

## Inter- and intra-specific variation of spider mite susceptibility to fungal infections: implications for the long-term success of biological control

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>

\*Joint first authors

# 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

Spider mites are severe pests of several annual and perennial crops worldwide, often causing important economic damages. Moreover, rapid evolution of pesticide resistance in this group hampers the efficiency of chemical control. Alternative control strategies, such as the use of entomopathogenic fungi, are thus being developed. However, while several studies have focussed on the evaluation of the control potential of different fungal species and/or isolates as well as their compatibility with other control methods (e.g. predators or chemical pesticides), knowledge on the extent of inter- and intraspecific variation in spider mite susceptibility to fungal infection is as yet incipient. Here, we measured the mortality induced by two generalist fungi, *Beauveria bassiana* and *Metarhizium brunneum*, in 12 spider mite populations belonging to different *Tetranychus* species: *T. evansi*, *T. ludeni*, the green form of *T. urticae* and the red form of *T. urticae*, within a full factorial experiment. We found that spider mite species differed in their susceptibility to infection to both fungal species. Moreover, we also found important intraspecific variation for this trait. These results draw caution on the development of single strains as biocontrol agents. Indeed, the high level of intraspecific variation suggests that (a) the one-size-fits-all strategy will probably fail to control spider-mite populations and (b) hosts resistance to infection may evolve at a rapid pace. Finally, we propose future directions to better understand this system and improve the long-term success of spider mite control strategies based on entomopathogenic fungi.

### KEY-WORDS

Tetranychidae; entomopathogenic fungi; parasite-induced mortality; resistance; tolerance; evolution.

## INTRODUCTION

Pesticides are still the main weapon used to control crop pests and disease vectors, despite the major threats they represent for food safety and for the environment (Bourguet and Guillemaud 2016). Moreover, the pervasive evolution and rapid spread of resistance to pesticides severely affect their efficiency in many taxa (Casida and Quistad 1998). Therefore, alternative control strategies are being sought to control disease epidemics and outbreaks of agricultural crop pests (Hajek et al. 2007; Lacey et al. 2001; Parolin et al. 2012; Zindel et al. 2011), including spider mites (Attia et al. 2013).

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), destroying annual and perennial crops. A few studies have evaluated the economic costs of spider mites, which vary among crops, seasons and plant age (Alatawi et al. 2007; Flamini 2006; Opit et al. 2005; Park and Lee 2002; Park and Lee 2005; Park and Lee 2007; Weihrauch 2005), and mathematical models suggest that detrimental effects of spider mites in agriculture will dramatically increase with increased global warming (Migeon et al. 2009). Moreover, due to their short generation time and high fecundity, spider mites rapidly develop resistance to pesticides (Van Leeuwen et al. 2010). Important efforts are thus being developed to evaluate the efficiency of different biological control methods, such as the use of essential oils, but also natural enemies such as predators, entomopathogenic bacteria and fungi (Attia et al. 2013). In particular, a plethora of studies have evaluated the virulence of many fungal species (e.g. *Neozygites* spp., *Metarhizium* spp., *Beauveria bassiana* and *Lecanicillium lecanii*) and/or strains to identify the best 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), as well as 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). However, these studies were conducted using a single host population and potential intraspecific variations in spider mites susceptibility have, to our knowledge, never been investigated within a single experiment (but see, for instance, Afifi et al. 2010; Fiedler and Sosnowska 2007; Ribeiro et al. 2009 for a comparison among spider mites and/or among other arthropod species; or Milner 1982; 1985; Perinotto et al. 2012; Uma Devi et al. 2008 for intraspecific variation within other arthropod species).

Both intra- and interspecific variability in host susceptibility to infection may modify epidemiological patterns of parasite in natural host populations (Dwyer et al. 1997; Hawley and Altizer 2011; Read 1995), thereby altering the efficiency and environmental persistence of biocontrol agents. Moreover, the use of such agents generates a strong selection pressure on the target pests (e.g. Fenner and Fantini 1999; see also Moscardi 1999; Tabashnik 1994) and, in general, variability in host susceptibility to infection may have

important consequences for the evolution of host resistance as well as parasite virulence and transmission (Elena 2017; Sorci et al. 1997; Stevens and Rizzo 2008). Hence, assessing both intra- and interspecific variability in spider mite susceptibility to infection by different potential biocontrol agents is a prerequisite for the development of efficient and long-lasting control strategies.

Here, we assessed the susceptibility to fungi infection of 12 different spider mite populations belonging to different species that are ubiquitous in Europe and often co-occur in the field (Migeon and Dorkeld 2006-2017; Z  l   et al. 2018b): 3 populations of the green form of *T. urticae*, 3 populations of the red form of *T. urticae* (also referred to as *T. cinnabarinus* by some authors; e.g. Li et al. 2009; Shi et al. 2005; Shi and Feng 2004), 3 populations of *T. ludeni*, and 3 populations of *T. evansi*. 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), and have wide geographical and host ranges (Greif and Currah 2007; Gurlek et al. 2018; Meyling and Eilenberg 2007; Rehner 2005; Roberts and Leger 2004). We then discuss the possible ecological and evolutionary causes and underlying mechanisms leading to the observed results, as well as their potential consequences for the evolution of both hosts susceptibility to infection and fungi virulence. Finally, we propose future directions to improve long-term success of spider mite control strategies using entomopathogenic fungi.

## MATERIALS AND METHODS

### *Spider mite populations and rearing*

Twelve populations of Tetranychid mites were used in this study. Three of *T. evansi* (called BR, GH and QL), three of *T. ludeni* (called Obi, Alval and Assaf), three of the red form of *T. urticae* (called AlRo, AMP.tet, FR.tet), and three of the green form of *T. urticae* (called TOM.rif, LS.tet, B6JS). Most of these populations were collected in Portugal from 2013 to 2016, FR.tet was collected in France and AlRo in Spain in 2013. The population BR of *T. evansi* was collected in a greenhouse in Brazil in 2002 (Godinho et al. 2016; Sarmiento et al. 2011), and the population LS.tet of the green form of *T. urticae* derived from the London strain, which was used to sequence the species genome (Grbic et al. 2011). These populations originated from various plant species in the field, and none of them carried bacterial endosymbionts (i.e. *Wolbachia*, *Cardinium*, *Rickettsia*, *Arsenophonus*, *Spiroplasma*), either because they were initially uninfected when collected in the field (Z  l   et al. 2018a), or following antibiotic treatment (3 generations with tetracycline hydrochloride, or 1 generation with rifampicin; all populations with ".tet" or ".rif" suffix, respectively; Breeuwer 1997; Gotoh et al. 2005; Li et al. 2014). All the information concerning these populations is summarized in Table S1. They were subsequently reared in the laboratory under standard conditions (25 ± 2°C, 60% RH, 16/8 h L/D) at high numbers (c.a. 500-

1000 females per cage) in insect-proof cages containing either bean cv. Contender seedlings (obtained from Germisem, Oliveira do Hospital, Portugal) for *T. urticae* and *T. ludeni*, or tomato cv. Money Maker seedlings (obtained from Mr. Fothergill's Seeds, Kentford, UK) for *T. evansi*.

### *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, age cohorts were created for each spider mite population by collecting ca. 100 females from each mass culture, allowing them to lay eggs during 4 days on detached bean leaves placed on water-soaked cotton. The offspring from these cohorts was used in the experiment.

One day prior to the onset of this experiment, 20 adult mated females with similar age were randomly collected from each cohort of each population and placed on a 9-cm<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 per population were performed within 7 temporal blocks (roughly 3 replicates of each treatment per block).

### *Statistical analysis*

Analyses were carried out using the R statistical package (version 3.5.3). Survival data were analysed using Cox proportional hazards mixed-effect models (coxme, kinship package). Spider-mite species, or populations within each species, and infection treatment (sprayed with *Beauveria bassiana*, with *Metarhizium brunneum*, or with Tween 20 only as control) were fit in as fixed explanatory variables, whereas discs nested within population, population (in the case of interspecific variation only) and block were fit as random explanatory variables.

Hazard ratios (HR) were 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 controls of each species/population (by changing the intercept of the model) and the BB or MB treatments.

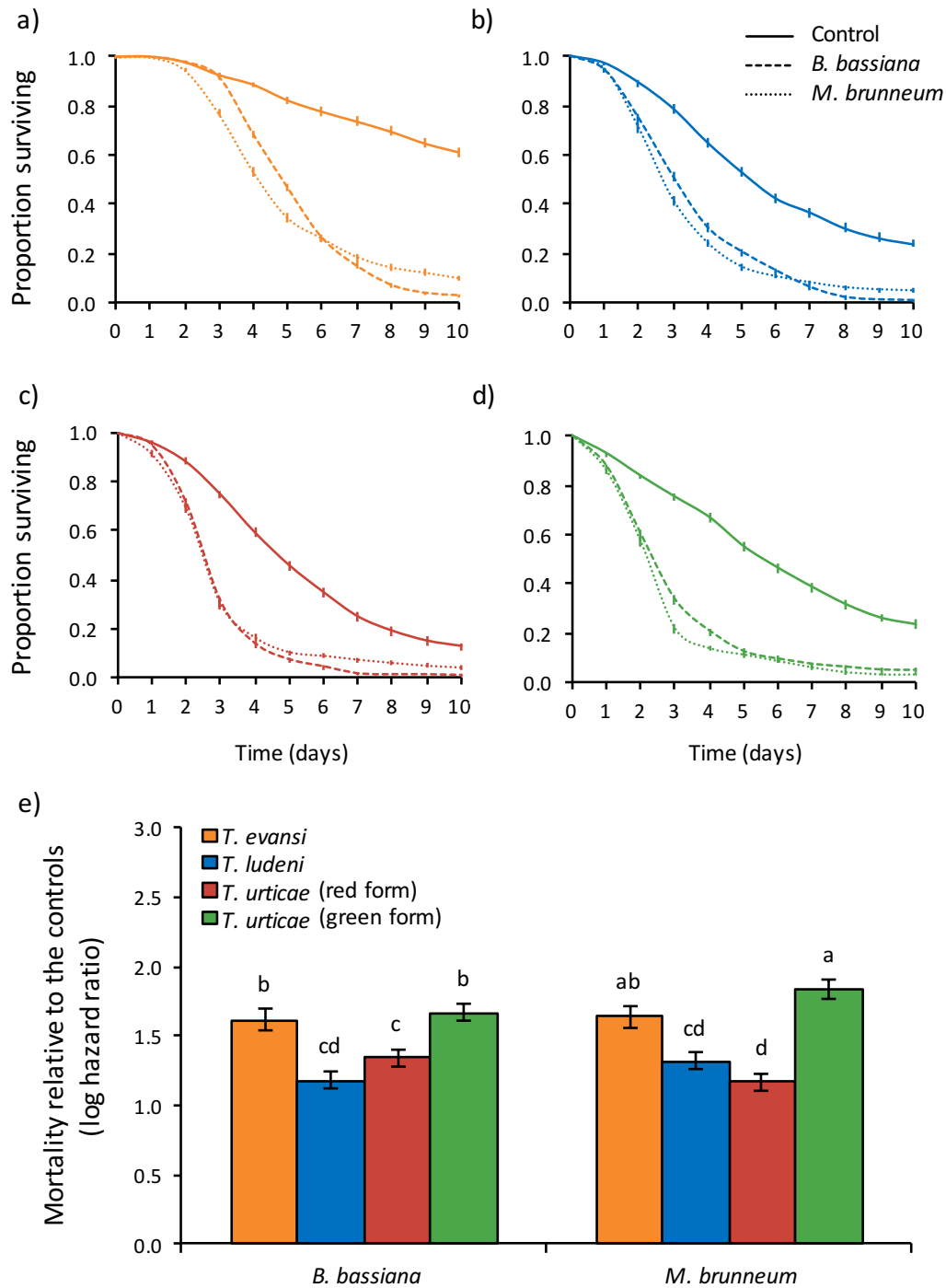
Maximal models, including all higher-order interactions, were simplified to establish a minimal model by sequentially eliminating non-significant terms and interactions (Crawley 2007). The significance of the explanatory variables was established using chi-squared tests (Bolker 2008). The significant 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 species/population and treatment effects on HR, the two factors were concatenated to fit a single fixed factor containing all species/population by treatments levels in the models. Multiple comparisons were then performed using General Linear Hypotheses (glht, package multcomp) with Holm corrections.

## RESULTS

### ***Interspecific variation of spider-mite susceptibility to infection by Beauveria bassiana and Metarhizium brunneum.***

The statistical analyses revealed a significant interaction between treatments (females sprayed with either Tween 20 only as control, *B. bassiana*, or *M. brunneum*) and species (*T. evansi*, *T. ludeni*, red and green form of *T. urticae*) on the survival of spider mites ( $\chi^2_6=80.61$ ,  $p<0.001$ ; Fig.1 a-d). Multiple comparisons of hazard ratios (HRs) obtained for each spider-mite species infected by each fungal species relative to the control revealed that all species were not equally affected by infection (Fig. 1e): both fungi induced a stronger mortality in *T. evansi* (HR=5.04 for *B. bassiana*, and HR=5.15 for *M. brunneum*) and in the green form of *T. urticae* (HR=5.30 for *B. bassiana*, and HR=6.27 for *M. brunneum*), than in *T. ludeni* (HR=3.25 for *B. bassiana*, and HR=3.73 for *M. brunneum*) and in the red form of *T. urticae* (HR=3.84 for *B. bassiana*, and HR=3.21 for *M. brunneum*); but their effect did not differ within these two groups of species (Fig. 1e; cf. Table S1 for the statistical results of all multiple comparisons). Moreover, while the two fungi induced similar levels of mortality in *T. evansi* ( $z=-0.39$ ,  $p=1.00$ ) and in *T. ludeni* ( $z=-0.14$ ,  $p=0.13$ ), infection with *B. bassiana* led to higher mortality than that with *M. brunneum* in the red form of *T. urticae* ( $z=0.18$ ,  $p=0.03$ ), while the reverse was found in the green form of *T. urticae* ( $z=-0.17$ ,  $p=0.04$ ). Note that survival in the *T. evansi* control was higher than in that of the three other species (Fig. S1 and Table S2).

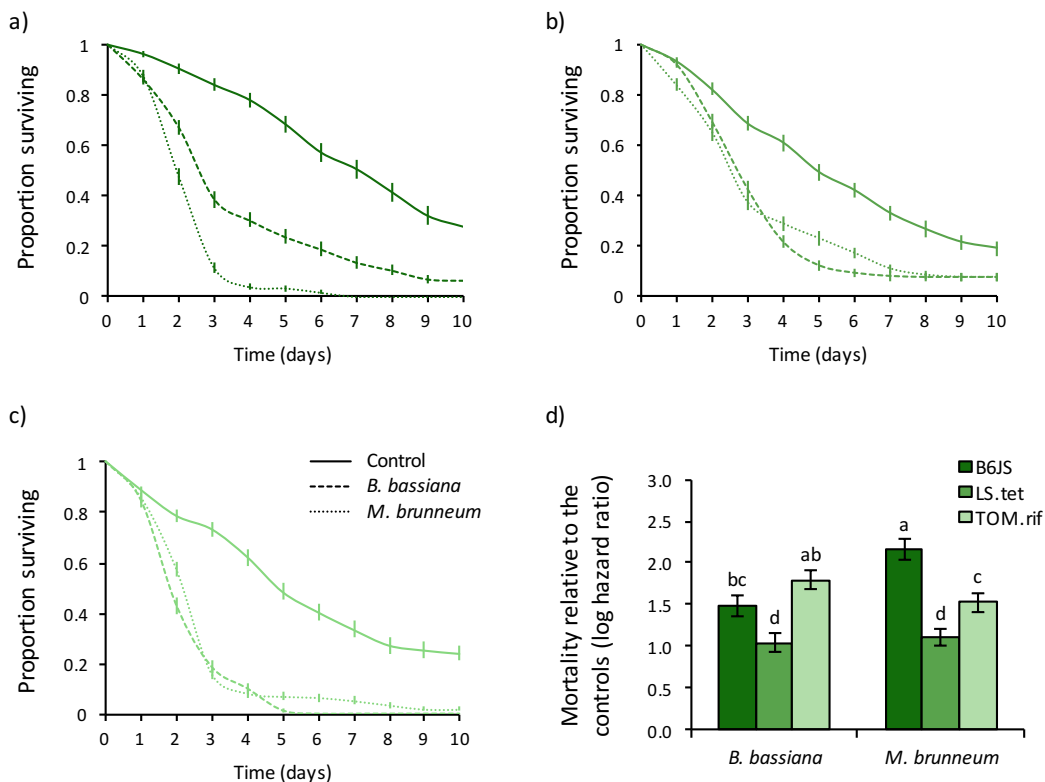


**Figure 1.** Survival curves of spider-mite females from (a) *T. evansi*, (b) *T. ludeni*, (c) red form of *T. urticae*, and (d) green form of *T. urticae*, sprayed with *B. bassiana* (dashed lines), *M. brunneum* (dotted lines), or with Tween 20 only (control; solid lines). (e) mortality upon infection by each fungus relative to the controls (log hazard ratio  $\pm$  s.e.); identical letter superscripts indicate non-significant differences between treatments at the 5% level (multiple comparisons with Holm correction).

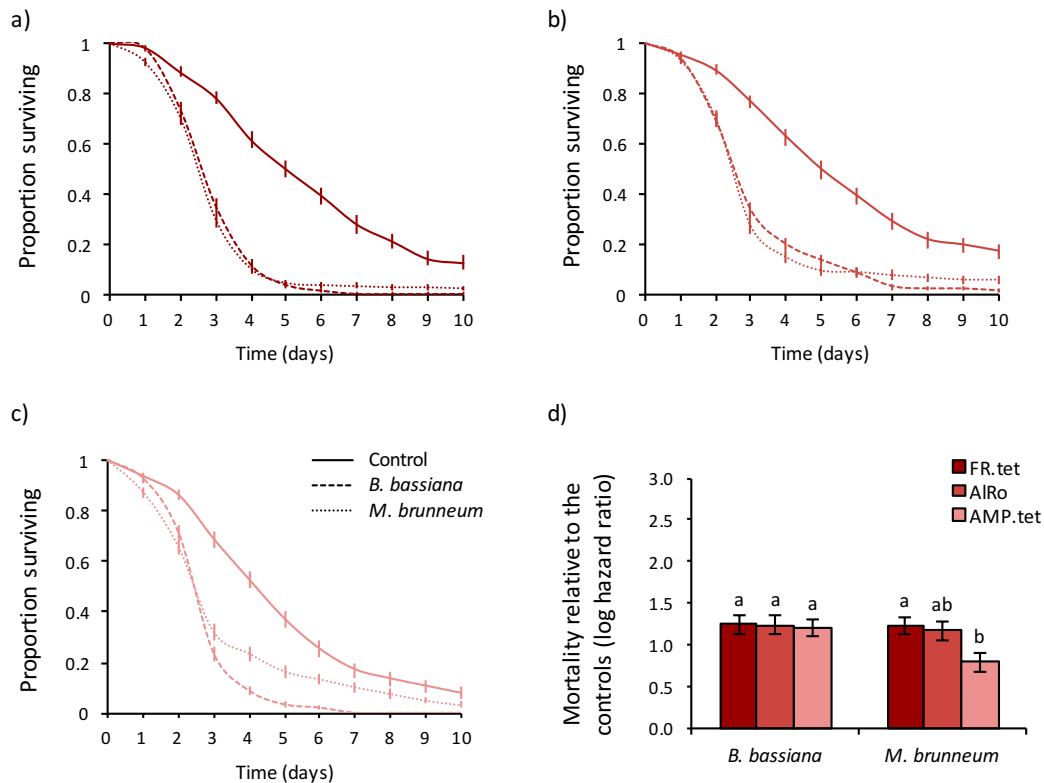
## Intraspecific variation of spider-mite susceptibility to infection by *Beauveria bassiana* and *Metarhizium brunneum*.

We also found a significant interaction between treatment and population on spider-mite survival within each of the species studied (in the green form of *T. urticae*:  $X^2_4=79.60$ ,  $p<0.0001$ ; in the red form *T. urticae*:  $X^2_4=12.12$ ,  $p<0.02$ ; in *T. ludeni*:  $X^2_4=17.41$ ,  $p<0.002$ ; in *T. evansi*:  $X^2_4=106.72$ ,  $p<0.0001$ ). Indeed, for all species, the spider mite populations differed in their susceptibility either to both fungi or to only one of them.

In the green form of *T. urticae*, *B. bassiana* induced a higher mortality in the populations TOM.rif and B6JS (a 6.01-fold and a 4.41-fold decrease in survival upon infection relative to the control, respectively) than in the population LS.tet (a 2.85-fold decrease in survival). Similarly, *M. brunneum* induced higher mortality in B6JS (a 8.67-fold decrease in survival) than in TOM.rif (a 4.60-fold decrease in survival), and the highest mortality was found in LS.tet (a 3.04-fold decrease in survival; Fig. 2a-d; see Table S3 for the statistical results of all comparisons). Moreover, while infection with both fungi led to similar mortality in LS.tet ( $z=-0.67$ ,  $p=1.00$ ), infection with *B. bassiana* induced a higher mortality rate than that with *M. brunneum* in TOM.rif ( $z=2.87$ ,  $p=0.04$ ), and the reverse was found in B6JS ( $z=-6.86$ ,  $p<0.0001$ ).



**Figure 2.** Survival curves of spider-mite females from different populations of the green form of *T. urticae*: (a) B6JS, (b) LS.tet, and (c) TOM.rif, sprayed with *B. bassiana* (dashed lines), *M. brunneum* (dotted lines), or with Tween 20 only (control; solid lines). (d) mortality relative to the controls (log hazard ratio  $\pm$  s.e.); identical letter superscripts indicate non-significant differences between treatments at the 5% level (multiple comparisons with Holm correction).



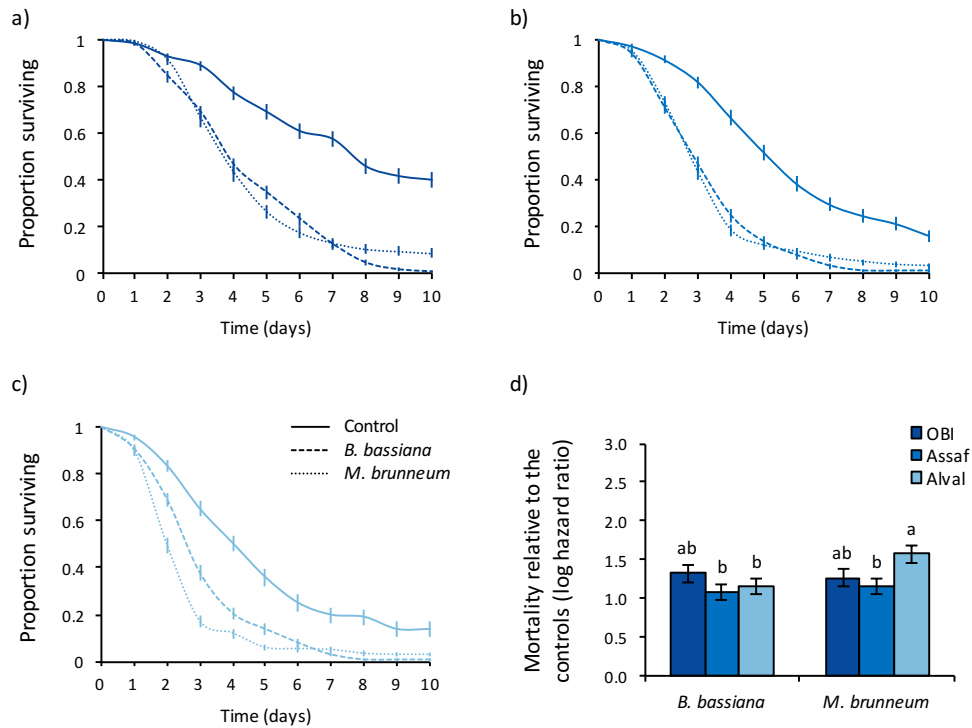
**Figure 3.** Survival curves of spider-mite females from different populations of the red form of *T. urticae*: (a) FR.tet, (b) AlRo, and (c) AMP.tet, sprayed with *B. bassiana* (dashed lines), *M. brunneum* (dotted lines), or with Tween 20 only (control; solid lines). (d) mortality relative to the controls (log hazard ratio  $\pm$  s.e.); identical letter superscripts indicate non-significant differences between treatments at the 5% level (multiple comparisons with Holm correction).

In the red form of *T. urticae*, both fungi had the same effect in all populations (HR ranged between 3.21 and 3.47) except in the population AMP.tet in which *M. brunneum* induced lower mortality (HR=2.21; Fig. 3a-d; see Table S4 for the statistical results of all comparisons).

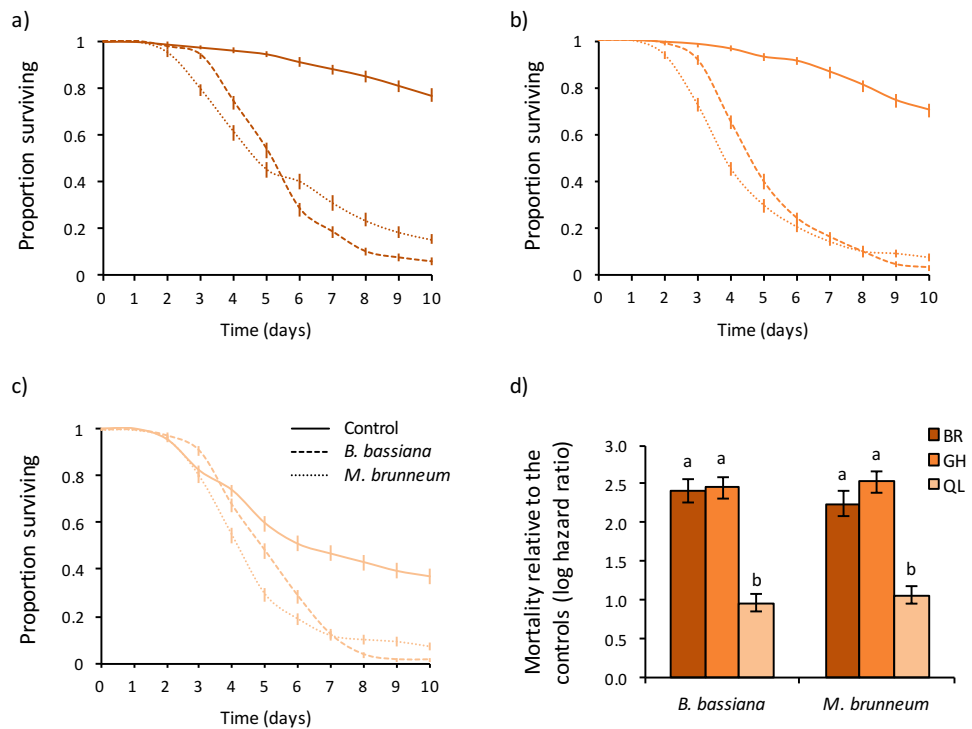
In *T. ludeni*, both fungi induced similar mortality in all populations except for the population Alval, in which *M. brunneum* induced higher mortality than in the population Assaf, with a 4.86-fold and 3.17-fold decreased survival upon infection relative to the control, respectively (HR ranged between 2.98 and 3.77 in the other treatments; Fig. 4a-d; see Table S7 for the statistical results of all comparisons). Note that, in this species, the population controls (not exposed to fungi) did not have the same survival (Fig. S1; Table S5).

In *T. evansi*, both fungi species induced similar mortality independently of the population tested, but they this induced mortality was higher in the populations GH and BR than in the population QL (c.a. 12-fold, 10-fold, and 2-fold decreased survival upon infection relative to the controls, respectively; Fig. 5a-d; see Table S6 for the statistical results of all comparisons). Note, however, that QL control had a much lower survival than that of the two other populations (Fig. S1; Tables S6).





**Figure 4.** Survival curves of spider-mite females from different *T. ludeni* populations: (a) OBI, (b) Assaf, and (c) Alval, sprayed with *B. bassiana* (dashed lines), *M. brunneum* (dotted lines), or with Tween 20 only (control; solid lines). (d) mortality relative to the controls (log hazard ratio  $\pm$  s.e.); identical letter superscripts indicate non-significant differences between treatments at the 5% level (multiple comparisons with Holm correction).



**Figure 5.** Survival curves of spider-mite females from different *T. evansi* populations: (a) BR, (b) GH, and (c) QL, sprayed with *B. bassiana* (dashed lines), *M. brunneum* (dotted lines), or with Tween 20 only (control; solid lines). (d) mortality relative to the controls (log hazard ratio  $\pm$  s.e.); identical letter superscripts indicate non-significant differences between treatments at the 5% level (multiple comparisons with Holm correction).

## DISCUSSION

In this study, we found both intra- and interspecific variability in the susceptibility of *Tetranychus* spider mite to infection by *B. bassiana* and *M. brunneum*. Overall, we observed a higher mortality upon infection in *T. evansi* and in the green form of *T. urticae*, than in *T. ludeni* and in the red form of *T. urticae*. These results, however, may not reflect accurately the virulence of both fungi in each of these spider mite species. Indeed, we further found important variation among populations within each species. Most variation was found among populations of *T. evansi* and of the green form of *T. urticae*, with, for instance, the mortality upon infection of two populations of *T. evansi* (BR and GH) being 5 times higher than that of another (QL). We also found variation among populations of *T. ludeni* and of the red form of *T. urticae*, although the amplitude of these effects was relatively smaller and depended on the fungal species.

Overall, our results suggest that spider mite susceptibility to infection is not a phylogenetically-conserved trait, and further corroborate the generalist status of both fungal species (Meyling and Eilenberg 2007; Rehner 2005; Roberts and Leger 2004). For instance, *B. bassiana* occurs naturally in more than 700 host species (Inglis et al. 2001), and this range is likely underestimated as prevalence estimates are usually done in arthropod species that are crop pests or predators and parasitoids used as biocontrol agents (Meyling and Eilenberg 2007). Moreover, differences in virulence between the two fungi shown here suggest population-specific responses to each fungus, instead of a more general response against infection. For instance, *M. brunneum* is more virulent than *B. bassiana* in the population B6JS of green *T. urticae* and in the population Alval of *T. ludeni*, while the reverse occurred in the population AMP.tet of the red form of *T. urticae*. Such differences in susceptibility to infection between populations independently of their phylogenetic relationship may thus reflect differences in exposure by each fungus species (i.e. different selection pressure for resistance mechanisms to evolve) throughout their evolutionary history.

Variations in the prevalence of each fungus leading to different exposure may, for instance, occur between different geographical areas due to several environmental factors, such as temperature, humidity and solar (UV) irradiation (Meyling and Eilenberg 2007). However, these fungi are known to have a cosmopolitan distribution and our results show no clear association between the susceptibility of a particular spider mite population and its country of origin. For instance, the *T. evansi* populations BR and GH come from Brazil and Portugal, respectively, but do not differ in susceptibility to infection by both fungi; similarly, the effect of *B. bassiana* do not differ between populations of red *T. urticae* collected in France (FR.tet), Spain (AIRo) and Portugal (AMP.tet). Instead, we found different susceptibility to infection between populations at small geographical scales, such as in the *T. evansi* populations GH and QL and in the green *T. urticae* populations B6JS and TOM.rif upon infection by both fungi; or in the *T. ludeni* populations Assaf and Alval upon infection by *M. brunneum*; while all of these populations were collected in the same region in Portugal. These results might

thus be explained by microhabitats-specific distribution of the fungi, as previously found for different isolates of *B. bassiana* (e.g. Ormond et al. 2010; Wang et al. 2003). Moreover, several studies suggest that both *B. bassiana* and *Metarhizium* spp. have the potential to interact directly with the host plants of arthropods (reviewed in Meyling and Eilenberg 2007), which may potentially lead to plant-specific distribution of the fungi. Indeed, *Metarhizium* spp. occur in the rhizosphere, which possibly provides a “refuge” where the fungus can survive outside insect hosts, and the presence of *B. bassiana* in internal plant tissue has been discussed as an adaptive protection against herbivorous insects (reviewed in Meyling and Eilenberg 2007). However, the host plant range of these fungi is, to our knowledge, as yet unknown. Moreover, no field survey of these fungi has been conducted to date in *Tetranychus* spp. (but see, for instance, Debnath and Sreerama Kumar 2017; Dick and Buschman 1995; Van Der Geest et al. 2002, for other fungi and/or spider mite species). Future evaluation of the prevalence of infection by *M. brunneum* and *B. bassiana* in natural populations of spider mites collected on different host plants would thus be necessary to further understand possible factors that could explain the patterns observed in our experiment (Boots et al. 2009).

Decreased host susceptibility to infection may be the result of two different (albeit nonexclusive) mechanisms (Boots et al. 2009; Read et al. 2008): resistance (i.e. reduction in parasite load) and/or tolerance (i.e. reduction of the damage incurred by a parasite). Differential host resistance to fungal infection might be due, for instance, to variability in different cuticular barriers. Such barriers include the absence of factors necessary for parasite recognition, or the presence of inhibitory compounds (phenols, quinones, and lipids) on the cuticle surface, but also the cuticle thickness, its degree of hardening by sclerotization, its resistance to enzymatic degradation and its permeability (reviewed in Hajek and St. Leger 1994). Subsequently, when a fungus bypass cuticular barriers, variability in systemic immunity may also lead to differential host resistance responses. This may include differential activation of the Toll and JAK/STAT pathways, which converge into the transcriptional activation of genes involved in phagocytosis, encapsulation and humoral responses (e.g. Dong et al. 2012). Interestingly, several, but not all, important genes described in these pathways in *Drosophila melanogaster* were absent in the genome of the green form of *T. urticae* (Grbic et al. 2011), and spider mites have high mortality upon bacterial infection (Santos-Matos et al. 2017). Whether the presence of such immune genes vary between or within spider mite species and their expression depend on different fungal species has not been explored to date. In particular, the absence of many important immune genes in *T. urticae* suggests that tolerance mechanisms (e.g. via a decrease of the immune response to avoid autophagy) rather than resistance have been favoured throughout their evolutionary history. However, such hypothesis remains to be tested and further studies are necessary to better understand the mechanisms of spider mite resistance and tolerance against fungal infection.

Independently of the underlying mechanisms at play, whether spider mite populations differ in resistance or tolerance to fungal infection may have different epidemiological and evolutionary consequences,

and, hence, different implications for the long-term success of spider mite control. On the one hand, resistance to infection might be rapidly selected following application of fungi to crops, and subsequently invade spider mite populations, thereby decreasing fungi prevalence and hampering the success of such control strategy. On the other hand, host tolerance should have neutral or even positive effect on parasite prevalence (Boots et al. 2009; Miller et al. 2006; Read et al. 2008), but as, by definition, tolerance minimizes the harm caused by pathogens, it may hamper the efficiency of fungi in controlling spider mites. Moreover, host resistance and tolerance may lead to different evolutionary outcomes for parasite virulence (Boots et al. 2009). Indeed, whereas host resistance is predicted to select for increased parasite virulence (e.g. (Gandon and Michalakis 2000)), host tolerance does not reduce parasite fitness and, therefore, will not lead to antagonistic counter-adaptation by pathogens (Raberg et al. 2007; Rausher 2001). Still, depending on the nature of the tolerance mechanism, it may lead to the evolution of more virulent and transmissible parasites (Miller et al. 2006), with potentially serious implications for non-tolerant populations (Boots et al. 2009), including non-target species such as crop auxiliaries or spider mite predators. Finally, although increased mortality due to infection should lead to a reduction in oviposition duration, spider mites may evolve the ability to compensate infection-driven fitness costs by changing the timing of their reproductive efforts (i.e. ‘fecundity compensation’; (Parker et al. 2011; Vezilier et al. 2015)), thereby limiting the efficiency of fungi applications for population control. Hence, assessing which of these evolutionary outcomes is more likely is timely. In particular, it is likely that the high level of intraspecific variation in susceptibility to infection found in our study is recapitulated within populations and is, at least partly, genetically determined. If this is the case, then this trait may evolve at a rapid pace.

In conclusion, our results show both intra- and interspecific variability in 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 effect of entomopathogenic fungi on the survival of multiple spider mite populations belonging to different species within a single full factorial experiment. In line with laboratory virulence tests that are not necessarily well correlated with field effectiveness (Roberts and Leger 2004), our results highlight the importance of studying several host populations/genomes when assessing the efficiency of a given biocontrol agent, and draw caution on the development of single strains as biocontrol agents as hosts resistance to infection may evolve at a rapid pace.

## **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 Diogo Godinho and Miguel Cruz 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

- Afifi A-AM, Mabrouk AM, Asran AA (2010) Effect of the entomopathogenic fungus *Beauveria bassiana* on three acarine pests. In: Trends in Acarology. Springer, pp 439-440
- Alatawi FJ, Margolies DC, Nechols JR (2007) Aesthetic damage thresholds for twospotted spider mites (Acari : Tetranychidae) on impatiens: Effect of plant age and level of infestation. J Econ Entomol 100:1904-1909 doi:10.1603/0022-0493(2007)100[1904:adtfts]2.0.co;2
- Attia S, Grissa KL, Lognay G, Bitume E, Hance T, Maillieux 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*. Journal of Pest Science 86:361-386 doi:10.1007/s10340-013-0503-0
- Bolker BM (2008) Ecological models and data in R Princeton University Press, New Jersey
- Boots M, Best A, Miller MR, White A (2009) The role of ecological feedbacks in the evolution of host defence: what does theory tell us? Philos Trans R Soc B Biol Sci 364:27-36 doi:10.1098/rstb.2008.0160
- Bourguet D, Guillemaud T (2016) The hidden and external costs of pesticide use. In: Sustainable Agriculture Reviews. Springer, pp 35-120
- Breeuwer JAJ (1997) *Wolbachia* and cytoplasmic incompatibility in the spider mites *Tetranychus urticae* and *T. turkestanii*. Heredity 79:41-47 doi:10.1038/hdy.1997.121
- Bugeme DM, Maniania NK, Knapp M, Boga HI (2008) Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to *Tetranychus evansi*. In: Diseases of Mites and Ticks. Springer, pp 275-285
- Casida JE, Quistad GB (1998) Golden age of insecticide research: Past, present, or future? Ann Rev Entomol 43:1-16 doi:10.1146/annurev.ento.43.1.1
- 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 Science and Technology 15:37-54  
doi:10.1080/09583150410001720617

Crawley MJ (2007) The R Book John Wiley & Sons, Ltd, Chichester, England

Debnath S, Sreerama Kumar P (2017) Fungi associated with mortality of the red spider mite, *Oligonychus coffeae* Nietner (Acari: Tetranychidae), a serious pest of tea in North-Eastern India. Egyptian Journal of Biological Pest Control 27:79-83

Dick GL, Buschman LL (1995) Seasonal occurrence of a fungal pathogen, *Neozygites adjarica* (Entomophthorales: Neozygitaceae), infecting banks grass mites, *Oligonychus pratensis*, and twospotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae), in field corn. Journal of the Kansas Entomological Society:425-436

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). Biological Control 111:6-12 doi:10.1016/j.biocontrol.2017.05.001

Dong Y, Morton Jr JC, Ramirez JL, Souza-Neto JA, Dimopoulos G (2012) The entomopathogenic fungus *Beauveria bassiana* activate toll and JAK-STAT pathway-controlled effector genes and anti-dengue activity in *Aedes aegypti*. Insect biochemistry and molecular biology 42:126-132

Dwyer G, Elkinton JS, Buonaccorsi JP (1997) Host heterogeneity in susceptibility and disease dynamics: tests of a mathematical model. The American Naturalist 150:685-707

Elena SF (2017) Local adaptation of plant viruses: lessons from experimental evolution. Mol Ecol 26:1711-1719 doi:10.1111/mec.13836

Fenner F, Fantini B (1999) Biological control of vertebrate pests: the history of myxomatosis, an experiment in evolution. CABI publishing,

Fiedler Ĺ, Sosnowska D (2007) Nematophagous fungus *Paecilomyces lilacinus* (Thom) Samson is also a biological agent for control of greenhouse insects and mite pests. BioControl 52:547-558

Flamini G (2006) Acaricides of natural origin. Part 2. Review of the literature (2002-2006). Natural Product Communications 1:1151-1158

Gandon S, Michalakis Y (2000) Evolution of parasite virulence against qualitative or quantitative host resistance. Proc R Soc B 267

Godinho DP, Janssen A, Dias T, Cruz C, Magalhães S (2016) Down-regulation of plant defence in a resident spider mite species and its effect upon con- and heterospecifics. Oecologia 180:161-167 doi:10.1007/s00442-015-3434-z

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.6800605

- Grbic M et al. (2011) The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature* 479:487-492 doi:10.1038/nature10640
- 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
- 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). *Egyptian Journal of Biological Pest Control* 28 doi:10.1186/s41938-018-0055-y
- Hajek A, St. Leger R (1994) Interactions between fungal pathogens and insect hosts. *Annual review of entomology* 39:293-322
- Hajek AE, McManus ML, Delalibera I (2007) A review of introductions of pathogens and nematodes for classical biological control of insects and mites. *Biological Control* 41:1-13 doi:10.1016/j.biocontrol.2006.11.003
- Hawley DM, Altizer SM (2011) Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. *Functional Ecology* 25:48-60 doi:10.1111/j.1365-2435.2010.01753.x
- Inglis GD, Goettel MS, Butt TM, Strasser H (2001) Use of hyphomycetous fungi for managing insect pests. *Fungi as biocontrol agents*:23-69
- 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*. *Biological Control* 26:168-173
- 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). *Biological control* 43:222-230
- Lacey LA, Frutos R, Kaya HK, Vail P (2001) Insect pathogens as biological control agents: Do they have a future? *Biological Control* 21:230-248
- Li T, Chen X-L, Hong X-Y (2009) Population genetic structure of *Tetranychus urticae* and its sibling species *Tetranychus cinnabaris* (Acari: Tetranychidae) in China as inferred from microsatellite data. *Annals of the Entomological Society of America* 102:674-683
- Li YY, Floate KD, Fields PG, Pang BP (2014) Review of treatment methods to remove *Wolbachia* bacteria from arthropods. *Symbiosis* 62:1-15 doi:10.1007/s13199-014-0267-1
- 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

- Meyling NV, Eilenberg J (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. *Biological 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/>.
- Migeon A et al. (2009) Modelling the potential distribution of the invasive tomato red spider mite, *Tetranychus evansi* (Acari: Tetranychidae). *Exp Appl Acarol* 48:199-212 doi:10.1007/s10493-008-9229-8
- Miller MR, White A, Boots M (2006) The evolution of parasites in response to tolerance in their hosts: The good, the bad, and apparent commensalism. *Evolution* 60:945-956 doi:10.1554/05-654.1
- Milner R (1982) On the occurrence of pea aphids, *Acyrtosiphon pisum*, resistant to isolates of the fungal pathogen *Erynia neoaphidis*. *Entomol Exp Appl* 32:23-27
- Milner R (1985) Distribution in time and space of resistance to the pathogenic fungus *Erynia neoaphidis* in the pea aphid *Acyrtosiphon pisum*. *Entomol Exp Appl* 37:235-240
- Moscardi F (1999) Assessment of the application of baculoviruses for control of Lepidoptera. *Ann Rev Entomol* 44:257-289 doi:10.1146/annurev.ento.44.1.257
- Opit GP, Chen Y, Williams KA, Nechols JR, Margolies DC (2005) Plant age, fertilization, and biological control affect damage caused by twospotted spider mites on ivy geranium: Development of an action threshold. *Journal of the American Society for Horticultural Science* 130:159-166
- Ormond EL, Thomas APM, Pugh PJA, Pell JK, Roy HE (2010) A fungal pathogen in time and space: the population dynamics of *Beauveria bassiana* in a conifer forest. *Fems Microbiology Ecology* 74:146-154 doi:10.1111/j.1574-6941.2010.00939.x
- Park YL, Lee JH (2002) Leaf cell and tissue damage of cucumber caused by twospotted spider mite (Acari : Tetranychidae). *J Econ Entomol* 95:952-957 doi:10.1603/0022-0493-95.5.952
- Park YL, Lee JH (2005) Impact of two-spotted spider mite (Acari : Tetranychidae) on growth and productivity of glasshouse cucumbers. *J Econ Entomol* 98:457-463
- Park YL, Lee JH (2007) Seasonal dynamics of economic injury levels for *Tetranychus urticae* Koch (Acari, Tetranychidae) on *Cucumis sativus* L. *J Appl Entomol* 131:588-592 doi:10.1111/j.1439-0418.2007.01217.x
- Parker BJ, Barribeau SM, Laughton AM, de Roode JC, Gerardo NM (2011) Non-immunological defense in an evolutionary framework. *Trends Ecol Evol* 26:242-248 doi:10.1016/j.tree.2011.02.005
- Parolin P, Bresch C, Desneux N, Brun R, Bout A, Boll R, Poncet C (2012) Secondary plants used in biological control: A review. *International Journal of Pest Management* 58:91-100 doi:10.1080/09670874.2012.659229



- Perinotto W, Angelo I, Golo P, Quinelato S, Camargo M, Sá F, Bittencourt V (2012) Susceptibility of different populations of ticks to entomopathogenic fungi. *Experimental parasitology* 130:257-260
- Raberg L, Sim D, Read AF (2007) Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* 318:812-814 doi:10.1126/science.1148526
- Rausher MD (2001) Co-evolution and plant resistance to natural enemies. *Nature* 411:857-864 doi:10.1038/35081193
- Read AF, Graham AL, Raberg L (2008) Animal Defenses against Infectious Agents: Is Damage Control More Important Than Pathogen Control? *PLoS Biol* 6:2638-2641 doi:10.1371/journal.pbio.1000004
- Read AP (1995) Genetics and evolution of infectious diseases in natural populations: group report. In: Grenfell BT, Dobson AP (eds) *Ecology of infectious diseases in natural populations*. Cambridge University Press, Cambridge, England, pp 450-477
- Rehner SA (2005) Phylogenetics of the insect pathogenic genus *Beauveria*. *Insect-Fungal Associations: Ecology and Evolution*:3-27
- Ribeiro AEL, Gondim Jr MGC, Calderan E, Delalibera Jr I (2009) Host range of *Neozygites floridana* isolates (Zygomycetes: Entomophthorales) to spider mites. *J Invertebr Pathol* 102:196-202
- 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, vol 54. *Advances in Applied Microbiology*. pp 1-70. doi:10.1016/s0065-2164(04)54001-7
- Santos-Matos G et al. (2017) *Tetranychus urticae* mites do not mount an induced immune response against bacteria. *Proc R Soc B* 284 doi:10.1098/rspb.2017.0401
- Sarmiento RA et al. (2011) A herbivore that manipulates plant defence. *Ecology Letters* 14:229-236 doi:10.1111/j.1461-0248.2010.01575.x
- 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. *Persian Journal of Acarology* 3
- 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 Science and Technology* 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
- 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

- 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. *Biological Control* 46:495-501
- 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. *Biological 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
- Sorci G, Moller AP, Boulinier T (1997) Genetics of host-parasite interactions. *Trends Ecol Evol* 12:196-200
- Stevens L, Rizzo DM (2008) Local adaptation to biocontrol agents: A multi-objective datadriven optimization model for the evolution of resistance. *Ecological Complexity* 5:252-259 doi:10.1016/j.ecocom.2008.04.002
- Tabashnik BE (1994) Evolution of resistance to *Bacillus thuringiensis*. *Ann Rev Entomol* 39:47-79
- Ullah MS, Lim UT (2017) Synergism of *Beauveria bassiana* and *Phytoseiulus persimilis* in control of *Tetranychus urticae* on bean plants. *Systematic and Applied Acarology* 22:1924-1935
- Uma Devi K, Padmavathi J, Uma Maheswara Rao C, Khan AAP, Mohan MC (2008) A study of host specificity in the entomopathogenic fungus *Beauveria bassiana* (Hypocreales, Clavicipitaceae). *Biocontrol Science and Technology* 18:975-989
- Van Der Geest LP, Moraes GJ, Navia D, Tanzini MR (2002) New records of pathogenic fungi in mites (Arachnida: Acari) from Brazil. *Neotropical Entomology* 31:493-495
- 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 Biochemistry and Molecular Biology* 40:563-572 doi:10.1016/j.ibmb.2010.05.008
- Vega FE et al. (2009) Fungal entomopathogens: new insights on their ecology. *Fungal Ecology* 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*. *Biological Control* 57:143-149 doi:10.1016/j.biocontrol.2011.02.007
- Vezilier J, Nicot A, Gandon S, Rivero A (2015) *Plasmodium* infection brings forward mosquito oviposition. *Biology Letters* 11 doi:10.1098/rsbl.2014.0840
- Wang CS, Shah FA, Patel N, Li ZZ, Butt TM (2003) Molecular investigation on strain genetic relatedness and population structure of *Beauveria bassiana*. *Environmental Microbiology* 5:908-915 doi:10.1046/j.1462-2920.2003.00485.x

- Weihrauch F (2005) Evaluation of a damage threshold for two-spotted spider mites, *Tetranychus urticae* Koch (Acari : Tetranychidae), in hop culture. *Annals of Applied Biology* 146:501-509 doi:10.1111/j.1744-7348.2005.040163.x
- 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
- Zélé F, Santos I, Olivieri I, Weill M, Duron O, Magalhães S (2018a) 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, Santos JL, Godinho DP, Magalhães S (2018b) *Wolbachia* both aids and hampers the performance of spider mites on different host plants. *FEMS Microbiol Ecol* 94:fiy187 doi:10.1093/femsec/fiy187
- Zindel R, Gottlieb Y, Aebi A (2011) Arthropod symbioses: a neglected parameter in pest- and disease-control programmes. *Journal of Applied Ecology* 48:864–872 doi:10.1111/j.1365-2664.2011.01984.x