, as they were previously shown to have the potential to suppress *T. urticae* populations (Dogan et al. 2017). The procedures used for fungal growth, inoculum preparation and spider mite infection are similar to that described in Dogan et al. (2017). Briefly, the two fungi were grown on Sabouraud Dextrose Agar (SDA) medium at 25 °C for 2 weeks. Conidia were harvested from sporulating cultures with the aid of a spatula, washed with sterile distilled water and filtered through 4 layers of gauze to remove any hyphae.

#### *Spider mite infection and survival*

The experiment was conducted in a growth chamber under standard conditions (25 ± 2°C, 80% RH, 16/8 h L/D). Roughly 2 weeks prior to the experiment, ca. 100 females were collected from each mass culture and allowed to lay eggs during 4 days on detached bean leaves placed on water-soaked cotton. One day prior to the onset of the experiment, 20 young adult mated females (hence with similar age) were randomly collected from these cohorts and placed on a 9cm<sup>2</sup> bean leaf disc placed on wet cotton (to ensure the leaf remained hydrated) with the abaxial (underside) surface facing upwards. On the first day of the experiment, the surface of the leaf discs was sprayed using a hand sprayer with 2.5 ml of a spore suspension of *M. brunneum* or *B. bassiana* in 0.03% (v/v) aqueous Tween 20 at 1 × 10<sup>7</sup> conidia/ml, or, as control, with 0.3% aqueous Tween 20 only. Twelve replicates per treatment and per population were performed within 2 experimental blocks of one day difference (6 replicates of each treatment per block).

#### *Statistical analysis*

Analyses were carried out using the R statistical package (version 3.5.3). The general procedure for building the statistical models was as follows. Survival data were analysed using Cox proportional hazards mixed-effect models (coxme, kinship package). Spider-mite populations (AIrO, AMP, DEF and TOM), antibiotic treatment (treated with rifampicin or not), and infection treatment (sprayed with BB: *Beauveria bassiana*, with MB: *Metarhizium brunneum*, or with Tween 20 only as control) were fitted in as fixed explanatory variables, whereas discs nested within population and block were fitted as random explanatory variables. To explore significant three-way interaction between the three fixed variables, each population was then analyzed

separately with the same model structure, except that the variable population was removed from the model. Hazard ratios (HR) were then obtained from these models as an estimate of the difference between the rates of dying (i.e. the instantaneous rate of change in the log number of survivors per unit time; (Crawley 2007) between the untreated controls and the BB or MB treatments for each population.

Because the timing of infection is an important parameter for the fitness of parasites, an additional early measurement of survival, the proportion of dead mites at 3 days post infection (dpi), was obtained from Kaplan–Maier estimates of the survival distribution for each disc. We chose this timing as it is close to (or already above) the median survival upon infection in most of the populations tested, and hence corresponds to a threshold time-point to unravel important differences between treatments. The numbers of dead and alive mites at 3 dpi were computed using the function `cbind` and analysed with a mixed model `glmmadmb` procedure (`glmmADMB` package) with a negative binomial error distribution (family “`nbinom1`” with a  $\emptyset\mu$  variance; chosen based on the Akaike information criterium) to correct for overdispersed errors. As above, populations, infection and antibiotic treatment were fitted in as fixed explanatory variables, whereas block was fitted as random explanatory variable.

Maximal models, including all higher-order interactions, were simplified by sequentially eliminating non-significant terms and interactions to establish a minimal model (Crawley, 2007). The significance of the explanatory variables was established using chi-squared tests (Bolker 2008). The significant chi-squared values given in the text are for the minimal model, whereas non-significant values correspond to those obtained before deletion of the variable from the model.

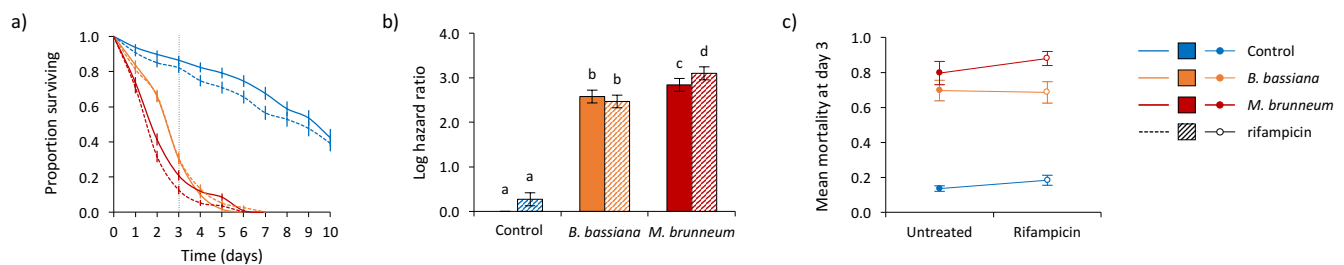
To further explore significant interactions between infection and antibiotic treatment effects on both HR and mortality at 3 dpi, the two factors were concatenated to fit a single fixed factor containing all treatments levels in the models. Multiple comparisons were then performed using General Linear Hypotheses (`glht`, package `multcomp`) with Bonferroni corrections.

## RESULTS

Overall, we found a significant three-way interaction between the effect of the infection by fungi (control females sprayed with Tween 20 only, BB: females sprayed with *B. bassiana*, or MB: females sprayed with *M. brunneum*), the rifampicin treatment, and the population tested, both on the overall survival of spider mites ( $X^2_6=36.16$ ,  $p<0.0001$ ), and on their mortality at 3 days post-infection (dpi;  $X^2_6=18.33$ ,  $p=0.005$ ). Indeed, the separate analyses of each population revealed that the survival of females from different populations, naturally infected or uninfected by *Wolbachia*, was not evenly affected by fungal infection and by rifampicin treatment.

### Effect of fungal infection and of antibiotic treatment in the naturally *Wolbachia*-uninfected population AIrO

In the population AIrO, we found a significant interaction between infection and rifampicin treatment ( $\chi^2_2=9.53$ ,  $p=0.009$ ; Fig. 1a), which was due to a stronger mortality induced by *M. brunneum* in rifampicin-treated mites than in untreated mites ( $z=2.80$ ,  $p=0.05$ ), while *B. bassiana* induced the same mortality in both rifampicin-treated and untreated mites ( $z=-1.20$ ,  $p=1.00$  Fig. 1b; Table S2). In both cases, however, *M. brunneum* induced a stronger mortality than *B. bassiana* (MB vs. BB:  $z=6.72$ ,  $p<0.0001$  and  $z=2.81$ ,  $p=0.05$  in rifampicin-treated and untreated mites, respectively). At 3 dpi, however, no significant interaction between infection and rifampicin treatment ( $\chi^2_2=1.78$ ,  $p=0.41$ ; Fig. 1c) and no effect of the antibiotics ( $\chi^2_1=0.90$ ,  $p=0.34$ ) were found. Only the effect of infection by the two fungi species was found to severely increase the mortality of both rifampicin-treated and untreated mites at this early age of infection ( $\chi^2_2=135.68$ ,  $p<0.00014$ ; see also Table S3 for multiple comparisons).

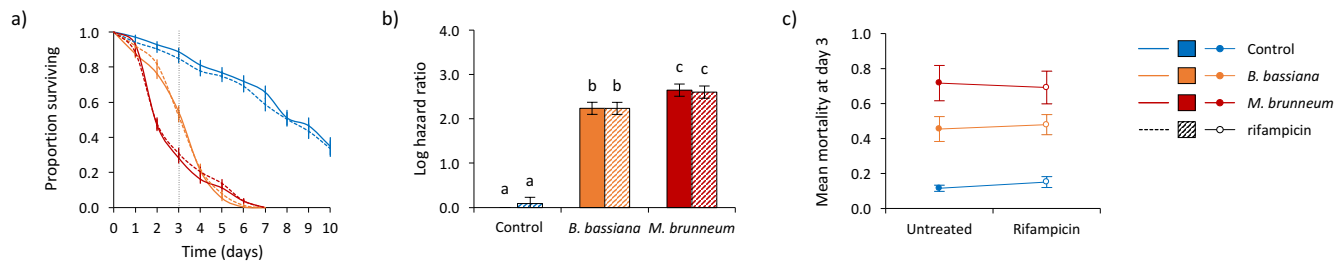


**Figure 1.** Survival curves (a), relative mortality (b), and survival at 3 dpi (c) of spider mites from the naturally *Wolbachia*-uninfected population AIrO. Adult females were treated (dashed lines, dashed bars and empty circles) or not (solid lines, filled bars and circles) with rifampicin, and sprayed with *B. bassiana* (orange), *M. brunneum* (red), or Tween 20 only as control (blue).

### Effect of fungal infection and of antibiotic treatment in the naturally *Wolbachia*-uninfected population DEF

In the population DEF, we did not find a significant interaction between infection and rifampicin treatment ( $\chi^2_2=0.65$ ,  $p=0.72$ ; Fig. 2a), neither a significant effect of rifampicin treatment ( $\chi^2_1=0.003$ ,  $p=0.96$ ), but only a significant effect of fungi infection ( $\chi^2_2=879.17$ ,  $p<0.0001$ ). Indeed, both fungi induced the same mortality in both rifampicin-treated and -untreated mites, with an overall stronger effect of *M. brunneum* than of *B. bassiana* (Fig. 2b; see Table S4 for the results of all comparisons). Similarly, at 3 dpi, no significant interaction between infection and rifampicin treatment ( $\chi^2_2=0.40$ ,  $p=0.82$ ; Fig. 2c), neither a significant effect of rifampicin treatment ( $\chi^2_1=0.14$ ,  $p=0.71$ ) was found. As for the population AIrO, only fungi infection affected the spider-mite survival ( $\chi^2_2=64.89$ ,  $p<0.0001$ ; see Table S5 for the results of all comparisons).

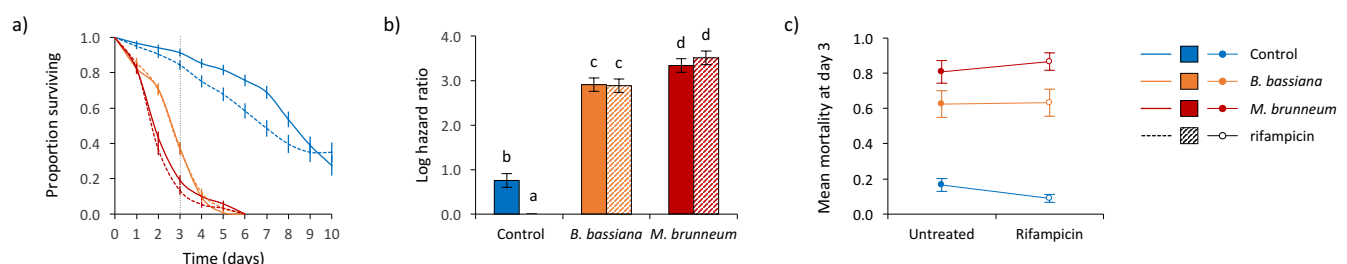




**Figure 2.** Survival curves (a), relative mortality (b), and survival at 3 dpi (c) of spider mites from the naturally *Wolbachia*-uninfected population DEF. Adult females were treated (dashed lines, dashed bars and empty circles) or not (solid lines, filled bars and circles) with rifampicin, and sprayed with *B. bassiana* (orange), *M. brunneum* (red), or Tween 20 only as control (blue).

### Effect of fungal infection and of antibiotic treatment in the naturally *Wolbachia*-infected population AMP

In the population AMP, we found a significant interaction between infection and rifampicin treatment ( $\chi^2=26.61$ ,  $p<0.0001$ ; Fig. 3a), which was due to a lower survival of *Wolbachia*-infected controls compared to uninfected controls ( $z=-4.92$ ,  $p<0.0001$ ), while *Wolbachia*-infected and uninfected mites had the same survival upon infection with both fungi species (for *B. bassiana*:  $z=-0.26$ ,  $p=1.00$ ; for *M. brunneum*:  $z=1.88$ ,  $p=0.55$ ; Fig. 3b and Table S6). Relative to their respective control, both fungi induced higher mortality in rifampicin-treated mites (HR=17.87 and HR=33.39, for BB and MB, respectively) than in *Wolbachia*-infected mites (HR=8.60 and HR=13.21, respectively). A significant interaction between infection and rifampicin treatment was also found at 3 dpi ( $\chi^2=6.5$ ,  $p=0.04$ ; Fig. 3c). However, this interaction was relatively weak at this time-point, and could not be explained by multiple comparisons between factor levels. Indeed, no differences were found between *Wolbachia*-infected and rifampicin-treated mites when sprayed with Tween 20 only, with *B. bassiana*, or with *M. brunneum* (see Table S7 for the results of all comparisons).

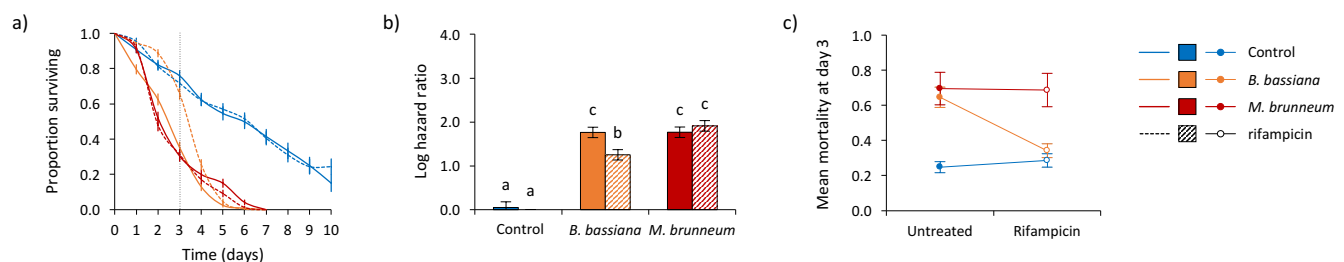


**Figure 3.** Survival curves (a), relative mortality (b), and survival at 3 dpi (c) of spider mites from the naturally *Wolbachia*-infected population AMP. Adult females were treated (dashed lines, dashed bars and empty circles) or not (solid lines, filled bars and circles) with rifampicin, and sprayed with *B. bassiana* (orange), *M. brunneum* (red), or Tween 20 only as control (blue).



### Effect of fungal infection and of antibiotic treatment in the naturally *Wolbachia*-infected population TOM

In the population TOM, we also found a significant interaction between infection and rifampicin treatment ( $X^2_2=26.00$ ,  $p<0.0001$ ; Fig. 4a). In this population, the effect of *B. bassiana* was weaker in rifampicin-treated mites (HR=3.49) than *Wolbachia*-infected mites (HR=5.53;  $z=-5.54$ ,  $p<0.0001$ ; Fig. 4b and Table S8), while *M. brunneum* had the same effect in both rifampicin-treated and untreated mites (HR=6.75 and HR=5.56, respectively;  $z=1.58$ ,  $p=1.00$ ). Moreover, whereas both fungi had the same effect on non-treated mites (MB vs. BB:  $z=0.05$ ,  $p=1.00$ ), *B. bassiana* decreased less the survival of rifampicin-treated mites than *M. brunneum* (MB vs. BB:  $z=-6.88$ ,  $p<0.0001$ ). This effect was even stronger at 3 dpi (interaction between infection and rifampicin:  $X^2_2=15.44$ ,  $p<0.001$ ). At this time-point, *B. bassiana* induced the same mortality than *M. brunneum* in *Wolbachia*-infected mites (BB vs. Control:  $z=6.24$ ,  $p<0.0001$ ), but did not affect significantly the survival of rifampicin-treated mites (BB vs. Control:  $z=0.75$ ,  $p=1.00$ ; Fig. 4c).



**Figure 4.** Survival curves (a), relative mortality (b), and survival at 3 dpi (c) of spider mites from the naturally *Wolbachia*-infected population TOM. Adult females were treated (dashed lines, dashed bars and empty circles) or not (solid lines, filled bars and circles) with rifampicin, and sprayed with *B. bassiana* (orange), *M. brunneum* (red), or Tween 20 only as control (blue).

## DISCUSSION

In this study, we found variable effects of infection by *B. bassiana* and *M. brunneum* following antibiotic treatment, depending on the spider mite populations and on whether they were naturally infected by *Wolbachia* or not. Indeed, the mortality induced by both fungi did not differ between *Wolbachia*-infected and uninfected mites in the population AMP, despite *Wolbachia* infection being costly in absence of fungal infection. Similarly, the mortality induced by *M. brunneum* was not affected by *Wolbachia* infection in the population TOM, but that induced by *B. bassiana* increased in presence of *Wolbachia*. These results suggest that *Wolbachia* may either buffer, or conversely increase, the effect of fungal infection depending on the fungi species, the *Wolbachia* strain and/or the host genetic background. Moreover, in absence of natural *Wolbachia*

infection, we found a relatively small effect of the antibiotic treatment on the susceptibility of the mites to infection. Indeed, the antibiotic treatment had no effect on the outcome of infection by fungi, with the exception of a higher mortality in antibiotic-treated mites from the population AI<sub>Ro</sub> when infected with *M. brunneum*. Still, this effect, although significant, is of relatively low amplitude and in the opposite direction than that observed in the *Wolbachia*-infected population TOM following *B. bassiana* infection. This suggests that the effect of *Wolbachia* in the population TOM may not be explained by an alteration of the whole bacterial community in mites following antibiotic treatment. However, because the effect of fungal infection and antibiotic treatment vary between populations independently of the presence of *Wolbachia*, we draw caution on the generalization of such results.

In different arthropod host species, *Wolbachia* may either protect (e.g. Braquart-Varnier et al. 2015; Cattel et al. 2016; Hughes et al. 2011; Kambris et al. 2009; Moreira et al. 2009; Panteleev et al. 2007; Teixeira et al. 2008; Ye et al. 2013), have no effect (e.g. Tortosa et al. 2008; Wong et al. 2011; Zouache et al. 2012), or even increase the susceptibility (e.g. Fytrou et al. 2006; Graham et al. 2012; reviewed in Hughes et al. 2014) of its arthropod hosts to infection depending on the pathogens tested, the *Wolbachia* strain (Chrostek et al. 2013; Martinez et al. 2017; Osborne et al. 2009), but also on the host genetic background, although to a lesser extent (Martinez et al. 2017). In several of these studies the effect of *Wolbachia* on host susceptibility to infection by other pathogens has been assessed following artificial *Wolbachia* infection (e.g. Frentiu et al. 2014; Joubert et al. 2016; Moreira et al. 2009; Walker et al. 2011; Yeap et al. 2011), which prevents a direct alteration of the host bacterial community but may not accurately reflect the effect of natural *Wolbachia* infections. Indeed, novel *Wolbachia* host associations are often costly for hosts (e.g. McGraw et al. 2002), mainly due to the activation of the host immune system following *Wolbachia* infection, which in turn prevents subsequent infections by other pathogens (reviewed in Zug and Hammerstein 2015). Conversely, the effect of natural *Wolbachia* infections on host susceptibility to infection by other pathogens is usually assessed by using antibiotic treatments. However, antibiotics do not affect *Wolbachia* only, but also the entire bacterial community in hosts (e.g. Lehman et al. 2009; Zhu et al. 2018; Zouache et al. 2009), which raises the necessity to assess the potential effect of the antibiotic treatment *per se*.

In *T. truncatus* spider mites, Zhu et al. (2018) showed that an antibiotic treatment (tetracycline hydrochloride during three generations) strongly affects the composition of the bacterial community even after more than 20 generations without antibiotics. In particular, these authors showed that bacteria from different families strongly increased in proportion in tetracycline-treated mites in absence of the Anaplasmataceae (which includes *Wolbachia*). Hence, the lower mortality observed for antibiotic-treated mites following infection by *B. bassiana* in the naturally *Wolbachia*-infected population TOM cannot be unambiguously attributable to *Wolbachia* only. This result could be explained, for instance, by *Wolbachia* outcompeting bacteria that participate to the host homeostasis and immunity (reviewed in Selosse et al. 2014; Shapira 2016;

Vavre and Kremer 2014; Weiss and Aksoy 2011), thereby increasing the success of *B. bassiana* infection (i.e., indirect facilitation; Zélé et al. 2018a). In contrast, in the *Wolbachia*-uninfected population AlRo, antibiotic-treated mites have a higher mortality than untreated mites when infected with *M. brunneum*. One possible explanation is that, in absence of natural *Wolbachia* infection, the antibiotic treatment affected differently the bacterial community, potentially eliminating bacteria that interfere with *M. brunneum*.

The apparent facilitation of *B. bassiana* by *Wolbachia* in the TOM population may also be due to *Wolbachia* interacting directly with the host immune system. Indeed, *Wolbachia* has been shown to downregulate autophagy-associated genes in naturally infected hosts, possibly as an immune evasion strategy (Chevalier et al. 2012; Kremer et al. 2009). Under such scenario, the elimination of *Wolbachia* with antibiotics may result in overall higher autophagic processes in the host, to which *B. bassiana* could be susceptible. Moreover, in diverse native hosts, including *T. urticae*, *Wolbachia* also plays a role in redox homeostasis (Zhang et al. 2015b; reviewed by Zug and Hammerstein 2015). The elimination of *Wolbachia* with antibiotics in coevolved *T. urticae* hosts may thus potentially lead to a disruption of redox homeostasis and higher production of reactive oxygen species (ROS), which are involved in host immunity (e.g. encapsulation, melanisation; (reviewed in Zug and Hammerstein 2015), thereby increasing host resistance to infection. However, all these different scenarios would only explain our results if such mechanisms affect differently the two fungus species and are specific to the *Wolbachia* strain and/or the host population.

As stated above, the host genetic background also plays a major role in determining host susceptibility to infection. First, as recently shown in several spider mite species (Zélé et al. 2019), not all populations (independently of their status of infection by *Wolbachia*) are equally affected by the infection by the two fungi (e.g. the mortality induced by both fungi is stronger in the population DEF than in the population TOM). Second, host susceptibility to infection may also result from G x G interactions with their endosymbionts, as shown, for instance, for *Wolbachia*-mediated protection against viruses across *Drosophila* species (Martinez et al. 2017). Here, the different effects of *Wolbachia* observed in the populations TOM and AMP cannot be unambiguously attributed to the *Wolbachia* strain only, but likely also result from their interaction with the host genetic background. Hence, although further investigations on the respective role of the *Wolbachia* strain and of the host genome, as well as on the composition of the bacterial communities in each of the population tested would be necessary to shed light on the mechanisms involved, these results show that the outcome of infection strongly depends on complex interactions between multiple microorganisms and their host.

In conclusion, our results show variable effects of *Wolbachia* on spider mite susceptibility to fungi-induced mortality using two generalist fungi, *B. bassiana* and *M. brunneum*. To our knowledge, this is the first study investigating the interaction between *Wolbachia* and entomopathogenic fungi on the survival of different spider mite populations within a single full factorial experiment. As *Wolbachia* was found to have

either no effect or to increase spider mite susceptibility to fungal infection, these results suggest that it may improve the success of biological control using entomopathogenic fungi. However, these results also highlight the complexity of within-host pathogens interaction, and draw caution on the generalization of the effects of *Wolbachia* as they may vary depending on both the *Wolbachia* strain and the host genetic background. Finally, our findings also point to the importance of considering the whole bacterial community of arthropods when assessing the effect of *Wolbachia* in a particular system.

## AUTHORS' CONTRIBUTIONS

Experimental conception and design: FZ, SM; maintenance of spider mite populations and plants: IS; acquisition of data: MA; statistical analyses: FZ; paper writing: FZ, SM, with input from all authors. Funding: IC, SM. All authors have read and approved the final version of the manuscript.

## ACKNOWLEDGMENTS

We thank Catarina Pinto and João Alpedrinha for their help in some parts of the experiment, as well as Marta Palma for technical support. We also thank all members of the SM lab for useful discussions and suggestions. This work was funded by an FCT-Tubitak agreement (FCT-TUBITAK/0001/2014 and TUBITAK TOVAG 1150610) to IC and SM, and by Adnan Menderes University Research Foundation (ZRF-17055) to IC. FZ was funded through an FCT Post-Doc fellowship (SFRH/BPD/125020/2016). Funding agencies did not participate in the design or analysis of experiments. We declare that we do not have any conflict of interest.

## REFERENCES

- Attia S, Grissa KL, Lognay G, Bitume E, Hance T, Mailleux AC (2013) A review of the major biological approaches to control the worldwide pest *Tetranychus urticae* (Acari: Tetranychidae) with special reference to natural pesticides: Biological approaches to control *Tetranychus urticae*. J Pest Sci 86:361-386 doi:10.1007/s10340-013-0503-0
- Baldo L et al. (2006) Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. Appl Environ Microbiol 72:7098-7110 doi:10.1128/aem.00731-06

- Ballard JWO, Melvin RG (2007) Tetracycline treatment influences mitochondrial metabolism and mtDNA density two generations after treatment in *Drosophila*. *Insect Mol Biol* 16:799-802 doi:10.1111/j.1365-2583.2007.00760.x
- Bolker BM (2008) Ecological models and data in R Princeton University Press, New Jersey. doi:10.2307/j.ctvc4m4g37
- Bourtzis K (2008) *Wolbachia*-based technologies for insect pest population control. In: Aksoy S (ed) Transgenesis and the Management of Vector-Borne Disease, vol 627. Advances in Experimental Medicine and Biology. Springer Science & Business Media, New York, pp 104-113. doi:10.1007/978-0-387-78225-6\_9.
- Bourtzis K et al. (2014) Harnessing mosquito-*Wolbachia* symbiosis for vector and disease control. *Acta Trop* 132:S150-S163 doi:10.1016/j.actatropica.2013.11.004
- Braquart-Varnier C, Altinli M, Pigeault R, Chevalier FD, Greve P, Bouchon D, Sicard M (2015) The mutualistic side of *Wolbachia*-isopod interactions: *Wolbachia* mediated protection against pathogenic intracellular bacteria. *Front Microbiol* 6 doi:10.3389/fmicb.2015.01388
- Bugeme DM, Maniania NK, Knapp M, Boga HI (2008) Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to *Tetranychus evansi*. *Exp Appl Acarol* 46:275-285 doi:10.1007/s10493-008-9179-1
- Calvitti M, Marini F, Desiderio A, Puggioli A, Moretti R (2015) *Wolbachia* density and cytoplasmic incompatibility in *Aedes albopictus*: concerns with using artificial *Wolbachia* infection as a vector suppression tool. *PLoS One* 10 doi:10.1371/journal.pone.0121813
- Cattell J, Martinez J, Jiggins F, Mouton L, Gibert P (2016) *Wolbachia*-mediated protection against viruses in the invasive pest *Drosophila suzukii*. *Insect Mol Biol* 25:595-603 doi:10.1111/imb.12245
- Chandler D, Davidson G, Jacobson RJ (2005) Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari : Tetranychidae), on tomato, *Lycopersicon esculentum*. *Biocontrol Sci Techn* 15:37-54 doi:10.1080/09583150410001720617
- Chevalier F et al. (2012) Feminizing *Wolbachia*: a transcriptomics approach with insights on the immune response genes in *Armadillidium vulgare*. *BMC Microbiol* 12 doi:10.1186/1471-2180-12-s1-s1
- Chrostek E, Marialva MS, Esteves SS, Weinert LA, Martinez J, Jiggins FM, Teixeira L (2013) *Wolbachia* variants induce differential protection to viruses in *Drosophila melanogaster*: a phenotypic and phylogenomic analysis. *PLoS Genet* 9:e1003896 doi:10.1371/journal.pgen.1003896
- Cook PE, McGraw EA (2010) *Wolbachia pipientis*: an expanding bag of tricks to explore for disease control. *Trends Parasitol* 26:373-375 doi:10.1016/j.pt.2010.05.006
- Crawley MJ (2007) The R Book John Wiley & Sons, Ltd, Chichester, England. doi:10.1002/9780470515075

- Dobson SL (2003) Reversing *Wolbachia*-based population replacement. Trends Parasitol 19:128-133 doi:10.1016/s1471-4922(03)00002-3
- Dobson SL, Marsland EJ, Rattanadechakul W (2002) Mutualistic *Wolbachia* infection in *Aedes albopictus*: accelerating cytoplasmic drive. Genetics 160:1087-1094
- Dogan YO, Hazir S, Yildiz A, Butt TM, Cakmak I (2017) Evaluation of entomopathogenic fungi for the control of *Tetranychus urticae* (Acari: Tetranychidae) and the effect of *Metarhizium brunneum* on the predatory mites (Acari: Phytoseiidae). Biol Control 111:6-12 doi:10.1016/j.biocontrol.2017.05.001
- Durvasula RV et al. (1997) Prevention of insect-borne disease: An approach using transgenic symbiotic bacteria. Proc Natl Acad Sci USA 94:3274-3278 doi:10.1073/pnas.94.7.3274
- Engelstadter J, Hurst GDD (2009) The ecology and evolution of microbes that manipulate host reproduction. Annu Rev Ecol Evol Syst 40:127-149 doi:10.1146/annurev.ecolsys.110308.120206
- Frentiu FD et al. (2014) Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with *Wolbachia*. PLoS Negl Trop Dis 8:e2688 doi:10.1371/journal.pntd.0002688
- Fytrou A, Schofield PG, Kraaijeveld AR, Hubbard SF (2006) *Wolbachia* infection suppresses both host defence and parasitoid counter-defence. Proc R Soc B 273:791-796 doi:10.1098/rspb.2005.3383
- Gotoh T, Noda H, Fujita T, Iwade K, Higo Y, Saito S, Ohtsuka S (2005) *Wolbachia* and nuclear-nuclear interactions contribute to reproductive incompatibility in the spider mite *Panonychus mori* (Acari : Tetranychidae). Heredity 94:237-246 doi:10.1038/sj.hdy.68000605
- Gotoh T, Sugawara J, Noda H, Kitashima Y (2007) *Wolbachia*-induced cytoplasmic incompatibility in Japanese populations of *Tetranychus urticae* (Acari : Tetranychidae). Exp Appl Acarol 42:1-16 doi:10.1007/s10493-007-9072-3
- Graham RI, Grzywacz D, Mushobozi WL, Wilson K (2012) *Wolbachia* in a major African crop pest increases susceptibility to viral disease rather than protects. Ecol Lett 15:993-1000 doi:10.1111/j.1461-0248.2012.01820.x
- Greif MD, Currah RS (2007) Patterns in the occurrence of saprophytic fungi carried by arthropods caught in traps baited with rotted wood and dung. Mycologia 99:7-19 doi:10.3852/mycologia.99.1.7
- Grenier S, Pintureau B, Heddi A, Lassabliere F, Jager C, Louis C, Khatchadourian C (1998) Successful horizontal transfer of *Wolbachia* symbionts between *Trichogramma* wasps. Proc R Soc B 265:1441-1445 doi:10.1098/rspb.1998.0455
- Gurlek S, Sevim A, Sezgin FM, Sevim E (2018) Isolation and characterization of *Beauveria* and *Metarhizium* spp. from walnut fields and their pathogenicity against the codling moth, *Cydia pomonella* (NL.) (Lepidoptera: Tortricidae). Egypt J Biol Pest Co 28 doi:10.1186/s41938-018-0055-y
- Hertig M, Wolbach SB (1924) Studies on *Rickettsia*-like micro-organisms in insects. J Med Res 44:329-374



- Hoffmann AA et al. (2014) Stability of the wMel *Wolbachia* infection following invasion into *Aedes aegypti* populations. PLoS Negl Trop Dis 8:e3115 doi:10.1371/journal.pntd.0003115
- Hoffmann AA et al. (2011) Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. Nature 476:454-457 doi:10.1038/nature10356
- Hughes GL, Koga R, Xue P, Fukatsu T, Rasgon JL (2011) *Wolbachia* infections are virulent and inhibit the human malaria parasite *Plasmodium falciparum* in *Anopheles gambiae*. PLoS Pathog 7:e1002043 doi:10.1371/journal.ppat.1002043
- Hughes GL, Rivero A, Rasgon JL (2014) *Wolbachia* can enhance *Plasmodium* infection in mosquitoes: implications for malaria control? PLoS Pathog 10:e1004182 doi:10.1371/journal.ppat.1004182
- Irigaray F, Marco-Mancebon V, Perez-Moreno I (2003) The entomopathogenic fungus *Beauveria bassiana* and its compatibility with triflumuron: effects on the two-spotted spider mite *Tetranychus urticae*. Biol Control 26:168-173 doi:10.1016/S1049-9644(02)00123-8
- Islam MS (2007) *Wolbachia*-mediated reproductive alterations in invertebrate hosts and biocontrol implications of the bacteria: an update. Univ J Zool Rajshahi University 26:1-19 doi:10.3329/ujzru.v26i0.691
- Iturbe-Ormaetxe I, Walker T, Neill SLO (2011) *Wolbachia* and the biological control of mosquito-borne disease. EMBO Rep 12:508-518 doi:10.1038/embor.2011.84
- Joubert DA et al. (2016) Establishment of a *Wolbachia* superinfection in *Aedes aegypti* mosquitoes as a potential approach for future resistance management. PLoS Pathog 12:e1005434 doi:10.1371/journal.ppat.1005434
- Kambris Z, Cook PE, Phuc HK, Sinkins SP (2009) Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. Science 326:134-136 doi:10.1126/science.1177531
- Klingen I, Westrum K (2007) The effect of pesticides used in strawberries on the phytophagous mite *Tetranychus urticae* (Acari: Tetranychidae) and its fungal natural enemy *Neozygites floridana* (Zygomycetes: Entomophthorales). Biol Control 43:222-230 doi:10.1016/j.biocontrol.2007.07.013
- Kremer N, Voronin D, Charif D, Mavingui P, Mollereau B, Vavre F (2009) *Wolbachia* interferes with ferritin expression and iron metabolism in insects. PLoS Pathog 5:e1000630 doi:10.1371/journal.ppat.1000630
- Laven H (1967) Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. Nature 216:383 - 384 doi:10.1038/216383a0
- Lehman RM, Lundgren JG, Petzke LM (2009) Bacterial communities associated with the digestive tract of the predatory ground beetle, *Poecilus chalcites*, and their modification by laboratory rearing and antibiotic treatment. Microb Ecol 57:349-358 doi:10.1007/s00248-008-9415-6



- Li T, Chen X-L, Hong X-Y (2009) Population genetic structure of *Tetranychus urticae* and its sibling species *Tetranychus cinnabarius* (Acari: Tetranychidae) in China as inferred from microsatellite data. *Ann Entomol Soc Am* 102:674-683 doi:10.1603/008.102.0412
- Liu Y, Miao H, Hong XY (2006) Distribution of the endosymbiotic bacterium *Cardinium* in Chinese populations of the carmine spider mite *Tetranychus cinnabarinus* (Acari : Tetranychidae). *J Appl Entomol* 130:523-529 doi:10.1111/j.1439-0418.2006.01112.x
- Maniania NK, Bugeme DM, Wekesa VW, Delalibera I, Knapp M (2008) Role of entomopathogenic fungi in the control of *Tetranychus evansi* and *Tetranychus urticae* (Acari: Tetranychidae), pests of horticultural crops. *Exp Appl Acarol* 46:259-274 doi:10.1007/s10493-008-9180-8
- Martinez J, Tolosana I, Ok S, Smith S, Snoeck K, Day JP, Jiggins FM (2017) Symbiont strain is the main determinant of variation in *Wolbachia*-mediated protection against viruses across *Drosophila species*. *Mol Ecol* 26:4072-4084 doi:10.1111/mec.14164
- McGraw EA, Merritt DJ, Droller JN, O'Neill SL (2002) *Wolbachia* density and virulence attenuation after transfer into a novel host. *Proc Natl Acad Sci USA* 99:2918-2923 doi:10.1073/pnas.052466499
- Meyling NV, Eilenberg J (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. *Biol Control* 43:145-155 doi:10.1016/j.biocontrol.2007.07.007
- Migeon A, Dorkeld F (2006-2017) Spider Mites Web: a comprehensive database for the Tetranychidae. <http://www.montpellier.inra.fr/CBGP/spmweb/>.
- Moreira LA et al. (2009) A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell* 139:1268-1278 doi:10.1016/j.cell.2009.11.042
- Nguyen TH et al. (2015) Field evaluation of the establishment potential of wMelPop *Wolbachia* in Australia and Vietnam for dengue control. *Parasite Vector* 8:563 doi:10.1186/s13071-015-1174-x
- O'Shea KL, Singh ND (2015) Tetracycline-exposed *Drosophila melanogaster* males produce fewer offspring but a relative excess of sons. *Ecol Evol* 5:3130-3139 doi:10.1002/ece3.1535
- Osborne SE, Leong YS, O'Neill SL, Johnson KN (2009) Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. *PLoS Pathog* 5:e1000656 doi:10.1371/journal.ppat.1000656
- Panteleev DY, Goryacheva II, Andrianov BV, Reznik NL, Lazebny OE, Kulikov AM (2007) The endosymbiotic bacterium *Wolbachia* enhances the nonspecific resistance to insect pathogens and alters behavior of *Drosophila melanogaster*. *Russ J Genet* 43:1066-1069 doi:10.1134/s1022795407090153
- Rehner SA (2005) Phylogenetics of the insect pathogenic genus *Beauveria*. In: Vega FE, Blackwell M (eds) *Insect-Fungal Associations: Ecology and Evolution*. Oxford University Press, New York, pp 3-27

- Roberts DW, Leger RJS (2004) *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: Mycological aspects. In: Laskin AI, Bennet JW, Gadd GM (eds) *Advances in Applied Microbiology*, vol 54. *Advances in Applied Microbiology*. pp 1-70. doi:10.1016/s0065-2164(04)54001-7
- Seiedy M (2014) Feeding preference of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) towards untreated and *Beauveria bassiana*-treated *Tetranychus urticae* (Acari: Tetranychidae) on cucumber leaves. *Pers J Acarol* 3:91-97 doi:10.22073/pja.v3i1.10134
- Seiedy M, Saboori A, Allahyari H (2012) Interactions of two natural enemies of *Tetranychus urticae*, the fungal entomopathogen *Beauveria bassiana* and the predatory mite, *Phytoseiulus persimilis*. *Biocontrol Sci Techn* 22:873-882 doi:10.1080/09583157.2012.695010
- Seiedy M, Saboori A, Zahedi-Golpayegani A (2013) Olfactory response of *Phytoseiulus persimilis* (Acari: Phytoseiidae) to untreated and *Beauveria bassiana*-treated *Tetranychus urticae* (Acari: Tetranychidae). *Exp Appl Acarol* 60:219-227 doi:10.1007/s10493-012-9652-8
- Selosse M-A, Bessis A, Pozo MJ (2014) Microbial priming of plant and animal immunity: symbionts as developmental signals. *Trends Microbiol* 22:607-613 doi:10.1016/j.tim.2014.07.003
- Shapira M (2016) Gut microbiotas and host evolution: scaling up symbiosis. *Trends Ecol Evol* 31:539-549 doi:10.1016/j.tree.2016.03.006
- Shi W-B, Jiang Y, Feng M-G (2005) Compatibility of ten acaricides with *Beauveria bassiana* and enhancement of fungal infection to *Tetranychus cinnabarinus* (Acari: Tetranychidae) eggs by sublethal application rates of pyridaben. *Appl Entomol Zool* 40:659-666 doi:10.1303/aez.2005.659
- Shi W-B, Zhang L, Feng M-G (2008) Time-concentration-mortality responses of carmine spider mite (Acari: Tetranychidae) females to three hypocrealean fungi as biocontrol agents. *Biol Control* 46:495-501 doi:10.1016/j.biocontrol.2008.04.006
- Shi WB, Feng MG (2004) Lethal effect of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces fumosoroseus* on the eggs of *Tetranychus cinnabarinus* (Acari : Tetranychidae) with a description of a mite egg bioassay system. *Biol Control* 30:165-173 doi:10.1016/j.biocontrol.2004.01.017
- Shin TY, Bae SM, Kim DJ, Yun HG, Woo SD (2017) Evaluation of virulence, tolerance to environmental factors and antimicrobial activities of entomopathogenic fungi against two-spotted spider mite, *Tetranychus urticae*. *Mycoscience* 58:204-212 doi:doi.org/10.1016/j.myc.2017.02.002
- Sinkins SP, Godfray HCJ (2004) Use of *Wolbachia* to drive nuclear transgenes through insect populations. *Proc R Soc B* 271:1421-1426 doi:10.1098/rspb.2004.2740
- Skinner M, Parker BL, Kim JS (2014) Role of entomopathogenic fungi in integrated pest management. In: Abrol DP (ed) *Integrated pest management: Current Concepts and Ecological Perspective*. Elsevier, San Diego, California, pp 169-191. doi:10.1016/C2012-0-00720-X

- Stouthamer R (1993) The use of sexual versus asexual wasps in biological-control. *Entomophaga* 38:3-6 doi:10.1007/bf02373133
- Stouthamer R, Breeuwer JAJ, Hurst GDD (1999) *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction. *Annu Rev Microbiol* 53:71-102 doi:10.1146/annurev.micro.53.1.71
- Teixeira L, Ferreira A, Ashburner M (2008) The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol* 6:2753-2763 doi:10.1371/journal.pbio.1000002
- Tortosa P, Courtiol A, Moutailler S, Failloux AB, Weill M (2008) Chikungunya-*Wolbachia* interplay in *Aedes albopictus*. *Insect Mol Biol* 17:677-684 doi:10.1111/j.1365-2583.2008.00842.x
- Turelli M, Hoffmann AA (1999) Microbe-induced cytoplasmic incompatibility as a mechanism for introducing transgenes into arthropod populations. *Insect Mol Biol* 8:243-255 doi:10.1046/j.1365-2583.1999.820243.x
- Ullah MS, Lim UT (2017) Synergism of *Beauveria bassiana* and *Phytoseiulus persimilis* in control of *Tetranychus urticae* on bean plants. *Syst Appl Acarol* 22:1924-1935 doi:10.11158/saa.22.11.11
- Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauw W, Tirry L (2010) Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. *Insect Biochem Mol Biol* 40:563-572 doi:10.1016/j.ibmb.2010.05.008
- Vavre F, Charlat S (2012) Making (good) use of *Wolbachia*: what the models say. *Curr Opin Microbiol* 15:263-268 doi:10.1016/j.mib.2012.03.005
- Vavre F, Kremer N (2014) Microbial impacts on insect evolutionary diversification: from patterns to mechanisms. *Curr Opin Insect Sci* 4:29-34 doi:10.1016/j.cois.2014.08.003
- Vega FE et al. (2009) Fungal entomopathogens: new insights on their ecology. *Fungal Ecol* 2:149-159 doi:10.1016/j.funeco.2009.05.001
- Vergel SJN, Bustos RA, Rodriguez CD, Cantor RF (2011) Laboratory and greenhouse evaluation of the entomopathogenic fungi and garlic-pepper extract on the predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* and their effect on the spider mite *Tetranychus urticae*. *Biol Control* 57:143-149 doi:10.1016/j.biocontrol.2011.02.007
- Walker T et al. (2011) The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* 476:450-453 doi:10.1038/nature10355
- Weinert LA, Araujo-Jnr EV, Ahmed MZ, Welch JJ (2015) The incidence of bacterial endosymbionts in terrestrial arthropods. *Proc R Soc B* 282:20150249 doi:10.1098/rspb.2015.0249
- Weiss B, Aksoy S (2011) Microbiome influences on insect host vector competence. *Trends Parasitol* 27:514-522 doi:10.1016/j.pt.2011.05.001
- Werren JH, Baldo L, Clark ME (2008) *Wolbachia*: master manipulators of invertebrate biology. *Nat Rev Microbiol* 6:741-751 doi:10.1038/nrmicro1969

- Wong ZS, Hedges LM, Brownlie JC, Johnson KN (2011) *Wolbachia*-mediated antibacterial protection and immune gene regulation in *Drosophila*. PLoS One 6:e25430 doi:10.1371/journal.pone.0025430
- Wu S, Xie H, Li M, Xu X, Lei Z (2016) Highly virulent *Beauveria bassiana* strains against the two-spotted spider mite, *Tetranychus urticae*, show no pathogenicity against five phytoseiid mite species. Exp Appl Acarol 70:421-435 doi:10.1007/s10493-016-0090-x
- Ye YH, Woolfit M, Rancès E, O'Neill SL, McGraw EA (2013) *Wolbachia*-associated bacterial protection in the mosquito *Aedes aegypti*. PLoS Negl Trop Dis 7:e2362 doi:10.1371/journal.pntd.0002362
- Yeap HL et al. (2011) Dynamics of the "Popcorn" *Wolbachia* infection in outbred *Aedes aegypti* informs prospects for mosquito vector control. Genetics 187:583-U346 doi:10.1534/genetics.110.122390
- Zeh JA, Bonilla MM, Adrian AJ, Mesfin S, Zeh DW (2012) From father to son: transgenerational effect of tetracycline on sperm viability. Sci Rep 2:375 doi:10.1038/srep00375
- Zélé F, Altıntaş M, Santos I, Cakmak I, Magalhães S (2019) Inter- and intra-specific variation of spider mite susceptibility to fungal infections: implications for the long-term success of biological control. bioRxiv doi:10.1101/615344
- Zélé F, Magalhães S, Kéfi S, Duncan AB (2018a) Ecology and evolution of facilitation among symbionts. Nat Commun 9 doi:10.1038/s41467-018-06779-w
- Zélé F, Santos I, Olivieri I, Weill M, Duron O, Magalhães S (2018b) Endosymbiont diversity and prevalence in herbivorous spider mite populations in South-Western Europe. FEMS Microbiol Ecol 94:fiy015 doi:10.1093/femsec/fiy015
- Zélé F, Weill M, Magalhães S (2018c) Identification of spider-mite species and their endosymbionts using multiplex PCR. Exp Appl Acarol 74:123-138 doi:10.1007/s10493-018-0224-4
- Zhang DJ, Lees RS, Xi ZY, Gilles JRL, Bourtzis K (2015a) Combining the sterile insect technique with *Wolbachia*-based approaches: II- A safer approach to *Aedes albopictus* population suppression programmes, designed to minimize the consequences of inadvertent female release. PLoS One 10 doi:10.1371/journal.pone.0135194
- Zhang YK, Chen YT, Yang K, Qiao GX, Hong XY (2016) Screening of spider mites (Acari: Tetranychidae) for reproductive endosymbionts reveals links between co-infection and evolutionary history. Sci Rep 6:27900 doi:10.1038/srep27900
- Zhang YK, Ding XL, Rong X, Hong XY (2015b) How do hosts react to endosymbionts? A new insight into the molecular mechanisms underlying the *Wolbachia*-host association. Insect Mol Biol 24:1-12 doi:10.1111/imb.12128
- Zhong Y, Li ZX (2014) Bidirectional cytoplasmic incompatibility induced by cross-order transfection of *Wolbachia*: Implications for control of the host population. Microb Ecol 68:463-471 doi:10.1007/s00248-014-0425-2

- Zhu YX, Song YL, Hoffmann AA, Jin PY, Huo SM, Hong XY (2018) A change in the bacterial community of spider mites decreases fecundity on multiple host plants. *MicrobiologyOpen*:e743 doi:10.1002/mbo3.743
- Zindel R, Gottlieb Y, Aebi A (2011) Arthropod symbioses: a neglected parameter in pest- and disease-control programmes. *J Appl Ecol* 48:864–872 doi:10.1111/j.1365-2664.2011.01984.x
- Zouache K, Michelland RJ, Failloux A-B, Grundmann GL, Mavingui P (2012) Chikungunya virus impacts the diversity of symbiotic bacteria in mosquito vector. *Mol Ecol* 21:2297-2309 doi:10.1111/j.1365-294X.2012.05526.x
- Zouache K, Voronin D, Tran-Van V, Mavingui P (2009) Composition of bacterial communities associated with natural and laboratory populations of *Asobara tabida* infected with *Wolbachia*. *Appl Environ Microbiol* 75 doi:10.1128/aem.02964-08
- Zug R, Hammerstein P (2015) *Wolbachia* and the insect immune system: what reactive oxygen species can tell us about the mechanisms of *Wolbachia*-host interactions. *Front Microbiol* 6 doi:10.3389/fmicb.2015.01201