

Article type: Original Article

Can interactions among predators alter the natural regulation of an herbivore in a climate change scenario? The case of *Tetranychus urticae* and its predators in citrus

Pablo Urbaneja-Bernat¹, Victoria Ibáñez-Gual², Marta Montserrat³, Ernestina Aguilar-Fenollosa^{1,4} and Josep A. Jaques¹

¹Unitat Associada d'Entomologia UJI-IVIA, Departament de Ciències Agràries i del Medi Natural, Universitat Jaume I, UJI, Campus del Riu Sec, 12071-Castelló de la Plana, Spain

²Departament de Matemàtiques, Universitat Jaume I, UJI, Campus del Riu Sec, 12071-Castelló de la Plana, Spain

³Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora"-Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Avda. Dr. Weinberg s/n, 29750-Algarrobo-Costa, Málaga, Spain

⁴Torres Hnos. y Suc. S.A.U., Departamento de Calidad, Pol. Ind. Agrícola, nº 2, 12590-Almenara, Spain

*Corresponding author:

Josep A. Jaques
Universitat Jaume I,
Departament de Ciències Agràries i del Medi Natural,
Campus del Riu Sec,
Av. de Vicent Sos Baynat, s/n,
12071 Castelló de la Plana
Tel.: +34 964 72 80 38
Fax: +34 964 72 82 16
E-mail: josep.jaques@camn.uji.es

Key Message

- Pest populations at climate change conditions can reach higher densities than forecasted according to its demographic parameters.
- Natural pest regulation provided by natural enemies depends on environmental conditions, is species-specific and is affected by interspecific interactions. Local extinctions may occur more frequently in a warmer future.
- Spider mite natural regulation can be seriously disrupted in a climate change scenario.
- Pest outbreaks may be increasingly more frequent in a warmer future.

Abstract

Climate change can dramatically affect the food web configuration of arthropod communities through its effects on species interactions. We have studied whether these effects could alter the probability of local extinction of three predatory mites naturally associated with the two spotted spider mite, *Tetranychus urticae*, in citrus in Spain and, therefore, disrupt the biological control of this phytophagous mite. Laboratory and semi-field results provide evidence that the natural regulation of *T. urticae* in citrus can be seriously disrupted in a warmer future. On the one hand, *T. urticae* populations at conditions representative of future hotter and drier summers, could reach higher population densities than forecasted according to its demographic parameters. On the other hand, *T. urticae* regulation provided by its predators depended on the environmental conditions, was species-specific and was affected by interspecific interactions. In some cases, one of the predatory mite species in the system could not be recovered. Because there is evidence that the composition of the community under scrutiny is highly sensitive to local habitat conditions, our results can be taken as evidence that local extinctions may occur more frequently in a warmer future and further contribute to an increasingly higher frequency of *T. urticae* outbreaks.

Keywords: Spider mite, *Phytoseiulus persimilis*, *Neoseiulus californicus*, *Euseius stipulatus*, global warming, food web, phytoseiidae, biological control

Introduction

Most climate change scenarios predict increases in mean temperatures and a rising frequency of extreme weather phenomena (IPCC, 2014). In agricultural systems, these events are often followed by herbivore pest outbreaks, which may be mediated through the impact on their natural enemies (Stireman et al. 2005; Hance et al. 2007; Thomson et al. 2010; Gillespie et al. 2012; Cock et al. 2013; Evans et al. 2013; Jeffs and Lewis 2013). This is the case of spider mites (Prostigmata: Tetranychidae), where outbreaks can follow droughts (English-Loeb 1990) and heat-waves (Montserrat et al. 2013). In general, spider mites are considered to be less heat-sensitive than their natural enemies, mostly predatory mites of the Phytoseiidae family, and these outbreaks can be partly attributed to the relative effects of extreme temperatures on the performance of spider mites and phytoseiids (Ali 1998; Roy et al. 2003; Gotoh et al. 2004), either directly or indirectly (Stavriniades et al. 2010). However, these effects are complex. To fully understand the possible consequences of climate change on agricultural systems, rather than setting the focus on the direct effects on individual species, the focus should be set on the effects on dispersal and the interactions between species. Indeed, these factors can dramatically alter individual fitness, geographic ranges, and the structure and dynamics of the communities where they occur (Davis et al. 1998; Gilman et al. 2010). Interestingly, though, most models of climate change effects on species ignore these interactions (Tylianakis et al. 2008; Blois et al. 2013; Urban et al. 2016).

The Mediterranean basin is considered as one of the most vulnerable areas to climate change (IPCC 2014). In this region, temperature increases between 1.5 and 2.0°C in winter and summer, respectively and 5-15% lower relative humidity values coupled with a 5% decrease in rainfall are forecasted for the mid-XXI century (Diffenbaugh et al. 2007; Giorgi and Lionello 2008; Gualdi et al. 2012; Navarra and Tubiana 2013; Jacob et al. 2014). These adverse climatic conditions combined with low water availability and an increasingly deteriorating quality of groundwater (Paranychianakis and Chartzoulakis 2005), make Mediterranean agriculture especially susceptible to climate change. An important crop in this region is citrus (FAO 2017).

Although there is abundant literature on the effects of climate change, especially drought stress, on the physiology of this crop (Anderson et al. 2004; Rodríguez-Gamir et al. 2010; García-Tejero et al. 2012), there is a lack of information on its effects on the arthropod communities inhabiting this agroecosystem (e.g., Aurambout et al., 2009; Narouei-Khandan et al., 2016). The guild of phytoseiid mites (Mesostigmata: Phytoseiidae) preying on *Tetranychus urticae* Koch (Prostigmata: Tetranychidae) (Abad-Moyano et al. 2009; Aguilar-Fenollosa et al. 2011b), a cosmopolitan pest of many crops (Helle and Sabelis 1985), which is a key pest of clementine mandarins, *Citrus clementina* Tanaka (Rutaceae), in the Mediterranean basin (Martínez-Ferrer et al. 2006; Aguilar-Fenollosa et al. 2011c; Pascual-Ruiz et al. 2014), is one of these communities. It consists of up to six phytoseiid species in commercial orchards of the Region of Valencia (Aguilar-Fenollosa et al. 2011b). These predators have different diet specializations (McMurtry and Croft 1997; McMurtry et al. 2013), ranging from specialized predators of *Tetranychus* spp. (e.g., *Phytoseiulus persimilis* Athias-Henriot), to extreme diet generalists, omnivores feeding on both animal and plant derived food (e.g., *Euseius stipulatus* (Athias-Henriot)), with intermediate diet specializations (e.g., *Neoseiulus californicus* (McGregor), which feeds on tetranychid mites). The conservation of this diversity is considered key for the management of *T. urticae* in clementines (Aguilar-Fenollosa et al., 2011b). On the one hand, *E. stipulatus* is the most abundant phytoseiid species in Spanish citrus orchards irrespective of the citrus cultivar and management practices used (Abad-Moyano et al. 2009a; Aguilar-Fenollosa et al. 2011a; Vela et al., 2017). This numerical superiority makes it an important predator of *T. urticae*. However, it is not the most effective one. A study of gut content analysis using molecular techniques, showed that this role was played by *P. persimilis*, which preyed on tetranychids almost five times more frequently than *E. stipulatus* (Pérez-Sayas et al., 2015). This specialist though, same as *N. californicus*, hardly represents 10% of total phytoseiid records in these orchards. The relevance of these two predators has been related to their ability to enter into the web colonies (Sabelis and Bakker, 1992; Montserrat et al., 2008). This complex situation and the fact that Abad-Moyano et al., (2010b) suggested the occurrence of lethal and non-lethal intraguild interactions between *E. stipulatus* and *P. persimilis* and between *E.*

stipulatus and *N. californicus*, respectively, make this community (*T. urticae* and the three phytoseiids) a suitable model to investigate how species interactions may alter the probability of local extinction in a climate change scenario and how these processes may affect the future natural regulation of a herbivore pest species. To achieve this goal, a semi-field assay including different community modules was run at hotter and drier spring and summer conditions representative of future climate change in the Mediterranean basin (Diffenbaugh et al. 2007; Giorgi and Lionello 2008; Gualdi et al. 2012; Navarra and Tubiana 2013; Jacob et al. 2014). Some demographic parameters of *T. urticae* were also determined under similar conditions in the laboratory.

Material and Methods

Plant material

Two-year-old clementine plants (*C. clementina* cv. Clementina de Nules grafted on citrange Carrizo) were used as experimental units in the semi-field experiment and as a source of leaves for laboratory assays. Fifty days before the beginning of each assay, 100 plants were defoliated and kept in a greenhouse at Universitat Jaume I (UTM: 39°59'10.883"N 0°3'4.769"W) set at $22 \pm 2^\circ\text{C}$, $55 \pm 10\%$ relative humidity and natural photoperiod. These plants were grown on vermiculite and peat (1:3; vol:vol) in 320 ml pots, were fertilized twice per week using a modified Hoagland's solution (Bañuls et al. 1997), and received no pesticide treatments. Bean leaves (*Phaseolus vulgaris* L. (Fabaceae)), lemon fruits (*Citrus lemon* Burm. f. (Rutaceae)) and *Carpobrotus edulis* (L.) (Aizoaceae) pollen (dried at 37°C , sieved, and frozen until use) obtained from pesticide-free plants were used to maintain mite stock colonies (see below).

Mite stock colonies

Four different mite species were used in our studies: the two-spotted spider mite *T. urticae*, and the phytoseiids *E. stipulatus*, *N. californicus*, and *P. persimilis*. These colonies were maintained in separate climatic chambers set at $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ relative humidity and a 16-hour light photoperiod.

Spider mites were originally collected in a Clementina de Nules orchard at Les Alqueries (UTM: 39°59'15.1"N 0°3'02.0"W) in 2010. This colony has been maintained ever since using standard procedures on detached leaves of clementine mandarins (Aguilar-Fenollosa et al. 2012) and, in some cases (see below), on pesticide-free lemon fruits (Abad-Moyano et al. 2010a). When spider mites were used to either feed the phytoseiid stock colonies or to start new cohorts for laboratory assays, bean leaflets were used. When used to feed phytoseiids, leaflets were infested by exposure to lemon fruit colonies. Cohorts were established by transferring 100 females to new rearing arenas on clementine leaves. Females were removed 1 day later and less than 24-h old eggs were further used in the assays.

Individuals of *N. californicus* were obtained from Koppert Biological Systems (SPICAL®) to initiate a laboratory colony. In contrast, *P. persimilis* and *E. stipulatus* were originally collected in 2012 in two clementine orchards in Les Alqueries (same location as *T. urticae*) and Montcada (UTM: 39° 32' 42.906" N 0° 23' 45.699" W), respectively. Phytoseiid stock colonies were maintained on detached leaf arenas using standard procedures (Pina et al. 2012). These arenas consisted of single bean leaflets placed upside down on moistened filter paper placed on top of a water saturated foam cube (3–4 cm thick) in an open plastic box half-filled with water. Colonies received detached bean leaves infested with *T. urticae* and *C. edulis* pollen as food.

Semi-field assay

Two experiments were carried out in spring (7 to 31 May) and summer (5 to 28 July) 2016 in a plot located in a commercial clementine orchard at Les Alqueries. Two lines of 12 trees each (N-S orientation) had been previously removed and replaced by 24 mesh cages (3 × 3 × 3 m) with a zipped door (1.5 m high) on one of their sides. Our intention was that by using these cages, environmental conditions inside the cage would match temperature and RH expected conditions representative of future climate change in the Mediterranean basin (Difflenbaugh et al. 2007; Giorgi and Lionello 2008; Gualdi et al. 2012; Navarra and Tubiana 2013; Jacob et al. 2014). Thirteen of these cages were randomly selected and used for different treatments (see below). Each cage received five potted clementine plants (i.e, five replicates per treatment)

regularly distributed within the cage without touching each other and the cage. To further prevent ambulatory mite movement between plants, the pots were set on top of a 9 cm diameter Petri dish placed in a round plate (16.5 cm in diameter) filled with water. Moreover, permanent glue (Tree Tanglefoot®; Grand Rapids, MI, USA) was applied at the base of the trunk and renewed twice per week. Plants were drip irrigated and the soil was kept weed-free. Four data loggers (model TESTO® 175-H, Madrid, Spain) were used to register hourly temperature and relative humidity at a height of 1.5 m above the ground inside and outside the cages.

At the beginning of the assay, each plant received 10 *T. urticae* adult females from the clementine stock colony. Each individual was transferred to a different leaf using a fine brush. One week later, the phytoseiids were released in different combinations corresponding to three different community modules (= treatments). One cage constituted the control and the five replicates within that cage received no phytoseiids. The other 12 treatments consisted of (a) the release of only one species of phytoseiid (trophic chain community module; three cages, one for each phytoseiid species), (b) the simultaneous release of two species (competition community module; three cages, one for each two-phytoseiid species combination), and (c) the sequential release in a 1-week interval of two phytoseiid species (sequential release, six cages, one for each two-phytoseiid species sequence). The release rate for all phytoseiids was 10 adult females per plant. These corresponded to 10 individuals of the same species for the trophic chain, and five mites of each species for the competition and the sequential release modules. Experiments were discontinued as soon as plants in the control treatment collapsed about eight weeks after the initial release of *T. urticae*.

Starting one week after the release of *T. urticae*, when first symptoms (chlorotic spots on leaves) were observed and prior to the release of phytoseiids, and then twice per week, *T. urticae* density was assessed on every plant. The numbers of (a) symptomatic leaves per plant and (b) females on up to eight of these leaves randomly selected without removing them from the plant (Agut et al. 2016) were counted. By multiplying both figures, an estimation of *T.*

urticae population per plant (i.e., density) was obtained. Additionally, as a measure of damage, cumulative mite days (CMD) per plant at the end of the assay were calculated as:

$$\sum \Delta t \frac{(X_1 + X_2)}{2}$$

Where \sum is summation over all sampling dates; Δt is the interval between two successive sampling dates (3-4 days); and X_1 and X_2 are *T. urticae* densities on those consecutive dates. At the end of the assay, to assess phytoseiid abundance and diversity, 25 leaves were randomly collected from trees in the same treatment. They were transported to the laboratory in a plastic bag in a cooler for mite extraction using Berlese funnels. Extracted arthropods were preserved in 70% ethanol and adult phytoseiids further processed for microscope observation in Hoyer's medium (Gutiérrez, 1985) and identification to species level following Ferragut et al. (2010). Immature forms of *N. californicus* and *E. stipulatus* in treatments where these two species had been released together could not be distinguished from each other. In the case of *P. persimilis*, because of the characteristic orange color of motiles, all forms, adult and immature, could be satisfactorily identified at species level.

Tetranychus urticae densities were analyzed using a generalized linear mixed model (GLMM) with a normal distribution of error and identity link function, with 'season' (spring and summer conditions of climate change in the Mediterranean) and 'treatment' (control, trophic chain, competition and sequential release) as fixed factors and 'time' (sampling date) as a random factor. Time was expressed as degree-days (DD) since initial *T. urticae* infestation using daily mean temperatures and a lower development threshold of 10.45°C according to Bounfour and Tanigoshi (2001). As our main goal was to identify possible seasonal differences (spring versus summer climate change conditions), we started the analysis of *T. urticae* densities by considering the main effect of the 'season' factor, as well as the interaction between 'season' and 'treatment'. Once the significance of the interaction between these two factors was verified, we continued the study of *T. urticae* density for each season separately. Akaike information

criterion (AIC) (Akaike 1974) was used to select the best model. When necessary, pairwise comparisons were made using the Bonferroni post-hoc test ($P < 0.05$).

Finally, we compared CMD and the variation of this parameter relative to control for every treatment at the end of the assay using a Generalized Linear Model (GLM), with a normal distribution of the error and identity link function (i.e, linear regression). When significant differences were found, we used Bonferroni post-hoc test ($P < 0.05$) for mean separation. Additionally, differences between seasonal values of CMD and their variation (difference between spring and summer climate change conditions) were analyzed using Student t -test ($P < 0.05$).

Laboratory assay

The intrinsic rate of increase (r_m) of *T. urticae* on clementine leaves was determined at 25°C and 70% RH (as a proxy of conditions in our hotter and drier spring experiment), and 30°C and 50% RH (as a proxy of conditions in our hotter and drier summer experiment). Less than 24-h old adult females obtained from a cohort were individually transferred to arenas consisting of a 5 cm diameter dish, with a 3 cm diameter hole in the cover. The base of the dish was filled with bacteriological agar (2.5% weight) to maintain the turgor of the leaves. As soon as agar was cold and solid enough, a fully expanded clementine leaf was placed upside down on top of the agar. The cover was subsequently put in place in such a way that the leaf substrate formed a 3 cm in diameter exposed area. To prevent *T. urticae* escape from the experimental arena, permanent glue (Tree Tanglefoot®; Grand Rapids, MI, USA) was applied along the rim of the arena. Finally, the dishes were sealed with Parafilm® (Pechiney Plastic Packaging, Menasha, WI, USA). Thirty arenas per environmental condition were assembled and a male was subsequently added to ensure mating. Males dying during the first 4 days were replaced. Oviposition and survival were scored daily until the female died. Non-ovipositing females and those escaping from the arenas were not considered for analyses. Egg hatching, immature survival, developmental time and sex ratio were assessed on 30 additional arenas per environmental condition. In this case, one egg less than 24 h-old was introduced into the setup.

Arenas were checked daily using a binocular microscope for hatching, molting and survival until the immature stages reached adulthood. With these data different demographic parameters, including egg hatching, immature survival, development time, sex ratio, and intrinsic rate of increase, were calculated. The r_m was calculated according to Birch (1948) and the Jackknife procedure was used to estimate the standard error of r_m values. Subsequently, r_m values were compared using a t -test (Maia et al. 2000). In order to facilitate comparison of the results obtained at the two environmental conditions considered, parameters depending on time (e.g., development time, r_m) were calculated with time expressed as days and degree-days (DD) as before.

All statistical analyses were performed using IBM SPSS Statistics 23.0.

Results

Semi-field assays

Daily mean temperatures inside the cages were 26.2 and 31.9°C during the spring and summer assays, respectively. These temperatures were on average 4°C higher than outside (Figure 1). Mean RH values within the cages were 64.7 and 45.2% in spring and summer, respectively and these values were on average 8 and 18% lower than outside the cages. At these conditions, control plants collapsed because of *T. urticae* damage nine and seven weeks after the initial infestation in spring and summer climate change conditions, respectively.

The results of the identification of the phytoseiid species found at the end of the assays are shown in Table 1. As expected, we did not find any alien phytoseiid in our treatments and the numbers of specimens recovered in summer were in general higher than in spring. We recovered a minimum of 3 and a maximum of 13 individuals per treatment in the trophic chain module, which corresponded to *E. stipulatus* and *P. persimilis*, respectively, in summer. In the case of competition, no specimens of *N. californicus* were recovered in spring irrespective of the combination considered. The same happened to this species and *E. stipulatus* when competing with each other in summer. *P. persimilis* was always the most abundant species. When

phytoseiids were sequentially released in spring, *E. stipulatus* and *N. californicus* could hardly be recovered. In these cases, less than two immature specimens were found. Same as in the competition module, *P. persimilis* was always the most abundant species. As a consequence, when looking at the grand totals recovered each season, *P. persimilis* was the most abundant species, regardless of being introduced first, second, or simultaneously with another predatory mite in the system.

The density of *T. urticae* along time was significantly affected by the factors 'season' and 'treatment' and their interaction (Table 2). Therefore, data were further analyzed separately for each season. Significant differences were observed for all treatments (Table 3 and 4) and the same occurred when we compared CMD values at the end of the assays (Table 5). Interestingly, for the same DD values, *T. urticae* densities observed in the control in summer were higher than in spring (Figure 2). Actually, *T. urticae* density in control reached 500 mites per plant at the end of the spring assay, when almost 500 DD had been accumulated, whereas the same density was reached at around 400 DD in summer. As a consequence, CMD values in the control at the end of the assay in summer were higher than in spring (Table 5).

The dynamics of *T. urticae* for the same treatment did not follow the same patterns in spring and summer (Figure 2). When singly released in the trophic chain module, all three phytoseiids affected *T. urticae* dynamics in spring, with *E. stipulatus* resulting in significantly different dynamics from those observed where *N. californicus* and *P. persimilis* were released (Figure 2a). These differences, though, did not affect mite damage, measured as CMD (Table 5), but resulted in *E. stipulatus* being more effective at reducing *T. urticae* densities than the other two species (Table 5). Remarkably, the dynamics of the herbivore were not significantly affected by the release of *E. stipulatus* in summer (Figure 2b). Therefore, plants where this phytoseiid was released showed the same level of damage as control (Table 5) and efficacy of *E. stipulatus* at summer climate change conditions was nil. On the contrary, same as in spring, the other two phytoseiid species significantly decreased the densities of the herbivore in a similar manner (Figure 2b), resulting in lower damage (Table 5), and similar efficacies. To sum up, hotter and

drier summer conditions representative of future climate change in the Mediterranean significantly reduced the efficacy of *E. stipulatus*, whereas they enhanced the performance of the other two phytoseiids.

When two phytoseiid species were simultaneously released in the competition module, differences between spring and summer dynamics were also observed. All combinations resulted in dynamics different from control in spring (Figure 2c). However, the combination including *E. stipulatus* and *N. californicus* did not differ from control in summer (Figure 2d). When these dynamics were translated into damage, the combination *P. persimilis* and *E. stipulatus* resulted in lowest damage values and highest efficacies both in spring and summer (Table 5). Interestingly, this combination in spring was the only case where the joint release resulted in higher efficacies than those corresponding to the same species separately (Table 6). Therefore, competition at summer hotter and drier conditions representative of future climate change significantly decreased the efficacy of phytoseiids, especially in those combinations where *N. californicus* was present (Table 5). Remarkably, this species was recovered only once in these treatments at the end of the assay (Table 1), whereas the number of specimens of *P. persimilis* recovered was similar irrespective of being released alone or together with another phytoseiid, in spite that the initial number of *P. persimilis* females when this species was released with another phytoseiid was half than when released alone.

The effects of the season on the dynamics of *T. urticae* when two phytoseiid species were released sequentially depended on the identity of the pair (Figure 2e-j). Only the combinations not including *P. persimilis* resulted in different dynamics in spring and summer climate change conditions and the release sequence was significant in summer only (Figure 2e and 2f). Moreover, in combinations including *P. persimilis* (Figures 2g-j) the sequence was irrelevant when released with *N. californicus* (Figure 2g and h). However, when *P. persimilis* was released with *E. stipulatus* (Figures 2i and j), the release of *P. persimilis* first consistently resulted in lower densities of *T. urticae*. In all cases these releases decreased the CMD at the end of the assays (Table 5), resulting in efficacies ranging from 10 to 90%. Lowest efficacies corresponded

the sequence *N. californicus* and *E. stipulatus*. The extremely low numbers of both predatory mite species recovered in spring at the end of the assay, just two immature stages (Table 1), may be related to this low efficacy. However, in summer *E. stipulatus* was five times more abundant than *N. californicus* and yet, efficacy was low.

The sequential release treatments allowed us to compare the effect of phytoseiid initial density on *T. urticae* dynamics during both seasons. Five and 10 females were initially released in the sequential release and the trophic chain treatments, respectively. Therefore, relatively higher initial efficacies were expected in the trophic chain module. In spring, at 233 DD (equivalent to 115 DD after the initial release of the phytoseiids), lower densities of *T. urticae* relative to control were observed (Table 6). However, only for *E. stipulatus*, the decrease achieved by the release of 10 individuals was higher than when releasing five. For the other two species, the same reduction was obtained irrespective of the release dose. In summer, though, the situation was much more variable. At 227 DD (63 DD after the release), lower densities relative to control were observed for all phytoseiids. However, at that date, the release of five predators resulted in higher reductions than 10. Just before the release of the second predator in the sequential release treatments (at 312 DD), as expected, lower densities of *T. urticae* were observed with the initial release of 10 predators (trophic chain treatments), except for *E. stipulatus*. In this case, efficacies were much lower than for the other phytoseiids and the release of 10 predators did not reduce *T. urticae* densities.

Laboratory assays

The durations in days of preoviposition, oviposition, and lifespan periods, and development time were longer at conditions representative of future hotter and drier spring than summer, whereas the postoviposition period was shorter. However, when these parameters were expressed in DD, there were no differences except for development time, which was still longer at spring climate change conditions (Table 7). No differences between conditions were observed for immature survival and sex ratio. However, fecundity was higher at spring conditions, same as oviposition rate and egg hatching at summer climate change conditions (Table 8). When

these values were combined to calculate net fecundity, generation time and r_m , differences appeared when the last two parameters were referred to days. However, they disappeared for r_m when time was expressed as DD (Table 9).

Discussion

The main objective of this study was to investigate how species interactions could alter the probability of local extinction in a climate change scenario and how these processes could affect the future natural regulation of *T. urticae*. As expected, the environmental conditions achieved during our assays inside the meshed cages fell within the 1.9-5.4°C higher mean temperature (Jacob et al. 2014) and 5-15% lower relative humidity (Diffenbaugh et al. 2007) predicted for the Mediterranean Basin for the period 2071-2100. Therefore, the spring and summer conditions during our assays inside the cages can be considered as representative of the predicted climate change scenario. Under these conditions, our results provide evidence that the natural regulation of *T. urticae* can be seriously disrupted. On the one hand, disproportionate population increases of *T. urticae* during the summer (see control curves in Figure 2) cannot be explained through the direct effects of temperature on spider mites (Table 9) and they are most probably mediated through the impact of climate change on the host plant. On the other hand, *T. urticae* regulation provided by its predators depends on environmental conditions (i.e., the season), is species-specific, and is affected by interspecific interactions. In some cases, the result of these interactions was the disappearance of one of the predators from the system (Table 1). Because there is evidence that the composition of the phytoseiid community under scrutiny is highly sensitive to local habitat conditions (Aguilar-Fenollosa et al. 2011a), our results can be taken as evidence that local extinctions may occur more frequently in future warmer seasons and further contribute to *T. urticae* outbreaks.

Although highly controversial (Koricheva et al., 1998; Larsson, 1989; Galway et al., 2003; White, 2009), the Plant Stress Hypothesis predicts that abiotic stress increases the suitability of plants as food for herbivores (White, 1969; 1993). This effect has been related to an increase of the nutritional quality of stressed plants (White 1984; Inbar et al. 2001) and a change in the

production of secondary metabolites and defensive proteins (Cornelissen et al. 2008). Plant leaves usually accumulate proline in response to environmental stresses (i.e., heat, drought) to regulate the osmotic potential. Indeed, heat tolerant plants, including citrange Carrizo, which is the rootstock used in our assays, accumulate higher amounts of this amino acid when exposed to thermal stress (Zandalinas et al. 2016). Interestingly, Ximénez-Embún et al. (2016) demonstrated the stimulating effect of this non-essential amino acid on feeding and egg-laying of *Tetranychus evansi* Baker & Pritchard (Prostigmata: Tetranychidae), a close relative of *T. urticae*, when added to tomato leaf disks. Should this effect occur in *T. urticae*, it could partly explain the higher than expected densities observed during the summer semi-field assay. Moreover, the salicylic acid defense pathway, which is involved in plant thermotolerance mechanisms (Larkindale and Huang 2005; Wang and Li 2006; Clarke et al. 2009), is induced by high temperatures in citrus (Zandalinas et al. 2016). This defense pathway often has a negative cross-talk with the jasmonic acid (JA) pathway (Pieterse et al. 2009), which is the key pathway in the defensive response of citrus to *T. urticae* (Agut et al. 2015). Therefore, both effects (proline accumulation in leaves and down-regulation of the JA pathway) provide a plausible explanation for the unexpected higher densities of *T. urticae* observed under summer conditions. Moreover, the stimulating effect of fluctuating temperatures (i.e., semi-field trial) relative to constant temperatures (i.e., laboratory assays) on the demographic parameters of *T. urticae* (Vangansbeke et al. 2013, 2015; Bayu et al. 2017) should not be neglected. These bottom-up effects alone could result in mite outbreaks in a warmer future. However, top-down effects are also at play.

In general, we observed a better natural regulation of *T. urticae* in spring than in summer climate change conditions (Figure 2). Although the average reductions in *T. urticae* populations observed in spring and summer were similar (55.9 ± 4.9 versus $59.7 \pm 5.1\%$, respectively), differences between treatments were larger in summer (ranging from 0 to 97.6% for *E. stipulatus* and *P. persimilis*, respectively), than in spring (ranging from 16.3 to 77.1% for the

sequential release of *N. californicus* plus *E. stipulatus* and *P. persimilis* plus *E. stipulatus*, respectively).

The three phytoseiid species under scrutiny provided a similar level of control when released singly in spring. However, *E. stipulatus* provided no control at climate change summer conditions, while the other two species were even more effective than in spring (Fig. 2a and 2b). This result may be partly explained by the low numbers of *E. stipulatus* recovered at the end of the summer assay (Table 1), which may be indicative of direct harmful effects of hotter and drier summer climate change conditions on the physiology and/or the behavioral responses of this predator. These altered behaviors could include increased intraspecific competition and cannibalism but also increased dispersal when seeking for shelter to avoid heat, a costly behavior in terms of lost foraging and reproduction opportunities (Gillespie et al. 2012). Grafton-Cardwell et al. (1997) also recognized *E. stipulatus* to significantly reduce *T. urticae* populations in 2-year-old orange trees in a semi-field experiment in spring. Conversely, during the summer both *N. californicus* (Palevsky et al., 2008; Walzer et al., 2007) and *P. persimilis* (Schausberger and Walzer 2001) alone have been considered as better suited for immediate suppression of local spider mite populations under glasshouse cropping conditions. Because *E. stipulatus* is the most abundant phytoseiid species in Valencian clementine orchards (Abad-Moyano et al. 2009; Aguilar-Fenollosa et al. 2011b) one might consequently expect *T. urticae* problems to worsen with future warmer and drier summers. However, as under these conditions *P. persimilis* appears the most abundant species (Table 1) and this phytoseiid is considered as the most efficient predator of *T. urticae* in clementines (Pérez-Sayas et al. 2015) the situation could be reverted. Notwithstanding, as *N. californicus* also endured these conditions, increased lethal and non-lethal intraspecific interactions between these two phytoseiids resulting in a deficient regulation of *T. urticae* populations may be anticipated. Indeed, a less efficient regulation of *T. urticae* was observed for this phytoseiid combination in the competition module in summer (Fig. 2d; 16.3 versus 43.6% decrease in CMD in summer and spring, respectively) in spite that the number of specimens of both species recovered at the end of the assay were higher than in spring (Table 1).

When further looking at the results of the simultaneous release in pairs of the three phytoseiids, the combination of *P. persimilis* and *E. stipulatus* was the only one that did not lose efficacy in summer compared to spring. Intriguingly, the numbers of *P. persimilis* at the end of the assay were similar independently of the phytoseiid species it had been released with (Table 1). Because the original populations of *P. persimilis* and *E. stipulatus* used in our assays were collected in the vicinity of the site where our assays took place, whereas the origin of *N. californicus* remains ignored (i.e., it was a commercial strain), we hypothesize that the former two local strains were adapted to coexist and endure present climatic fluctuations at the site where our assays took place (Aguilar Fenollosa and Jacas 2014). In fact, the two competition treatments where *N. californicus* was released resulted in efficacies below 21% in summer (Table 4; Figure 2d) and the disappearance of both predators when released with *E. stipulatus* (Table 1). These results may be indicative that contrary to the results of Abad-Moyano et al. (2009), where *E. stipulatus* was pointed out as the most aggressive phytoseiid of the three included in this study, under climatic change conditions this hierarchy may change. Such a change has been described for aphid predators (Barton and Ives 2014) and fish communities (Matthews and Wong 2015; Kerry and Bellwood 2016).

In the case of sequential releases, natural regulation was remarkably impaired when *N. californicus* was first released and followed by *E. stipulatus*, especially in summer (Fig. 2e and 2f). However, for the remaining combinations sequential releases resulted in a better regulation of *T. urticae* populations than the simultaneous release of the two species (Fig. 2). Besides, in summer, at least one specimen per species was found at the end of the assay in all six combinations considered, whereas in summer no specimens of *N. californicus* and *E. stipulatus* were found when these species were simultaneously released in the competition module (Table 1). The week elapsed between the release of the first and the second phytoseiid when sequentially released most likely allowed the first predator in the system to oviposit and produce some immature motile stages free of the negative interactions between adult heterospecific females (competition, intraguild predation). This was also the case for *N. californicus* and *E.*

stipulatus. However, in this case, only one adult specimen of *N. californicus* was found at the end of the assay and this may be indicative of a preference of *E. stipulatus* for the immature stages of the former species. In this case, the difficulties encountered by *E. stipulatus* to cope with the web produced by *T. urticae* (Shimoda et al. 2009) may explain a preference for the immature stages of heterospecific phytoseiids.

The comparison of the results obtained in the trophic chain and the sequential release treatments during the first week after the release of the first predator species (Table 5) reveals the occurrence of some type of intraspecific interference, either competition or cannibalism, resulting in lower efficacies for release numbers above a certain threshold at summer climate change conditions. This result should be taken into account in future management tactics exploiting augmentative biological control, as it may be relevant for the calculation of the numbers of these predators necessary for release in citrus orchards (Abad-Moyano et al. 2010b, a).

In general, *P. persimilis* alone or in combination with the other predators, produced the highest efficacies both in spring and summer climate change conditions. Therefore, our results reinforce the idea that this is the key species to maintain in the system to ensure natural regulation of *T. urticae* populations in clementines (Aguilar-Fenollosa et al., 2011b). Nevertheless, some authors (Skirvin and Fenlon 2003; Walzer et al. 2007), consider this *T. urticae* specialist a suitable predator until 25°C but not at higher temperatures. The use of a local strain of *P. persimilis* presumably adapted to prevailing climatic conditions in clementine orchards may explain our results, which highlights the importance of locally adapted natural enemies to cope with climate change (Aguilar-Fenollosa and Jacas 2014). To further confirm this hypothesis, it would be important to test a local strain of *N. californicus* occurring in clementines.

One striking result of our experiments is that in almost half of the combinations including *N. californicus* and one third of those including *E. stipulatus*, we could not recover any motile at the end of the assay whereas this situation was never encountered for *P. persimilis* (Table 1). Even though we assume that our samplings were representative of the abundance of these

predators in our trees, we cannot exclude some circadian rhythms occurring in this system, which may have hindered the estimation of their real abundance (Pérez-Sayas et al. 2015). Moreover, as *N. californicus* is prone to seek refuge in citrus branches and trunks (Pérez-Sayas et al. 2017), the results of the number phytoseiids recovered at the end of our assays should be taken with caution and, if possible, further assays digging on the same issue should consider not only the sampling of leaves but also branches and/or different times.

To sum up, our results are indicative that climate change may actually disrupt existing natural regulation of *T. urticae* in citrus, especially in increasingly hotter and drier summers. Further laboratory research under controlled conditions could help explain some of the results obtained. This will allow a better prediction of future outcomes of the interactions considered in this study and also the design of fit-for-purpose tactics for the effective future management of this pest, ideally aimed at maintaining phytoseiid diversity which guarantees natural regulation of this pest.

Author contribution statement

JAJ and MM conceived and designed research. JAJ and PUB conducted the experiments. All authors analyzed results and wrote the manuscript.

Acknowledgments

This research was partially funded by MINECO (grants AGL2011-30538-C03-01 and AGL2014-55616-C3-3-R). The authors thank M. Piquer (UJI) and A. Sanz (UJI) for technical assistance and J. Calvo (KOPPERT BS, Spain) for supplying *N. californicus*. PUB received a predoctoral grant from MCINN (EEBB-I-14-08555).

Compliance with Ethical Standards

Funding: this study was partially funded by the Spanish Ministry of Economy and Competitiveness, MINECO, (grants AGL2011-30538-C03-01 and AGL2014-55616-C3-3-R) and the Spanish Ministry of Science and Innovation, MCINN, (grant EEBB-I-14-08555).

Conflict of interest: no conflict exists.

Ethical approval: this article does not contain any studies with human participants performed by any of the authors.

References

- Abad-Moyano R, Pina T, Dembilio Ó, et al (2009) Survey of natural enemies of spider mites (Acari: Tetranychidae) in citrus orchards in eastern Spain. *Exp Appl Acarol* 47:49–61. doi: 10.1007/s10493-008-9193-3
- Abad-Moyano R, Pina T, Pérez-Panadés J, et al (2010a) Efficacy of *Neoseiulus californicus* and *Phytoseiulus persimilis* in suppression of *Tetranychus urticae* in young clementine plants. *Exp Appl Acarol* 50:317–28. doi: 10.1007/s10493-009-9318-3
- Abad-Moyano R, Urbaneja A, Hoffmann D, Schausberger P (2010b) Effects of *Euseius stipulatus* on establishment and efficacy in spider mite suppression of *Neoseiulus californicus* and *Phytoseiulus persimilis* in clementine. *Exp Appl Acarol* 50:329–341. doi: 10.1007/s10493-009-9320-9
- Abad-Moyano R, Urbaneja A, Schausberger P (2010c) Intraguild interactions between *Euseius stipulatus* and the candidate biocontrol agents of *Tetranychus urticae* in Spanish clementine orchards: *Phytoseiulus persimilis* and *Neoseiulus californicus*. *Exp Appl Acarol* 50:23–34. doi: 10.1007/s10493-009-9278-7
- Aguilar-Fenollosa E, Ibáñez-Gual V, Pascual-Ruiz S, et al (2011a) Effect of ground-cover management on spider mites and their phytoseiid natural enemies in clementine mandarin orchards (I): Bottom-up regulation mechanisms. *Biol Control* 59:158–170. doi: 10.1016/j.biocontrol.2011.06.013
- Aguilar-Fenollosa E, Ibáñez-Gual V, Pascual-Ruiz S, Jacas JA (2011b) Effect of ground-cover management on spider mites and their phytoseiid natural enemies in clementine mandarin orchards (II): Top-down regulation mechanisms. *Biol Control* 59:171–179. doi: 10.1016/j.biocontrol.2011.06.012
- Aguilar-Fenollosa E, Jacas JA (2014) Can we forecast the effects of climate change on entomophagous biological control agents? *Pest Manag Sci* 70:853–859. doi: 10.1002/ps.3678
- Aguilar-Fenollosa E, Pascual-Ruiz S, Hurtado MA, Jacas JA (2011c) Efficacy and economics of ground cover management as a conservation biological control strategy against *Tetranychus urticae* in clementine mandarin orchards. *Crop Prot* 30:1328–1333. doi: 10.1016/j.cropro.2011.05.011
- Aguilar-Fenollosa E, Pina T, Gómez-Martínez MA, Jacas JA (2012) Does host adaptation of *Tetranychus urticae* populations in clementine orchards with a *Festuca arundinacea* cover contribute to a better natural regulation of this pest mite? *Entomol Exp Appl* 144:181–190.

doi: 10.1111/j.1570-7458.2012.01276.x

- Agut B, Gamir J, Jaques JA, Flors V (2015) *Tetranychus urticae* -triggered responses promote genotype- dependent conspecific repellence or attractiveness in citrus. *New Phytol* 207:790–804. doi: 10.1111/nph.13357
- Ali FS (1998) Life tables of *Phytoseiulus macropilis* (Banks) (Gamasida: Phytoseiidae) at different temperatures. *Exp. Appl. Acarol.* 22:335–342.
- Anderson PK, Cunningham AA, Patel NG, et al (2004) Emerging infectious diseases of plants: Pathogen pollution, climate change and agrotechnology drivers. *Trends Ecol Evol* 19:535–544. doi: 10.1016/j.tree.2004.07.021
- Aurambout JP, Finlay KJ, Luck J, Beattie GAC (2009) A concept model to estimate the potential distribution of the Asiatic citrus psyllid (*Diaphorina citri* Kuwayama) in Australia under climate change-A means for assessing biosecurity risk. *Ecol Modell* 220:2512–2524. doi: 10.1016/j.ecolmodel.2009.05.010
- Bañuls J, Serna MD, Legaz F, et al (1997) Growth and gas exchange parameters of Citrus plants stressed with different salts. *J Plant Physiol* 150:194–199. doi: 10.1016/S0176-1617(97)80202-7
- Barton BT, Ives AR (2014) Direct and indirect effects of warming on aphids, their predators, and ant mutualists. *Ecology* 95:2069–2076. doi: 10.1002/ecy.1851
- Bayu MSYI, Ullah MS, Takano Y, Gotoh T (2017) Impact of constant versus fluctuating temperatures on the development and life history parameters of *Tetranychus urticae* (Acari: Tetranychidae). *Exp Appl Acarol* 72:205–227. doi: 10.1007/s10493-017-0151-9
- Blois JL, Zarnetske PL, Fitzpatrick MC, Finnegan S (2013) Climate Change and the Past, Present, and Future of Biotic Interactions. *Science* (80-) 341:499–504. doi: 10.1126/science.1237184
- Bounfour M, Tanigoshi LK (2001) Effect of Temperature on Development and Demographic Parameters of *Tetranychus urticae* and *Eotetranychus carpini* (Acari: Tetranychidae). *Ecol Popul Ecol* 400–404. doi:10.1603/0013-8746
- Clarke SM, Cristescu SM, Miersch O, et al (2009) Jasmonates act with salicylic acid to confer basal thermotolerance in *Arabidopsis thaliana*. *New Phytol* 182:175–187. doi: 10.1111/j.1469-8137.2008.02735.x
- Cock MJW, Biesmeijer JC, Cannon RJC, et al (2013) The implications of climate change for positive contributions of invertebrates to world agriculture. *CAB Reviews* 8: 1-47. doi: 10-1079/PAVSNR20138028
- Cornelissen T, Fernandes GW, Vasconcellos-Neto J (2008) Nordic Society Oikos Size Does Matter: Variation in Herbivory between and within Plants and the Plant Vigor. *Source: Oikos* 117:1121–1130. doi: 10.1111/j.2008.0030-1299.16588.x
- Davis AJ, Lawton JH, Shorrocks B, Jenkinson LS (1998) Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. *J Anim Ecol* 67:600–612. doi: 10.1046/j.1365-2656.1998.00223.x
- Diffenbaugh NS, Pal JS, Giorgi F, Gao X (2007) Heat stress intensification in the Mediterranean climate change hotspot. *Geophys Res Lett* 34:1–6. doi: 10.1029/2007GL030000
- English-Loeb GM (1990) Plant Drought Stress and Outbreaks of Spider Mites. *Ecology* 71:1401–1411.
- Evans EW, Carlile NR, Innes MB, Pitigala N (2013) Warm springs reduce parasitism of the

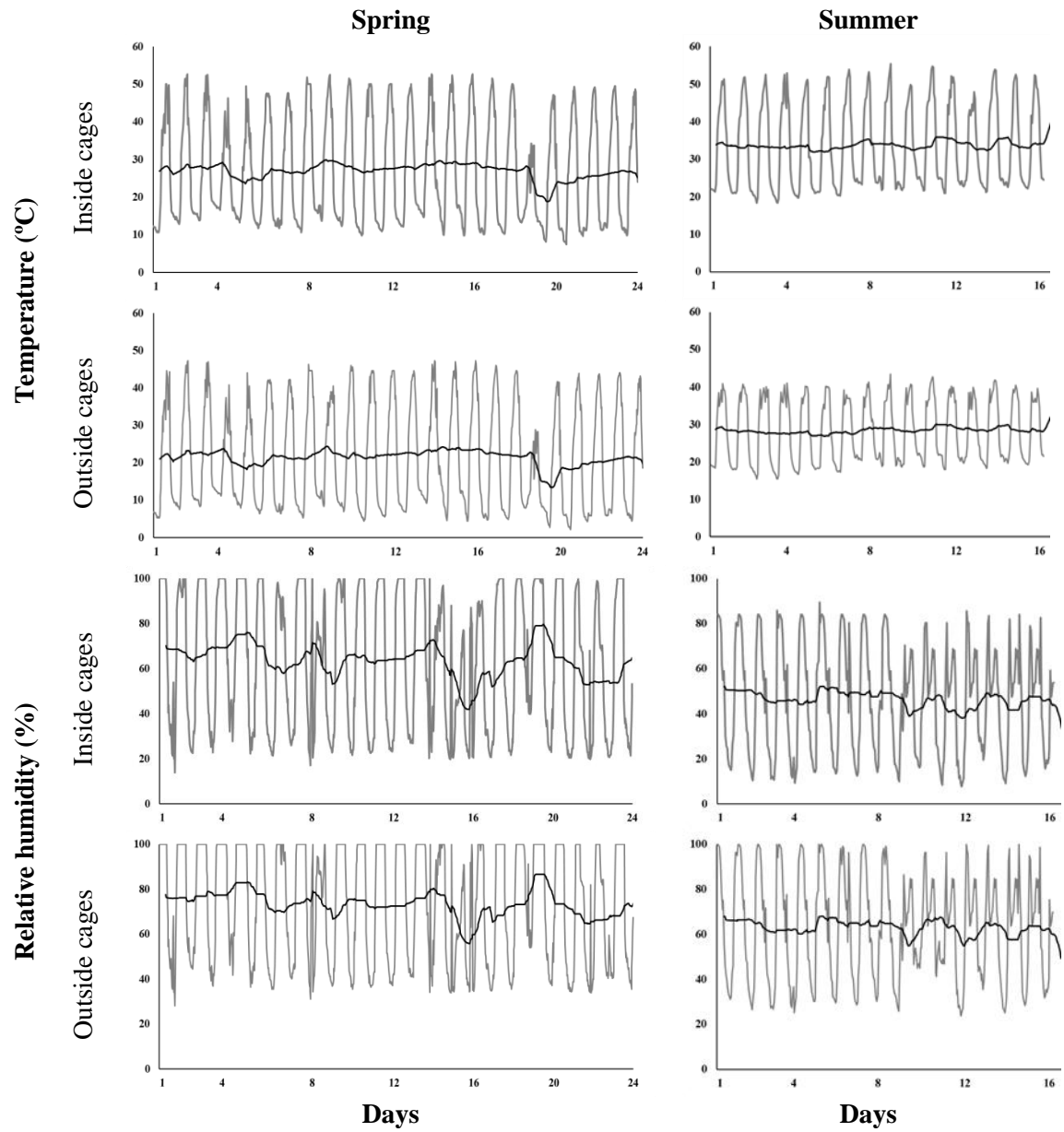
- cereal leaf beetle through phenological mismatch. *J Appl Entomol* 137:383–391. doi: 10.1111/jen.12028
- FAO (2017) FAOSTAT. Food and Agriculture Organization of the United Nations. In: <http://www.fao.org/faostat/en/#data/QC>. Accessed 21 Dec 2017
- García-Tejero I, Durán-Zuazo VH, Javier AS, Muriel-Fernández JL (2012) Impact of water stress on citrus yield. *Agron Sustain Dev* 32:651–659. doi: 10.1007/s13593-011-0060-y
- Gillespie DR, Nasreen A, Moffat CE, et al (2012) Effects of simulated heat waves on an experimental community of pepper plants, green peach aphids and two parasitoid species. *Oikos* 121:149–159. doi: 10.1111/j.1600-0706.2011.19512.x
- Gilman SE, Urban MC, Tewksbury J, et al (2010) A framework for community interactions under climate change. *Trends Ecol Evol* 25:325–331. doi: 10.1016/j.tree.2010.03.002
- Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. *Glob Planet Change* 63:90–104. doi: <https://doi.org/10.1016/j.gloplacha.2007.09.005>
- Gotoh T, Nozawa M, Yamaguchi K (2004) Prey consumption and functional response of three acarophagous species to eggs of the two-spotted spider mite in the laboratory. *Appl Entomol Zool* 39:97–105. doi: 10.1303/aez.2004.97
- Grafton-Cardwell EE, Ouyang Y, Striggow RA (1997) Predaceous Mites (Acari: Phytoseiidae) for Control of Spider Mites (Acari: Tetranychidae) in Nursery Citrus. *Environ Entomol* 26:121–130. doi: 10.1093/ee/26.1.121
- Gualdi S, Somot S, Li L, et al (2012) The CIRCE simulations: a new set of regional climate change projections performed with a realistic representation of the Mediterranean Sea. *Bull Am Meteorol Soc* 94: 65-81. doi: 10.1175/BAMS-D-11-00136.1
- Hance T, van Baaren J, Vernon P, Boivin G (2007) Impact of Extreme Temperatures on Parasitoids in a Climate Change Perspective. *Annu Rev Entomol* 52:107–126. doi: 10.1146/annurev.ento.52.110405.091333
- Helle H, Sabelis MW (1985) Spider mites: their biology, natural enemies and control. Elsevier, Amsterdam, The Netherlands.
- Inbar M, Doostdar H, Mayer RT (2001) Suitability of Stressed and Vigorous Plants to Various Insect Herbivores. *Oikos* 94:228–235. doi: 10.2307/3547567
- Jacob D, Petersen J, Eggert B, et al (2014) EURO-CORDEX: New high-resolution climate change projections for European impact research. *Reg Environ Chang* 14:563–578. doi: 10.1007/s10113-013-0499-2
- Jefferies CT, Lewis OT (2013) Effects of climate warming on host-parasitoid interactions. *Ecol Entomol* 38:209–218. doi: 10.1111/een.12026
- Kerry JT, Bellwood DR (2016) Competition for shelter in a high-diversity system: structure use by large reef fishes. *Coral Reefs* 35:245–252. doi: 10.1007/s00338-015-1362-3
- Koricheva J, Larsson S, Haukioja E (1998) Insect Performance on Experimentally Stressed Woody Plants: A Meta-Analysis. *Annu Rev Entomol* 43:195–216. doi: 10.1146/annurev.ento.43.1.195
- Larkindale J, Huang B (2005) Effects of abscisic acid, salicylic acid, ethylene and hydrogen peroxide in thermotolerance and recovery for creeping bentgrass. *Plant Growth Regul* 47:17–28. doi: 10.1007/s10725-005-1536-z
- Larsson S (1989) Stressful Times for the Plant Stress: Insect Performance Hypothesis. *Oikos* 56:277–283. doi: 10.2307/3565348

- Maia A de HN, Luiz AJB, Campanhola C (2000) Statistical Inference on Associated Fertility Life Table Parameters Using Jackknife Technique: Computational Aspects. *J Econ Entomol* 93:511–518. doi: 10.1603/0022-0493-93.2.511
- Martínez-Ferrer MT, Jacas JA, Ripollés-Moles JL, Aucejo-Romero S (2006) Approaches for sampling the twospotted spider mite (Acari: Tetranychidae) on clementines in Spain. *J Econ Entomol* 99:1490–1499. doi: 10.1603/0022-0493-99.4.1490
- Matthews SA, Wong MYL (2015) Temperature-dependent resolution of conflict over rank within a size-based dominance hierarchy. *Behav Ecol* 26:947–958. doi: 10.1093/beheco/arv042
- McMurtry JA, Croft B a (1997) Life-styles of Phytoseiid mites and their roles in biological control. *Annu Rev Entomol* 42:291–321. doi: 10.1146/annurev.ento.42.1.291
- McMurtry JA, Moraes GJDE, Sourassou NF (2013) Revision of the lifestyles of phytoseiid mites (Acari : Phytoseiidae). *Syst Appl Acarol* 18:297–320. doi: 10.11158/saa.18.4.1
- Montserrat M, de la Peña F, Hormaza JI et al (2008) How do *Neoseiulus californicus* (Acari: Phytoseiidae) females penetrate densely webbed spider mite nests? *Experimental and Applied Acarology* 44:101–106. doi: <https://doi.org/10.1007/s10493-008-9137-y>
- Montserrat M, Sahún R, Guzmán C (2013) Can climate change jeopardize predator control of invasive herbivore species? A case study in avocado agro-ecosystems in Spain. *Exp Appl Acarol* 59:27–42. doi: 10.1007/s10493-012-9560-y
- Narouei-Khandan HA, Halbert SE, Worner SP, van Bruggen AHC (2016) Global climate suitability of citrus huanglongbing and its vector, the Asian citrus psyllid, using two correlative species distribution modeling approaches, with emphasis on the USA. *Eur J Plant Pathol* 144:655–670. doi: 10.1007/s10658-015-0804-7
- Navarra A, Tubiana L (2013) Regional Assessment of Climate Change in the Mediterranean. Volume 2: Agriculture, Forests and Ecosystem Services and People, Springer. Springer Netherlands, Dordrecht, The Netherlands
- Paranychianakis N V., Chartzoulakis KS (2005) Irrigation of Mediterranean crops with saline water: From physiology to management practices. *Agric Ecosyst Environ* 106:171–187. doi: 10.1016/j.agee.2004.10.006
- Pascual-Ruiz S, Aguilar-Fenollosa E, Ibáñez-Gual V, et al (2014) Economic threshold for *Tetranychus urticae* (Acari: Tetranychidae) in clementine mandarins *Citrus clementina*. *Exp Appl Acarol* 62:337–362. doi: 10.1007/s10493-013-9744-0
- Pérez-Sayas C, Aguilar-Fenollosa E, Hurtado MA, et al (2017) When do predatory mites (Phytoseiidae) attack? Understanding their diel and seasonal predation patterns. *Insect Sci.* doi: 10.1111/1744-7917.12495
- Pérez-Sayas C, Pina T, Gómez-Martínez MA, et al (2015) Disentangling mite predator-prey relationships by multiplex PCR. *Mol Ecol Resour* 15:1330–1345. doi: 10.1111/1755-0998.12409
- Pieterse CMJ, Leon-Reyes A, Van Der Ent S, Van Wees SCM (2009) Networking by small-molecule hormones in plant immunity. *Nat Chem Biol* 5:308–316. doi: 10.1038/nchembio.164
- Pina T, Argolo PS, Urbaneja A, Jacas JA. (2012) Effect of pollen quality on the efficacy of two different life-style predatory mites against *Tetranychus urticae* in citrus. *Biol Control* 61:176–183. doi: 10.1016/j.biocontrol.2012.02.003
- Rodríguez-Gamir J, Primo-Millo E, Forner JB, Forner-Giner MA (2010) Citrus rootstock

- responses to water stress. *Sci Hortic (Amsterdam)* 126:95–102. doi: 10.1016/j.scienta.2010.06.015
- Roy M, Brodeur J, Cloutier C (2003) Temperature and sex allocation in a spider mite. *Oecologia* 135:322–326. doi: 10.1007/s00442-002-1160-9
- Sabelis MW, Bakker FM (1992) How predatory mites cope with the web of their tetranychid prey: a functional view on dorsal chaetotaxy in the Phytoseiidae. *Experimental and Applied Acarology* 16:203–225. doi: 10.1007/BF01193804
- Schausberger P, Walzer A (2001) Combined versus Single Species Release of Predaceous Mites: Predator–Predator Interactions and Pest Suppression. *Biol Control* 20:269–278. doi: 10.1006/bcon.2000.0908
- Shimoda T, Kishimoto H, Takabayashi J, et al (2009) Comparison of thread-cutting behavior in three specialist predatory mites to cope with complex webs of *Tetranychus* spider mites. *Exp Appl Acarol* 47:111–120. doi: 10.1007/s10493-008-9205-3
- Skirvin DJ, Fenlon JS (2003) The effect of temperature on the functional response of *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Exp Appl Acarol* 31:37–49.
- Stavrinides MC, Daane KM, Lampinen BD, Mills NJ (2010) Plant Water Stress, Leaf Temperature, and Spider Mite (Acari: Tetranychidae) Outbreaks in California Vineyards. *Environ Entomol* 39:1232–1241. doi: 10.1603/EN09288
- Stireman JO, Dyer LA, Janzen DH, et al (2005) Climatic unpredictability and parasitism of caterpillars: Implications of global warming. *Proc Natl Acad Sci* 102:17384–17387. doi: 10.1073/pnas.0508839102
- Thomson LJ, Macfadyen S, Hoffmann A. (2010) Predicting the effects of climate change on natural enemies of agricultural pests. *Biol Control* 52:296–306. doi: 10.1016/j.biocontrol.2009.01.022
- Tylianakis JM, Didham RK, Bascompte J, Wardle DA (2008) Global change and species interactions in terrestrial ecosystems. *Ecol Lett* 11:1351–1363. doi: 10.1111/j.1461-0248.2008.01250.x
- Urban MC, Bocedi G, Hendry AP, et al (2016) Improving the forecast for biodiversity under climate change. *Science* 353: 1114–1122. doi: 10.1126/science.aad8466
- Vangansbeke D, Audenaert J, Nguyen DT, et al (2015) Diurnal temperature variations affect development of a herbivorous arthropod pest and its predators. *PLoS One* 10:e0124898. doi: 10.1371/journal.pone.0124898
- Vangansbeke D, Schrijver L De, Sprangers T, et al (2013) Alternating temperatures affect life table parameters of *Phytoseiulus persimilis*, *Neoseiulus californicus* (Acari: Phytoseiidae) and their prey *Tetranychus urticae* (Acari: Tetranychidae). *Exp Appl Acarol* 61:285–298. doi: 10.1007/s10493-013-9704-8
- Vela JM, Wong E, Jaques JA et al (2017) Mite diversity (Acari: Tetranychidae, Tydeidae, Iolinidae, Phytoseiidae) and within-tree distribution in citrus orchards in southern Spain, with special reference to *Eutetranychus orientalis*. *Exp Appl Acarol* 73: 191–207. doi: 10.1007/s10493-017-0180-4
- Walzer A, Castagnoli M, Simoni S, et al (2007) Intraspecific variation in humidity susceptibility of the predatory mite *Neoseiulus californicus*: Survival, development and reproduction. *Biol Control* 41:42–52. doi: 10.1016/j.biocontrol.2006.11.012
- Wang L, Li L (2006) Salicylic acid-induced heat or cold tolerance in relation to Ca²⁺ homeostasis and antioxidant systems in young grape plants. *Plant Sci* v. 170:685–694–2006 doi: 10.1016/j.plantsci.2005.09.005

- White TCR (1969) An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology* 50:905–909. doi: 10.2307/1933707
- White TCR (2009) Plant vigour versus plant stress: A false dichotomy. *Oikos* 118:807–808. doi: 10.1111/j.1600-0706.2009.17495.x
- White TCR (1984) The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63:90–105. doi: 10.1007/BF00379790
- Ximénez-Embún MG, Ortego F, Castañera P (2016) Drought-stressed tomato plants trigger bottom-up effects on the invasive *Tetranychus evansi*. *PLoS One* 11:1–19. doi: 10.1371/journal.pone.0145275
- Zandalinas SI, Rivero RM, Martínez V, et al (2016) Tolerance of citrus plants to the combination of high temperatures and drought is associated to the increase in transpiration modulated by a reduction in abscisic acid levels. *BMC Plant Biol* 16:1–16. doi: 10.1186/s12870-016-0791-7

Figure 1. Hourly (grey line) and daily (black line) mean temperature and relative humidity during the spring (May 7 to 31) and summer (July 5 to 28) assays inside and outside the cages.



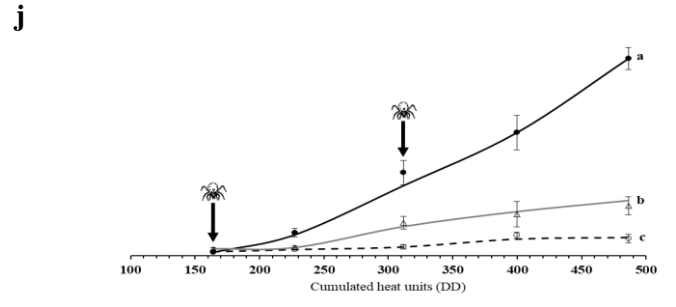
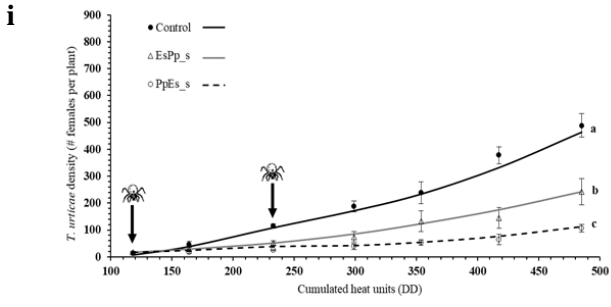
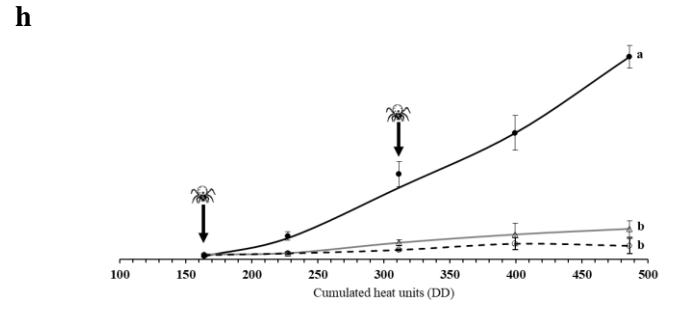
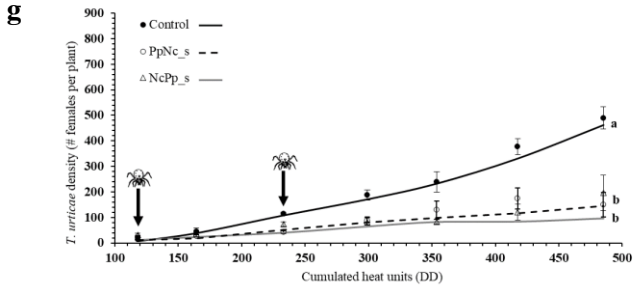


Table 1. Number of adult females and motile immature stages (in brackets) recovered at the end of the assay for each treatment in spring and summer.

Treatment	Spring			Summer		
	<i>E. stipulatus</i> (Es)	<i>P. persimilis</i> (Pp)	<i>N. californicus</i> (Nc)	<i>E. stipulatus</i> (Es)	<i>P. persimilis</i> (Pp)	<i>N. californicus</i> (Nc)
Control	0	0	0	0	0	0
Trophic chain	Es	1 (5)	-	1 (2)	-	-
	Nc	-	-	1 (4)	-	2 (6)
	Pp	-	1 (4)	-	5 (8)	-
Competition	EsNc	1 (0)	-	0 (0)	-	0 (0)
	NcPp	-	3 (0)	0 (0)	10 (4)	2 (5)
	PpEs	1 (0)	5 (1)	-	2 (1)	11 (3)
Sequential release	NcEs*	0 (1)	-	0 (1)	-	1 (0)
	EsNc*	0 (0)	-	0 (0)	-	5 (6)
	PpNc	-	2 (0)	0 (0)	-	10 (0)
	NcPp	-	1 (0)	0 (0)	-	4 (0)
	EsPp	0 (0)	8 (4)	-	0 (2)	5 (2)
	PpEs	0 (1)	1 (0)	-	1 (0)	2 (0)
Grand Total	3 (7)	21 (9)	1 (5)	12 (9)	47 (17)	15 (18)

*The number of immature motile stages found was distributed between species based on the proportion of adult females found

Table 2. Statistics (F ; df ; P -value) of GLMM adjusted to *T. urticae* density, as well as those corresponding to the factor season and its interaction with treatment. AIC was 8965.5.

Effect	Statistics
Model	11.88; 25; < 0.001
Season	30.99; 1; < 0.001
Season * Treatment	11.07; 24; < 0.001

Table 3. Statistics (F; *df*; *P*-value) of the GLMM adjusted to *T. urticae* density in spring and summer, as well as those corresponding to the factor treatment and its interaction with time. AIC were 4137.5 and 3230.6 for spring and summer, respectively.

Effect	Statistics	
	Spring	Summer
Model	4.43; 90; < 0.001	12.82; 90; < 0.001
Treatment	15.65; 12; < 0.001	36.45; 12; < 0.001
Treatment* time	2.71; 78; < 0.001	7.34; 78; < 0.001

Table 4. Results of the pairwise comparisons of the GLMM adjusted to the density of *T. urticae* corresponding to different treatments in spring and summer. The treatments consisted of the release of the phytoseiids *E. stipulatus* (Es), *N. californicus* (Nc) and *P. persimilis* (Pp) either singly (trophic chain), simultaneously (competition), or sequentially one week apart (sequential release).

Treatment		Spring	Summer
Control		a	ab
Trophic chain	Es	d	ab
	Nc	c	f
	Pp	c	f
Competition	EsNc	c	a
	NcPp	c	b
	PpEs	e	def
Sequential release	NcEs	b	c
	EsNc	cf	def
	PpNc	c	f
	NcPp	c	def
	EsPp	c	d
	PpEs	ef	def

Table 5. Cumulative Mite Days (CMD) and percentage reduction of *T. urticae* density (mean \pm SE) compared with control (no phytoseiid release) in spring and summer. The phytoseiids *E. stipulatus* (Es), *N. californicus* (Nc) and *P. persimilis* (Pp) were released either singly (trophic chain), simultaneously (competition), or sequentially one week apart (sequential release). Spring and summer values were compared using a *t*-test. For each season, CMD and percentage reductions followed by the same letter are not significantly different (Bonferroni post hoc test at $P < 0.05$).

Treatment	Spring		Summer		Spring vs. Summer (<i>t</i> ; <i>P</i> -value)		
	CMD	Reduction	CMD	Reduction	CMD	Reduction	
Control	1693.2 \pm 31.7 a	-	2511.6 \pm 61.6 a	-	4.14; < 0.001	-	
Trophic chain	<i>Es</i>	671.8 \pm 41.5 d	60.7 \pm 6.1 b	2684.5 \pm 44.6 a	0 d	11.82; < 0.001	9.36; < 0.001
	<i>Nc</i>	764.4 \pm 89.2 d	51.6 \pm 9.3 bc	63.5 \pm 6.5 f	96.7 \pm 1.9 a	1.95; < 0.001	2.23; < 0.001
	<i>Pp</i>	797.3 \pm 13.3 d	52.8 \pm 1.5 bc	57.2 \pm 4.8 f	97.6 \pm 1.2 a	17.36; < 0.001	49.56; < 0.001
Competition	EsNc	727.4 \pm 64.8 d	60.6 \pm 8.0 b	2156.3 \pm 67.6 b	20.10 \pm 10.0 c	5.47; < 0.001	2.69; < 0.001
	NcPp	1012.7 \pm 61.8 c	43.6 \pm 8.8 c	2208.1 \pm 105.3 b	16.3 \pm 4.5 c	1.99; < 0.001	1.12; < 0.001
	PpEs	307.8 \pm 23.9 e	75.4 \pm 3.3 a	612.2 \pm 79.3 c	75.9 \pm 9.5 b	1.31; 0.224	0.05; 0.962
Sequential release	NcEs	1382.5 \pm 79.1 b	16.3 \pm 7.4 d	2149.5 \pm 52.7 b	10.8 \pm 7.6 c	2.89; 0.020	0.62; 0.457
	EsNc	704.9 \pm 50.9 d	56.3 \pm 8.6 bc	304.4 \pm 71.9 d	86.9 \pm 2.6 b	2.45; 0.040	2.61; < 0.001
	PpNc	649.6 \pm 61.3 d	66.0 \pm 7.6 ab	226.1 \pm 34.0 d	90.1 \pm 3.8 a	2.16; 0.003	2.92; 0.019
	NcPp	634.7 \pm 60.8 d	62.3 \pm 7.5 ab	424.3 \pm 51.5 c	83.4 \pm 14.8 b	0.95; 0.372	1.95; 0.084
	EsPp	773.9 \pm 59.3 d	53.8 \pm 5.4 bc	716.2 \pm 14.3 c	71.8 \pm 9.7 b	0.24; 0.815	1.41; < 0.001
	PpEs	456.3 \pm 22.1 e	77.1 \pm 3.8 a	291.6 \pm 10.8 d	87.61 \pm 6.8 b	11.83; < 0.001	3.18; 0.024
Statistics	AIC	960.77	754.32	1045.07	896.35		
	<i>F</i> ; <i>df</i> ; <i>P</i>	52.68; 12; < 0.001	68.56; 11; < 0.001	83.07; 12; < 0.001	124.93; 11; < 0.001		

Table 6. Densities of *T. urticae* (individuals per plant) during the first three sampling dates in spring and summer. The first date per season coincides with the release of either 10 (trophic chain) or 5 (sequential release) individuals of one phytoseiid species per plant. The third date per season corresponds to the release of a second species in the sequential release treatments. Therefore, in between these two dates, the effects of either 10 or 5 individuals of one phytoseiid species on *T. urticae* density were measured. The phytoseiids were *E. stipulatus* (Es), *N. californicus* (Nc) and *P. persimilis* (Pp). Within each treatment and sampling date, values followed by the same letter are not significantly different (Bonferroni post-hoc test at $P < 0.05$).

Treatments	Spring sampling date (DD)			Summer sampling date (DD)		
	118 (1 st release)	164	233 (2 nd release)	164 (1 st release)	227	312 (2 nd release)
Control (no phytoseiid)	12.9 ± 4.5	44.8 ± 13.6	114.4 ± 7.3	13.5 ± 9.2	89.6 ± 16.0	325.1 ± 47.6
Es trophic chain	14.5 ± 3.4 a	35.8 ± 8.1 a	23.5 ± 4.4 c	20.3 ± 3.7 a	53.9 ± 6.1 b	297.4 ± 22.6 a
EsNc sequential	18.7 ± 3.5 a	32.5 ± 5.5 a	42.8 ± 8.8 b	17.1 ± 1.6 a	26.3 ± 8.3 c	114.8 ± 26.3 b
EsPp sequential	16.1 ± 1.5 a	28.3 ± 6.9 a	50.7 ± 8.3 b	26.3 ± 6.5 a	31.1 ± 6.1 c	129.6 ± 24.8 b
Control	a	a	a	a	a	a
Nc trophic chain	17.3 ± 7.4 a	45.6 ± 13.6 a	56.8 ± 19.8 b	21.7 ± 3.1 a	35.7 ± 8.1 b	22.11 ± 6.2 c
NcEs sequential	21.5 ± 3.4 a	40.6 ± 6.5 a	72.2 ± 6.9 b	14.7 ± 2.3 a	24.1 ± 0.8 c	53.6 ± 8.3 b
NcPp sequential	31.8 ± 6.5 a	35.7 ± 4.2 a	73.9 ± 9.9 b	20.1 ± 4.5 a	24.6 ± 5.7 c	64.15 ± 11.3 b
Control	a	a	a	a	a	a
Pp trophic chain	20.1 ± 3.1 a	33.5 ± 6.9 a	40.8 ± 11.1 b	22.6 ± 4.6 a	36.6 ± 3.5 b	16.8 ± 3.8 c
PpEs sequential	17.5 ± 1.5 a	24.9 ± 5.6 a	38.8 ± 1.7 b	16.1 ± 2.2 a	24.4 ± 1.5 c	34.3 ± 8.7 b
PpNc sequential	24.5 ± 2.7 a	27.6 ± 4.2 a	42.3 ± 5.2 b	17.3 ± 1.1 a	23.6 ± 2.9 c	36.8 ± 2.8 b
Control	a	a	a	a	a	a

Table 7. *Tetranychus urticae* preoviposition, oviposition, and postoviposition periods, development time, and life span (in days, d, and degree-days, DD, mean \pm standard error), at mild (25°C and 70 % RH) and hot-dry (30°C and 50% RH) laboratory conditions. For each parameter and unit, comparisons were made with Student *t*-test.

Environmental conditions	Pre-oviposition period		Oviposition period		Post-oviposition period		Development time		Lifespan	
	d	DD	d	DD	d	DD	d	DD	d	DD
Mild	1.84 \pm 0.03	26.76 \pm 0.45	10.65 \pm 0.14	155.03 \pm 2.16	1.89 \pm 0.02	36.83 \pm 0.27	11.52 \pm 0.05	167.66 \pm 0.78	15.03 \pm 0.14	222.42 \pm 0.81
Hot-dry	1.04 \pm 0.02	20.31 \pm 0.43	8.44 \pm 0.08	165.05 \pm 1.45	2.53 \pm 0.02	37.07 \pm 0.34	8.00 \pm 0.01	156.40 \pm 0.28	11.37 \pm 0.07	218.62 \pm 0.28
<i>t; P*</i>	3.98; < 0.001	1.98; 0.052	2.48; 0.016	0.73; 0.470	4.65; < 0.001	0.11; 0.914	11.25; < 0.001	2.42; 0.019	4.26; < 0.001	0.28; 0.776

*degrees of freedom (*df*) were 53 in all cases

Table 8. *Tetranychus urticae* egg hatching (%), immature survival (%), sex ratio (% females), fecundity (# eggs), and oviposition rate (eggs d⁻¹) at mild (25°C and 70 % RH) and hot-dry (30°C and 50% RH) laboratory conditions. For each parameter, comparisons were made using Student *t*-test.

Environmental conditions	Egg hatching	Immature survival	Sex ratio	Fecundity	Oviposition rate
Mild	95.9 ± 0.1	76.3 ± 2.1	81.4 ± 0.2	32.8 ± 0.2	2.2 ± 0.1
Hot-dry	98.9 ± 0.1	78.5 ± 1.1	83.5 ± 0.1	29.6 ± 0.2	3.0 ± 0.1
<i>t</i>; <i>P</i> *	3.37; 0.001	0.53; 0.606	1.47; 0.148	2.72; 0.027	6.55; <0.001

*degrees of freedom (*df*) were 53 in all cases

Table 9. Selected life history parameters of *T. urticae*: net fecundity (female eggs per female), generation time (in days, d, and degree-days, DD), intrinsic rate of increase (r_m , females per female per either day, d, or degree-day, DD) when exposed to mild (25°C and 70 % RH) and hot-dry (30°C and 50% RH) laboratory conditions. For each parameter and unit, comparisons were made with Student *t*-test.

Environmental conditions	Net fecundity	Generation time		r_m	
		d	DD	d ⁻¹	DD ⁻¹
Mild	26.55 ± 0.12	13.36 ± 0.06	194.42 ± 0.82	0.169 ± 0.001	0.011 ± 0.001
Hot-dry	24.84 ± 0.21	9.04 ± 0.02	176.77 ± 0.45	0.219 ± 0.001	0.011 ± 0.001
<i>t</i>; <i>P</i> *	1.45; 0.154	12.73; <0.001	3.44; 0.001	12.54; <0.001	1.36; 0.185

*degrees of freedom (*df*) were 53 in all cases