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Effect of pollen provision on life-history parameters of phytoseiid predators under hot and dry environmental conditions

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38 **The name(s) of the author(s):**

39 Pablo Urbaneja-Bernat[1] and Josep A. Jaques[1]

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45 **The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country:**

46

47 [1]Departament de Ciències Agràries i del Medi Natural, Unitat Associada d'Entomologia UJI-IVIA,

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

49

50 **A clear indication and an active e-mail address of the corresponding author:**

51

52 Pablo Urbaneja-Bernat

53 Universitat Jaume I, Departament de Ciències Agràries i del Medi Natural,

54 E-mail: paurbaneja@gmail.com

55

56 **If available, the 16-digit ORCID of the author(s):**

57

58 Pablo Urbaneja-Bernat: 0000-0002-6995-5468

59 Josep A. Jaques: 0000-0003-1353-1727

60

Key Message

- Climate change may trigger outbreaks of *T. urticae* and disrupt its biological control

- The response of phytoseiids to hot and dry conditions is species-specific

- Whether the observed harmful effects could be compensated by pollen supply was evaluated

- Pollen increased survival, predation, and oviposition of omnivorous *E. stipulatus*

- Pollen boosted survival and reduced performance of specialists *N. californicus* and *P. persimilis*

- Pollen may not be enough to compensate for the observed adverse effects of hot and dry conditions

Abstract

Biological control can be severely disrupted under climate change conditions. This is the case of the spider mite *Tetranychus urticae* in Spanish citrus orchards, where the omnivorous phytoseiid *Euseius stipulatus*, the most abundant predator in the system, was highly impacted by hot and dry conditions mimicking future warmer summers. Such a situation can often be compensated by the provision of alternative food to support generalist predators. As a first step to studying whether such a technique could be applied in this case, we studied at laboratory conditions whether pollen could mitigate the negative effects of hotter and drier conditions derived of climate change on three phytoseiids with different diet specializations. In addition to *E. stipulatus*, these predators, which all together, are considered key for the biological control of *T. urticae* in citrus, are *Neoseiulus californicus* and *Phytoseiulus persimilis*. Our results confirm the extremely fine-tuning of *T. urticae* to hot-dry conditions. They also provide evidence of the poor performance of *E. stipulatus*, especially in terms of reproduction, compared to the other two phytoseiids at these conditions, even when high-quality pollen was available. Moreover, access to pollen in combination with *T. urticae* eggs enhanced survival but reduced predation and oviposition relative to a *T. urticae*-only diet for *N. californicus* and *P. persimilis*. Therefore, whether the overall effect of pollen would justify its use in citrus to counteract the deleterious effects of a hotter and drier climate on the natural regulation of *T. urticae* is still controversial.

Introduction

The Mediterranean region is considered one of the most vulnerable areas to climate change worldwide (IPCC, 2014). Temperature increases between 1.5 and 2.0ºC in winter and summer, respectively, coupled with a 5% decrease in rainfall are forecasted for the mid 21th century (Giorgi and Lionello, 2008; Gualdi et al., 2012; Navarra and Tubiana, 2013; IPCC 2018). This increase, together with increasingly frequent extreme events (IPCC 2018) such as drought and heatwaves (Hansen et al., 2012), can have detrimental effects on ecosystem dynamics (Ummenhofer and Meehl, 2017). Many authors have analyzed, designed, categorized, and represented the growing risks of this weather phenomenon (Beck and Mahony 2017; O'Neill et al. 2017), which can trigger disruptions of natural control (Aguilar-Fenollosa and Jacas, 2014; Montserrat et al., 2013a) and failure of biological control (Roy et al. 2003; Stavrinides et al. 2010; Montserrat et al. 2013b). This is the case for spider mites (Prostigmata: Tetranychidae), where outbreaks can follow droughts (English-loeb 1990) and heatwaves (Montserrat et al., 2013a). Contrary to the progressive increase in average temperature, which is a long-term process, extreme climatic events, such as heatwaves, can induce immediate consequences for poikilothermic animals as mites with further effects at higher organizational levels (i.e., population, communities) (Ciais et al. 2005; Jentsch et al. 2007; De Boeck et al. 2010; Bannerman et al. 2011; Smith 2011; Gillespie et al. 2012; Sentis et al. 2013).

In the Mediterranean basin, the two-spotted spider mite, *Tetranychus urticae* Koch, is a key pest of clementine mandarins, *Citrus clementina* Tanaka (Rutaceae) (Martínez-Ferrer et al. 2006; Aguilar-Fenollosa et al. 2011a; Pascual-Ruiz et al. 2014). Its main natural enemies are different phytoseiid predatory mite species (Mesostigmata: Phytoseiidae), which are present in this system and have different diet specializations (McMurtry and Croft 1997; McMurtry et al. 2013). The most abundant phytoseiid in Spanish citrus orchards, irrespective of the citrus cultivar and management practices used, is the omnivorous *Euseius stipulatus* (Athias-Henriot) (Abad-Moyano et al. 2009a; Aguilar-Fenollosa et al. 2011b; Vela et al. 2017). However, this phytoseiid, is not the most effective predator of *T. urticae*. This role is played by the *T. urticae*-specialist *Phytoseiulus persimilis* (Athias-Henriot), which preys on this herbivore almost five times more frequently than *E. stipulatus* (Pérez-Sayas et al. 2015). The Tetranychidae-specialist *Neoseiulus californicus* (McGregor) is also commonly found in these citrus orchards (Abad-Moyano et al. 2009a; Aguilar-Fenollosa et al. 2011b; Vela et al. 2017). These specialists are consistently found in clementine orchards grown in association with a grass cover, where the abundance

120 of *E. stipulatus* relative to other ground covers diminishes (Aguilar-Fenollosa et al. 2011c), resulting in

121 enhanced biological control of *T. urticae* (Aguilar-Fenollosa et al. 2011b).

122 Urbaneja-Bernat et al., (2019) showed under semi-field conditions representative of hotter and

123 drier environmental conditions in the Mediterranean basin that the regulation of *T. urticae* in clementine

124 trees provided by *E. stipulatus, N. californicus,* and *P. persimilis* could be seriously disrupted. The

125 dynamics of *T. urticae* in simple trophic chain modules (Bascompte and Melián 2005) including these

126 predators were species-specific and did not follow the same patterns in spring and summer. This study

127 showed that these predators provided similar control levels of *T. urticae* when released singly in conditions

128 mimicking spring climate change conditions. Although, it is generally acknowledged that species with the

129 highest specializations in lifestyle or habitat are typically most threatened by climate change (Aguilar-

130 Fenollosa and Jacas 2014), the omnivorous *E. stipulatus* provided no control at hotter and drier summer

131 conditions representative of climate change, whereas, the other two prey-specialized species were even

132 more effective in summer than in spring. These unexpected results suggest that future warmer and drier

133 summers could result in a deficient control of *T. urticae* in citrus orchards because of the high impact on

134 most abundant *E. stipulatus*. However, this could be compensated by (1) a better performance of less

135 abundant but more efficient *T. urticae*-specialists *P. persimilis* and *N. californicus*, which could reverse the

136 situation (Urbaneja-Bernat et al., 2019) and/or (2) the addition of supplementary food to the system, as the

137 importance of such a supply to support generalist predatory mite populations, like *E. stipulatus*, has been

138 widely recognized (González-Fernández et al. 2009; Pozzebon et al. 2009; Maoz et al. 2011; McMurtry et

139 al. 2013; Janssen and Sabelis 2015; Khanamani et al., 2017). Indeed, *E. stipulatus* and *N. californicus* can

140 persist in citrus when *T. urticae* is scarce, feeding on other food sources including pollen (Pina et al. 2012).

141 Moreover, Beltrà et al. (2017) demonstrated that the provisioning of pollen and sugars in Spanish citrus

142 orchards could boost phytoseiid natural populations in spring and fall. However, this supply had no effect

143 from June to September. Therefore, there are doubts on whether pollen supply could be an effective measure

144 to mitigate the effects of climate change in this system.

145 To challenge the hypotheses that (1) the specialist predators *N. californicus* and *E. stipulatus* can

146 do better than the generalist omnivore *E. stipulatus* at hotter and drier conditions and (2) pollen supply can

147 compensate the adverse effects of these conditions on these natural enemies, we performed a series of short-

148 term experiments under laboratory conditions. This type of assays, which are commonly used to assess the

149 effect of extreme climatic events such as heatwaves (Ciais et al. 2005; Jentsch et al. 2007; De Boeck et al.

150   2010; Bannerman et al. 2011; Smith 2011; Gillespie et al. 2012; Sentis et al. 2013), allowed us to explore

151   how different combinations of temperature ('T') and relative humidity ('RH'), including those typical of

152   hotter and drier abiotic conditions associated with climate change, affect the key biological parameters (i.e.

153   survival, oviposition, and predation) of *T. urticae* and its predators *E. stipulatus, N. californicus* and *P.*

154   *persimilis* with and without provision of pollen as a supplementary food. The results of this work should

155   help to explain the semi-field results observed (Urbaneja-Bernat et al., 2019) and provide evidence of

156   whether pollen supply could be a tactic allowing the conservation of these natural enemies in a rapidly

157   approaching warmer future.

158

159   **Material and Methods**

160   **Plant material**

161   Two-year-old clementine plants (*Citrus clementina* Tanaka cv. Clementina de Nules (Rutaceae)

162   grafted on citrange Carrizo) were used as a source of leaves for the assays. Fifty days before the beginning

163   of each assay, 25 plants were defoliated and kept in a greenhouse at Universitat Jaume I (UTM: 39°

164   59'10.883 "N 0°3'4.769"W) set at 22 ± 2ºC, 55 ± 10% relative humidity, and natural photoperiod. These

165   plants were grown on vermiculite and peat (1:3; vol: vol) in 320 ml pots, were fertilized twice per week

166   using a modified Hoagland's solution (Bañuls et al. 1997) and received no pesticide treatments. When

167   necessary for the rearing of mites, bean leaves (*Phaseolus vulgaris* L. (Fabaceae)), lemon fruits (*Citrus*

168   *lemon* Burm. f. (Rutaceae)), and *Carpobrotus edulis* (L.) (Aizoaceae) pollen (dried at 37ºC, sieved, and

169   frozen until use) obtained from pesticide-free plants were used. This pollen is considered as high-quality

170   for phytoseiid mites. At laboratory conditions, it can sustain and even boost populations of *N. californicus*

171   and *E. stipulatus*, respectively (Pina et al., 2012)

172   **Mite stock colonies**

173   Four different mite species were used in our studies: the two-spotted spider mite *T. urticae*, and

174   the Phytoseiidae *E. stipulatus*, *N. californicus*, and *P. persimilis*. These colonies were maintained in

175   separate climatic chambers set at 25 ± 1ºC, 65 ± 5 % 'RH', and a 16-hour light photoperiod.

176   Spider mites were collected in a Clementina de Nules orchard at Les Alqueries (UTM:

177   39°59'15.1"N 0°3'02.0"W) in 2010. This colony has been maintained ever since using standard procedures

178   on detached leaves of clementine mandarins (Aguilar-Fenollosa et al. 2012) and, in some cases (see below),

179   on pesticide-free lemon fruits (Abad-Moyano et al. 2010). Spider mites were used to either feed the

180 Phytoseiidae stock colonies or to start new cohorts for our assays. When used to feed Phytoseiids, bean

181 leaflets were infested by exposure to lemon fruit colonies. New cohorts were established by transferring

182 100 females to new rearing arenas on clementine leaves. Females were removed one day later, and these

183 units containing less than 24-hour old eggs were held separately in a climatic chamber (25ºC, 65 % 'RH')

184 and constituted the cohorts used in our assays.

185 Individuals of *N. californicus* were obtained from Koppert Biological Systems (SPICAL®) to

186 initiate a laboratory colony. Contrarily, *P. persimilis* and *E. stipulatus* were collected in 2012 in two

187 clementine orchards in Les Alqueries (same location as *T. urticae*) and Montcada (UTM: 39° 32' 42.906"

188 N 0° 23' 45.699" W), respectively. Phytoseiid stock colonies were maintained on detached leaf arenas.

189 These arenas consisted of single bean leaflets placed upside down on moistened filter paper placed on top

190 of a water-saturated foam cube (3–4 cm thick) in an open plastic box half-filled with water. Phytoseiid

191 colonies received twice a week detached bean leaflets infested with *T. urticae* and *C. edulis* pollen as food.

192 **Experimental arenas**

193 Arenas consisted of a petri dish (5 cm in diameter) with a 3 cm in diameter hole in the cover. The

194 base of the dish was filled with bacteriological agar (2.5 % weight). As soon as agar was cold and solid

195 enough, a fully expanded clementine leaf was placed upside down on top of the agar to maintain its turgor.

196 The cover was subsequently put in place so that the leaf substrate formed a 3 cm in diameter exposed area.

197 The upper and lower parts of the dishes were sealed with a strip Parafilm® (Pechiney Plastic Packaging,

198 Menasha, WI, USA). Finally, to prevent mite escape from the arena, permanent glue (Tree Tanglefoot®;

199 Grand Rapids, MI, USA) was applied along the rim of the cover hole (Guzmán et al. 2016).

200 **Effect of temperature and relative humidity on *T. urticae* performance: survival and oviposition**

201 Less than 24 hours old presumably mated females (i.e., those reaching the adult stage immediately

202 after the quiescent teliochrysalis stage) were selected and individually moved into a clean experimental

203 arena. Survival (i.e., alive, dead specimens, and escapees) and oviposition (number of eggs laid during the

204 experiment) were assessed under a binocular microscope 24 hours after the onset of the assay.

205 Different 'T' (10 to 40ºC in 5ºC steps) and 'RH' values (30, 50, and 70 %) were combined in our assays.

206 Constant 'RH' values were obtained by using different salt solutions (Winston and Bates, 1960) in

207 desiccators kept inside environmental chambers (Sanyo Electric Co., Ltd., Japan) set at a photoperiod of

208 16:8 hours L:D and the different target temperatures. We performed five replicates of six arenas per

209 environmental condition (i.e., a total of 30 replicates per environmental condition).

Effect of temperature and relative humidity on phytoseiid performance: survival, predation, and oviposition

A fully expanded healthy clementine leaf was introduced onto a *T. urticae*-infested lemon stock colony. Twenty-four hours later, the infested leaf was moved into a phytoseiid colony and left there for an additional 24-hour period. Then, leaves were inspected under a binocular microscope to remove all motile stages. A separate phytoseiid colony was started with every single leaf, and they constituted the cohorts used in our assays. As this method did not work for *E. stipulatus*, the eggs of this species were obtained by exposing a few cotton threads to an existing colony. 24 hours later all motile forms on these threads were removed, and the remaining eggs were used to start a new cohort. Phytoseiids were reared up to the adult stage following the same procedure as for the stock colonies.

In our assays, we used gravid adult phytoseiid females at their peak oviposition rate (12 to 14 days from egg hatching) (Aucejo-Romero et al., 2004; Janssen & Sabelis, 1992). To ensure the same level of starvation in all females tested, these were randomly selected from a cohort and individualized in plastic arenas (same as for the stock colonies but substituting the plant material by a plastic board) placed on top of a sponge in a water-containing tray where they starved for 24 hours. The edges of these plastic boards were covered with tissue paper in contact with the sponge and the water, which served as both a barrier and a water source for mites.

Experimental arenas received 15 *T. urticae* females, which fed, laid eggs, and produced a web for 48 hours. At that time, we removed all mobile forms of *T. urticae,* and only ≤ 48 hours of old eggs were left. The mean number of *T. urticae* eggs per arena was 75. Immediately after, a starved phytoseiid female was introduced into the arena. These units were then transferred to a desiccator, where the desired 'RH' was achieved as above. Likewise, these desiccators were introduced into an environmental chamber set at the target temperature (same 'T' and 'RH' combinations as for *T. urticae*). Arenas were checked 24 hours after the onset of the assay (i.e., 48 hours after the onset of the starvation period for adult phytoseiid females) under a binocular microscope. This period was selected because *T. urticae* eggs used in the arenas could start hatching in 48 hours, especially at high temperatures (>30ºC). Survival, oviposition, and predation (number of *T. urticae* eggs eaten) were scored. Same as with *T. urticae*, we performed five replicates of six arenas per environmental condition and mite species.

Effect of alternative food on phytoseiid performance: survival, predation, and oviposition

240 In addition to arenas containing ≤ 48 hours old *T. urticae* eggs, two more diets were considered: (1) pollen

241 of *C. edulis*, and (2) a combination of the former two. Arenas containing ≤ 48 hours of old eggs were

242 obtained as before. Arenas containing pollen were prepared by adding *C. edulis* pollen *ad libitum* in a single

243 point in the center of the arena. As soon as the arenas were ready, one starved phytoseiid female was

244 introduced. As before, the arenas were checked 24 hours later when survival, oviposition, and predation

245 were scored. In this assay, the combination of three 'T' (15, 25, and 30 ºC) and three 'RH' (30, 50, and 70

246 %) was considered. We performed a total of 15 replicates per environmental condition, diet, and phytoseiid

247 species.

248

249 **Statistical methods**

250   To study the effects of the 'T' and 'RH' 'on *T. urticae* and phytoseiid performance, we used general

251 linear models (GLM) and separately analyzed *T. urticae* and phytoseiids. In the case of survival, which had

252 three different possible outputs (i.e., live and dead specimens and escapees), we used a GLM with a

253 multinomial distribution of the error and a generalized logit link function. For predation (only for

254 phytoseiids) and oviposition, we used a GLM with a Poisson distribution of the error and a logistic link

255 function. The factors 'species', 'T', and 'RH' were used as fixed effects in all cases. As one of our main

256 goals was to identify phytoseiid species-specific differences, in the case of predators, we started our

257 analyses by considering all combinations, including 'species' as a factor. Once the statistical significance

258 of the 'species' factor was clear, we similarly continued the analyses of survival, predation, and oviposition

259 by studying the effect of 'T', 'RH', and their interaction. We included the factor 'replicate' (1-5) as a

260 random factor.

261   To study the effects of alternative food on the performance of phytoseiids, we used the same

262 general linear models (GLM) as above. For survival, oviposition, and predation, the factors 'species', 'diet',

263 'T', and 'RH' were used as fixed factors. As our main goal was to identify species-specific patterns of

264 response, same as above, we started our analyses by considering all combinations, including the 'species'

265 factor. Then, we studied the effect of 'diet'. Eventually, we separately analyzed for each species and diet,

266 the effects of 'T', 'RH', and their interaction. The factor 'replicate' (1-3) was included in our analyses as a

267 random factor. In both cases, when necessary, we used the Bonferroni post-hoc test for mean separation at

268 $P < 0.05$. All data were analyzed using SPSS 23.0 software.

269

Results

Effect of temperature and relative humidity on *T. urticae* performance: survival and oviposition

The factor 'T' and the interaction 'T'*'RH' significantly affected survival and oviposition (Table 1). The absolute highest survival (i.e., the percentage of live specimens) was observed at 25ºC and 30% relative humidity (100% survival) (Figure 1a). Survival was always above 60%, even at the extreme temperatures tested (10 and 40ºC) during the experiment.

Oviposition (eggs / day) was significantly affected by 'T', 'RH', and their interaction (Table 1), with minimum values observed at 10 and 40ºC (0.3 ± 0.1 and 0.4 ± 0.1 eggs, respectively; Figure 1b). Between these temperatures, oviposition increased with no clear trends for relative humidity. Indeed, at 35ºC maximum oviposition rates were attained independently of 'RH' (mean of 7.4 ± 0.9 eggs per female).

Effect of temperature and relative humidity on phytoseiid performance: survival, predation, and oviposition when preying on *T. urticae*

We observed significant differences ($P < 0.05$) between phytoseiid species for all parameters considered (survival, predation, and oviposition; supplementary materials Table 1). Consequently, we analyzed the effect of 'T' and 'RH' for each phytoseiid species separately.

The GLM to analyze the survival 24 hours after the onset of the assay for the three predators (Figure 2) included 'T' and 'RH'. For *E. stipulatus*, 'T' and its interaction with 'RH' were significant (Table 2). This species could not survive temperatures above 30ºC. Below this threshold, survival usually increased with 'RH'. However, the percentage of escapees was around 50% in all combinations of 'T' and 'RH' considered except at 25ºC and either 50 or 75% 'RH', when more than 60% of the specimens tested escaped from the arenas. For *N. californicus,* only the interaction between 'T' and 'RH' was significant (Table 2). This species could not survive 40ºC at any of the three 'RH' values considered. Same as *E. stipulatus*, around half of the individuals tried to escape from the experimental setup at this temperature. However, for the remaining conditions, survival was similar and relatively high (Figure 2). At 25ºC and 70%, relative humidity survival was 100%. For *P. persimilis,* 'RH' and the interaction 'T'*'RH' significantly affected survival (Table 2). Like *N. californicus*, this species did not survive 40ºC, and in line with the previous species, almost half of these individuals tried to escape and seek refuge elsewhere. For the remaining conditions, maximal survival was observed in the range 15-20ºC. Compared to the other two species, in this case, survival at 35ºC was maximal at the lowest relative humidity tested (Figure 2).

10

300   The number of eggs preyed was significantly affected by 'T', 'RH', and their interaction for the

301   three phytoseiid species (Table 2). In the case of *E. stipulatus*, the lowest predation rates were observed at

302   10ºC irrespective of relative humidity (Figure 2). Above this temperature and up to 25ºC, predation

303   increased, and maximum rates were usually associated with 70% relative humidity. A maximum of 15.6 ±

304   1.9 eggs eaten per female was observed at 15 and 25ºC at this relative humidity. Beyond 30ºC there was no

305   survival and so no predation was observed. Predation rates for *N. californicus* (Figure 2) were minimal at

306   10ºC irrespective of relative humidity. Above this temperature, they increased up to 25-30ºC, then

307   decreased at 35ºC and were zero at 40ºC because of no survival at this temperature. Interestingly, at 15 and

308   35ºC, predation was maximal at 50% relative humidity, whereas, at 25 and 30ºC, the highest predation rates

309   were associated with the highest relative humidity values tested, with a mean of 21.6 ± 1.2 *T. urticae* eggs

310   eaten per female. *Phytoseiulus persimilis* was the most voracious mite at any of the temperatures and

311   relative humidity combinations tested and presented a trend closely matching what we observed for *N.*

312   *californcius* (Figure 2). In this case, maximum predation rates were reached at 30ºC independent of relative

313   humidity and at 25ºC with 70% relative humidity with a mean of 39.3 ± 2.5 eggs per female. The number

314   of eggs consumed per female decreased dramatically to 16.3 ± 2.5 eggs at 35ºC, but these values were still

315   higher than those observed at 10ºC.

316   During the first 24 hours of the assay, the number of eggs laid was affected both by 'T' and 'RH' in

317   *N. californicus,* by 'T' and the interaction of this factor with 'RH' for *P. persimilis*, and it was independent

318   of these factors for *E. stipulatus* (Table 2). This independence could be attributed to the meager oviposition

319   rates observed for this phytoseiid at all combinations tested (0 to 0.2 eggs per female and day; Figure 2).

320   The oviposition rate of *N. californicus* (Figure 2) increased from about 0.1 to 2.5 eggs per female between

321   15 and 30ºC with the absolute maximum number of eggs laid per female at 30ºC and 50% relative humidity

322   (3.2 ± 0.1 eggs). Below 20 and above 30ºC, oviposition was minimal, and at 10ºC only a few eggs could

323   be collected in the arenas kept at 70% relative humidity. Intriguingly, oviposition at 20ºC and 70% relative

324   humidity was as low as the reported minimum values. A similar trend was observed for the response of *P.*

325   *persimilis* to temperature (Figure 2). In this case, oviposition increased from about 0.7 to around 4.1 eggs

326   per female between 10 and 30ºC. However, in this case, the effect of relative humidity changed direction

327   depending on the temperature. Maximum oviposition rates at 25 and 30ºC were associated with lowest and

328   intermediate relative humidity values (around 4.2 ± 0.2 eggs per female).

329

Effect of alternative food on phytoseiid performance: survival, predation, and oviposition

There were significant differences between the three phytoseiids for survival (Supplementary materials Table 2). Consequently, we further analyzed the influence of the factor 'diet' for each species separately (Supplementary materials Table 3). We found that this factor was significant ($P < 0.001$) in all cases. Consequently, these results led us to eventually analyze the influence of 'T', 'RH', and their interaction for each mite species and diet separately.

For the omnivorous *E. stipulatus*, survival was highest when it had access to the pollen only diet (means of 77.1 versus 49.3 and 33.8 % for pollen only, mixed, and *T. urticae* eggs only diets, respectively) (Figure 3). The pollen (either alone or with *T. urticae* eggs) made survival independent of relative humidity (Table 3). This result should be probably attributed to the zero death rates observed at 25ºC in the pollen only diet and at 15 and 25ºC in the mixed diet. Contrarily, in the *T. urticae* eggs only diet, the effect of temperature and relative humidity did not follow any clear trend and, although 30% relative humidity provided the lowest survival rates at 15 and 25ºC, it provided the highest at 30ºC. Remarkably, the percentage of escapees was similar in the two diets supplemented with T. *urticae* eggs (up to 52%) and higher than when pollen only was available. The number of dead individuals was highest when only *T. urticae* eggs were offered as a food source (around 11%).

In the case of the tetranychid-specialist predator *N. californicus*, survival was higher when the mite had access to *T. urticae* eggs, either alone (86.5%) or combined with pollen (85.9%), and decreased when pollen was the only food source available (31.6%) (Figure 3). However, when we analyzed the survival for each diet, we observed that the GLM model provided a good fit ($P < 0.05$) only in the case of pollen alone (Table 3). This failure was attributed to the fact that all individuals survived (no dead or stuck-in-the-glue specimens) at the combinations 25 and 30 ºC with 50% relative humidity for *T. urticae* eggs and mixed diets, respectively. For the pollen only food, survival was highest at 25ºC, much lower at 15ºC, and became zero at 30ºC. For the other two diets, survival never dropped below 60.0%.

For the *Tetranychus* sp.-specialist predator *P. persimilis*, survival (Figure 3) was significantly affected by diet (Table 3). The highest survival occurred when this phytoseiid had access to *T. urticae* eggs only (47.7%), closely followed by the mixed diet (44.4%). Lowest survival was observed when this phytoseiid had access to pollen only (21.5%). Interestingly, the mixed diet was the only one resulting in no dead specimens. However, same as the other two phytoseiids, moderate to high escapee rates were observed even in this case. 'T' was the single factor affecting survival for all diets. 'RH' was significant for the mixed

12

360 diet only (Table 3). This mixed diet resulted in the absolute highest survival at 25ºC tested, and 70% relative

361 humidity (93.8% survival), with no dead individuals observed at any of the different combinations of

362 temperature and relative humidity. When we offered pollen and *T. urticae* eggs separately, survival was

363 highest at 15ºC (31.2 and 63%, respectively).

364   The number of eggs preyed by *E. stipulatus* was affected by 'T' and 'RH' in both diets (Table 4).

365 The highest predation rates were observed when *E. stipulatus* was offered a mixture of pollen and *T. urticae*

366 eggs (average of 11.4 ± 1.4 eggs eaten versus 7.9 ± 1.6 for the *T. urticae* eggs only diet) (Figure 4).

367 However, we observed similar predation rates at 15 and 25ºC and 70% relative humidity when *T. urticae*

368 eggs constituted the only food source available. The predation rate of *N. californicus* also was significantly

369 affected by 'T' and 'RH' for the two diets considered (Table 4). In general, highest predation rates were

370 observed at 30ºC irrespective of relative humidity and, within each temperature, at 70% relative humidity

371 (Figure 4). The number of eggs preyed by *P. persimilis* (Figure 4) was affected by 'T,' 'RH' their interaction

372 in both diets (Table 4). Pollen provision affected predation rates because the number of eggs preyed

373 decreased when offered a mixed diet (20.6 ± 1.6) compared with *T. urticae* eggs only (28.5 ± 2.1). For both

374 diets, predation significantly decreased at 15ºC independently of relative humidity, and there were no

375 differences between 25 and 30ºC.

376   The number of eggs laid by *E. stipulatus* at the different temperature and relative humidity

377 combinations was significantly different in diets with either *T. urticae* or pollen alone (Table 5). However,

378 when we offered a mixture of *T. urticae* eggs and pollen, these differences disappeared (Table 5). These

379 results should probably be attributed to this species' extremely low fecundity in our assays, which was

380 always below one egg per female and day (Figure 5).

381   For *N. californicus*, oviposition was affected by 'T' and 'RH' (Table 5), and it was lowest when

382 the phytoseiid had access to pollen only (it was zero at 15 and 30ºC irrespective of relative humidity)

383 (Figure 5). Interestingly, this species was especially fecund at 30ºC with much lower values at 15ºC and

384 intermediate at 25ºC.

385   Same as *N. californicus*, in the case of *P. persimilis*, 'T' and 'RH' significantly affected oviposition

386 (Table 5), which became almost zero when this mite had access to pollen only (Figure 5). From the other

387 two food sources, the *T. urticae* eggs alone presented higher values (mean 2.8 ± 0.3 eggs) than the mixed

388 diet (1.3 ± 0.2). For these food sources, oviposition was lowest at 15ºC and increased along with

13

389   temperature, with maximum values usually associated with intermediate relative humidity rather than to

390   70%.

391

392   **Discussion**

393   We aimed to determine whether, in agreement with previous semi-field work (Urbaneja-Bernat et

394   al., 2019), (1) *N. californicus* and *P. persimilis* were better adapted than *E. stipulatus* to hotter and drier

395   conditions expected for Spanish citrus orchards under climate change, and (2) pollen could compensate this

396   suspected poor adaptation of *E. stipulatus* to hot and dry conditions. Our results provide evidence of the

397   extremely poor performance of *E. stipulatus*, especially in terms of reproduction compared to the other two

398   phytoseiids, even when pollen was available under hot and dry conditions. Although pollen supply

399   significantly increased survival, predation, and oviposition of *E. stipulatus* under hot and dry conditions

400   (Figures 3-5), these increases rarely exceeded or even matched the values observed for *N. californicus* and

401   *P. persimilis*, especially for predation and oviposition,. Because *T. urticae* could survive temperatures of

402   40ºC and even achieve maximum oviposition rates at 35ºC (Figure 1), a temperature that only *N.*

403   *californicus* and *P. persimilis* could survive with limited reproduction (Figure 2), our results confirm that

404   *T. urticae* outbreaks in citrus could become increasingly more frequent in the future.

405   We hypothesized that climate change could differentially impact second and third trophic levels

406   of the mite community established around *T. urticae* in clementines. Our results demonstrate that the

407   different parameters studied (survival, oviposition, and predation) depend on both temperature and relative

408   humidity and are affected by the available food source. Moreover, the magnitude of the impact was species-

409   specific. Mean temperatures above 25ºC, which can be taken as a proxy of summer climate change

410   conditions in Spanish citrus-growing areas (Urbaneja-Bernat et al., 2019), were more detrimental to

411   phytoseiids than to *T. urticae*, which presents maximum survival and oviposition at temperatures about 5ºC

412   higher than best-adapted phytoseiids, independently of relative humidity (Figure 1).

413   Probably because of the experimental setup used, where escapees could not seek shelter but instead

414   died stuck in the glue, the highest impact of hot and dry conditions among phytoseiids was observed for the

415   omnivore *E. stipulatus*. Theory would not predict, the highest impact of climate change on generalist instead

416   of specialist predators, but this is what we were expecting based on previous semi-field assays (Urbaneja-

417   Bernat et al., 2019). The survival of *T. urticae* in our experimental conditions was always above 60%, and

418   it even reached 100% at 25ºC and 30% relative humidity (Figure 1). This means that the strain we worked

419   with, originally collected in a clementine orchard of La Plana Region, is quite tolerant to hot and dry

420   conditions. Other authors had also reported high survival rates for *T. urticae*, at either constant or fluctuating

421   temperatures, similar to ours (Gotoh et al., 2004a and b; Vangansbeke et al., 2013). Likewise, the maximal

422   oviposition rate (8.7 eggs) obtained at 35ºC independently of relative humidity falls into the range of what

423   other authors had previously reported (7.1 to 9.1 eggs) (Bounfour & Tanigoshi, 2001; Vangansbeke et al.,

424   2013). These values contrast with what was observed for the three phytoseiids. Only *N. californicus*

425   presented a survival comparable to *T. urticae* at high temperatures (except when pollen was the only food

426   source available) (Figure 3). This species was initially purchased from a commercial producer and this may

427   explain the tolerance to a wide range of temperature and relative humidity conditions representative of the

428   new environments where it may be released. For the other two phytoseiids, survival barely exceeded 60%

429   in the best case, which was always at temperatures below 25º C, except in the case of *E. stipulatus* when

430   fed pollen only. In this case, survival also exceeded 60% irrespective of temperature and relative humidity

431   (Figure 3).

432   Although, *N. californicus* and, particularly, *P. persimilis* could increase their predation rates on *T.*

433   *urticae* eggs up to 30ºC, and probably effectively regulate the herbivore below this temperature (i.e.

434   predation rate > *T. urticae* oviposition rate) (Figures 1, 2), at higher temperatures this regulation would

435   probably disappear. The combined effect of lower predation and meager oviposition rates for both

436   phytoseiids plus the lower survival rate for *P. persimilis*, would probably release *T. urticae* from effective

437   natural regulation. However, this was not what we found when the same trophic chain were studied under

438   semi-field conditions at spring and summer climate change conditions (Urbaneja-Bernat et al., 2019).

439   Indeed, *E. stipulatus* lost effectiveness during hot and dry conditions, and this is in agreement with what

440   we have found in the present study. However, both *N. californicus* and *P. persimilis* could keep *T. urticae*

441   under control within the fluctuating temperature and relative humidity regimes registered in these semi-

442   field assays (Urbaneja-Bernat et al., 2019). Some authors (Ferragut et al. 1987) do not consider *E. stipulatus*

443   as a suitable biological control agent for *T. urticae*. However, both field (Pérez-Sayas et al., 2015) and semi-

444   field (Grafton-Cardwell et al., 1997) assays in citrus point at the important role of this phytoseiid, which

445   should be attributed to its higher abundance relative to other co-occurring phytoseiids rather than to its

446   effectiveness, in the regulation of *T. urticae* populations in clementines. This predator is known to be poorly

447   adapted to prey on *T. urticae* because it cannot invade the web produced by this spider mite (Ferragut et al.

448   1992; Abad-Moyano et al. 2010). However, we observed a non-negligible predation rate at low

449   temperatures and high relative humidity regimes (15.6 eggs at 15-20ºC and 70% relative humidity), which

450   improved when *E. stipulatus* had access to pollen (Figure 4). As this is an omnivorous phytoseiid,

451   considered a specialized pollen feeder (Ferragut et al. 1987; González-Fernández et al. 2009; Pina et al.

452   2012; Guzmán et al. 2016), the provision of pollen partly compensated the negative results observed in the

453   laboratory when *T. urticae* was the only food source available (Figures 2-5). Whether this positive effect

454   would translate into better control of the populations of *T. urticae*, though, remains doubtful. As already

455   mentioned, Beltrà et al., (2017) demonstrated that the provisioning of pollen and sugars in Spanish citrus

456   orchards had no impact on phytoseiids from June to September. Moreover, Montserrat et al., (2013a) found

457   that although pollen supply in avocadoes could boost the populations of omnivorous predatory mites

458   (including *E. stipulatus*), this increase did not result in better control of the populations of another

459   tetranychid pest mite, the persea mite, *Oligonychus perseae* Turtle, Baker, and Abbatiello (Acari:

460   Tetranychidae).

461   *Neoseiulus californicus* has been traditionally associated with higher tolerance to heat and drought

462   (Escudero and Ferragut 2005; Walzer et al. 2007; Abad-Moyano et al. 2009b; Ahn et al. 2010). However,

463   this has not always been the case (Castagnoli et al., 2001; Castagnoli & Simoni, 1999; Croft et al., 1998;

464   Ghazy et al., 2014; Nguyen et al., 2015), and this may be partly attributed to the use of different strains

465   which may differ in their tolerance to harsh conditions. As this species was also the most tolerant to climate

466   change conditions in our laboratory assays, its good performance in the field (Urbaneja-Bernat et al., 2019)

467   was not a surprise, and it indeed performed better than what could be deduced based on these laboratory

468   assays. This enhanced performance could be the result of two factors acting synergistically. On the one

469   hand, in the laboratory, we worked at constant temperature regimes, which can be taken as a worst-case

470   scenario that does not allow the mite to recover, most likely at night, from maximum temperatures attained

471   during the day at field conditions. It is known that fluctuating temperatures have usually a lower impact on

472   arthropod physiology and behavior than a constant temperature equivalent to their mean (Nguyen &

473   Amano, 2010; Vangansbeke et al., 2013; Gotoh et al., 2014; Bayu et al., 2017). On the other, we observed

474   that the number of phytoseiid escapees in our assays was more extensive than that of dead specimens

475   (Figure 2, 3), and this may be taken as indicative that in the real world, these individuals would have been

476   able to survive in refuges (i.e., crevices or cracks in branches). This behavior, which may impact predator

477   fitness in terms of lost foraging time and reproduction opportunities when looking for shelter (Gillespie et

478   al. 2012), may increase its survival under field conditions. These two factors could also apply to the third

479 predator considered in this study, *P. persimilis*, as the number of escapees for this mite was even higher

480 than observed for *N. californicus* (Figure 1b and c). Indeed, *P. persimilis* spends relatively more time

481 searching and moving around the leaf than the other predatory mite species (Sabelis & Dicke 1985; Gontijo

482 et al., 2012). However, Skirvin & Fenlon (2003) showed that the mobility of *P. persimilis* is reduced at

483 temperatures above 25°C. Nonetheless, we observed the highest number of escapees at 30 and 35°C.

484 Therefore, the local *P. persimilis* strain used in our assays generally performed better than other strains of

485 this phytoseiid at higher temperatures and lower relative humidity regimes (Skirvin & Fenlon, 2003;

486 Escudero & Ferragut 2005; Abad-Moyano et al. 2009; Vangansbeke et al. 2015). Consequently, *P.*

487 *persimilis* would most likely result in a good regulation of *T. urticae* populations, as it was actually observed

488 in our semi-field assays (Urbaneja-Bernat et al., 2019).

489   To sum up, our results partly support the results observed under semi-field climate change

490 conditions (Urbaneja-Bernat et al., 2019). On the one hand, they confirm the extremely fine-tuning of *T.*

491 *urticae* response to hot and dry conditions. On the other, the extremely poor adaptation of *E. stipulatus* and

492 the intermediate effect of hot and dry conditions on *N. californicus* and *P. persimilis*. This poor adaptation,

493 combined with some behavioral adaptations that could not be considered in our laboratory assays, may

494 explain semi-field results (Urbaneja-Bernat et al., 2019) where *T. urticae* was still naturally regulated at

495 temperature and relative humidity conditions matching predicted hotter and drier conditions in the

496 Mediterranean. Although the enormous impact observed on *E. stipulatus* at laboratory conditions when *T.*

497 *urticae* eggs was the only food source available could be partly compensated by the provision of

498 supplementary food (e.g., pollen), this effect does not seem enough to allow *E. stipulatus* to continue to

499 play the essential role in natural regulation of *T. urticae* which it is playing nowadays at spring conditions.

500 Importantly, our results also show that for the other two phytoseiids, access to pollen in combination with

501 *T. urticae* eggs decreased the number of specimens found dead in the arenas and reduced predation and

502 oviposition relative to the *T. urticae*-only diet. Further implications of this supplementary food on

503 interspecific relationships between these predatory species, as *E. stipulatus* is considered a superior

504 intraguild competitor (Abad-Moyano et al., 2010a and b), could shed light on whether pollen supply in this

505 particular system could be advisable or not.

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526   **References**

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Figure legends

Figure 1. Fraction of T. urticae females (a) (stuck in the glue, dead, or alive), and oviposition (b) (number

of eggs laid per female) when exposed to temperatures in the range 10 to 40 °C in combination with 30

(black bars), 50 (grey bars) and 70 % (white bars) relative humidity values during the first 24-hour periods

after the onset of the assay. For and oviposition, bars with the same letter are not statistically different

(Bonferroni P < 0.05).

Figure 2. Survival (stuck in the glue (grey bars), dead (black bars) and alive (white bars)), predation

(number of T. urticae eggs eaten per female), and oviposition (number of eggs laid per female) for Euseius

stipulatus, Neoseiulus californicus and Phytoseiulus persimilis when exposed to temperatures in the range

10 to 40 °C in combination with 30 (black bars), 50 (grey bars) and 70 % (white bars) relative humidity

values during the first 24-hour period after the onset of the assay. For each phytoseiid species, predation

and oviposition bars with the same letter are not statistically different (Bonferroni P < 0.05).

Figure 3. Survival (stuck in the glue (grey bars), dead (black bars) and alive (white bars)) of (a) Euseius

stipulatus, (b) Neoseiulus californicus, and (c) Phytoseiulus persimilis when offered three different diets

(pollen, T. urticae eggs and T. urticae eggs + pollen) considering three temperatures (15, 25 and 30 ºC)

combined with three relative humidity values (30, 50 and 70 % RH).

Figure 4. Predation of (a) Euseius stipulatus, (b) Neoseiulus californicus and (c) Phytoseiulus persimilis

when offered two different diets, (1) T. urticae eggs and (2) T. urticae eggs+ pollen, at 15º, 25º and 30° C

combined with 30, 50 and 70 % of RH. For each figure, bars with the same letter are not statistically

different (Bonferroni P < 0.05).

**Figure 5**. Oviposition of (a) Euseius stipulatus, (b) Neoseiulus californicus and (c) Phytoseiulus persimilis

when offered three different diets: (1) T. urticae eggs, (2) T. urticae eggs + pollen and (3) pollen, at 15º,

25º and 30° C combined with 30, 50 and 70 % of RH. For each figure, bars with the same letter are not

statistically different (Bonferroni P < 0.05).

733

734
735   **Figure 1.**

Euseius stipulatus   *Neoseiulus californicus*   *Phytoseiulus persimilis*

736

737   **Figure 2.**

27

738

Fraction of females

a

Euseius stipulatus

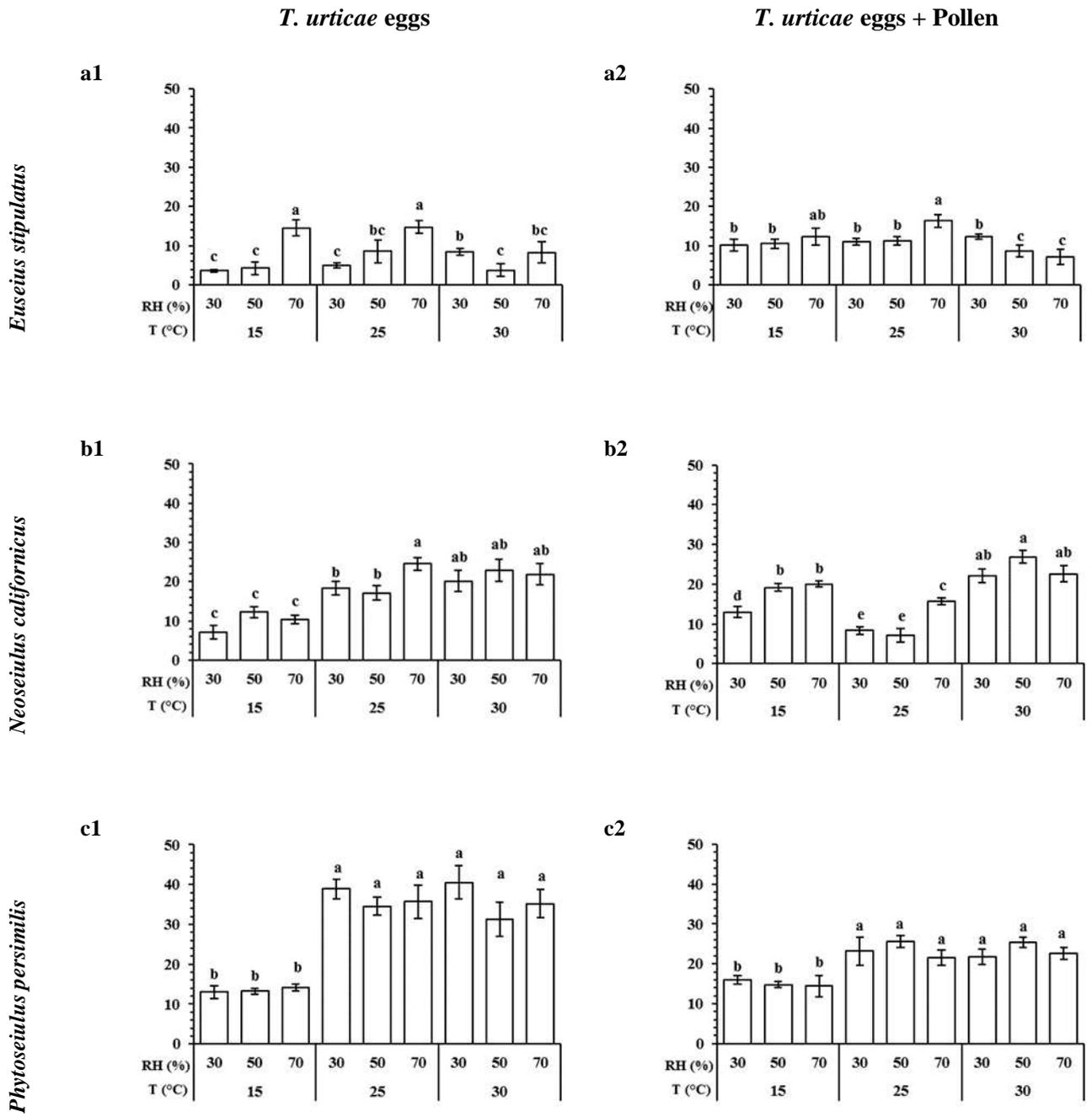
b

Neoseiulus californicus

c
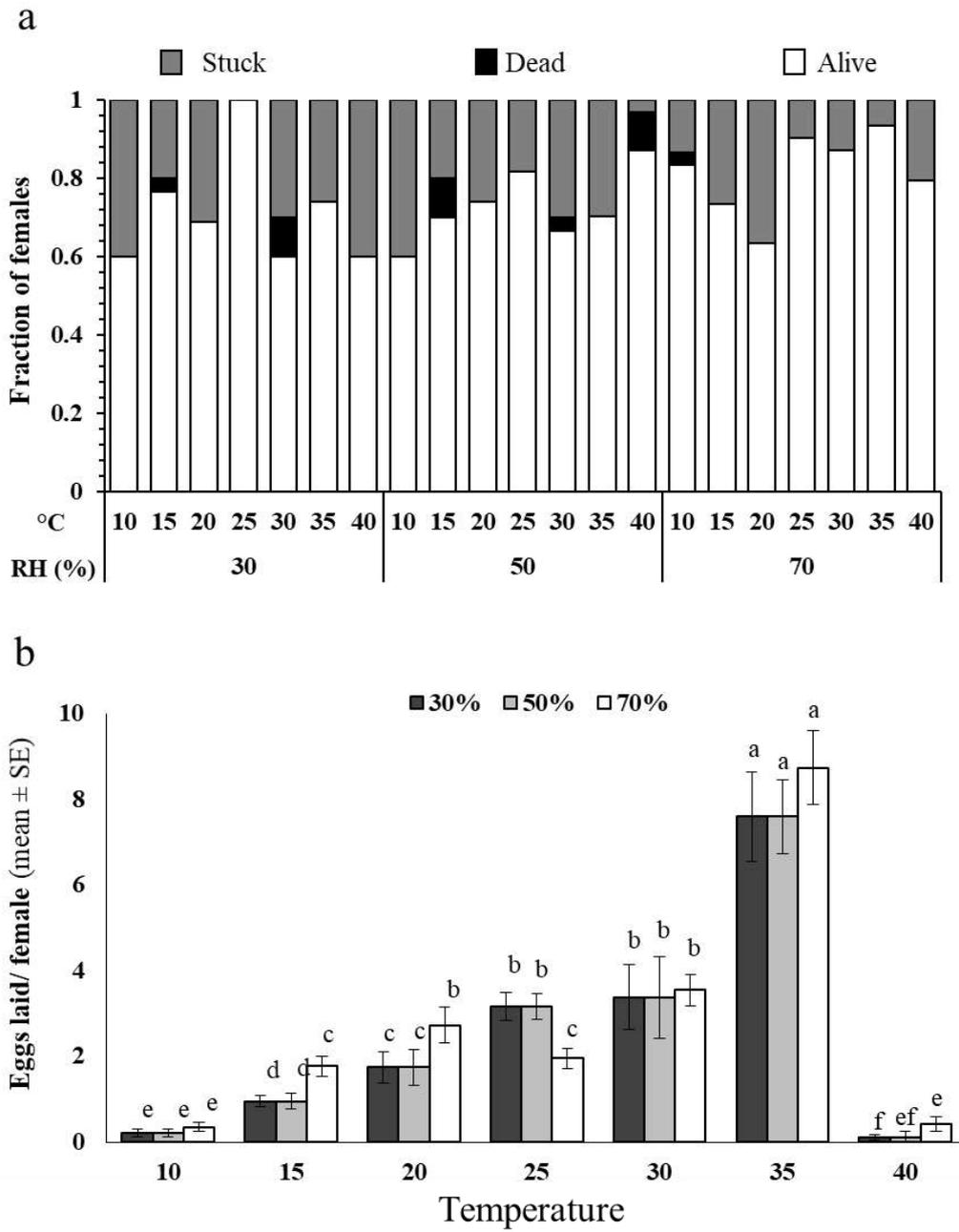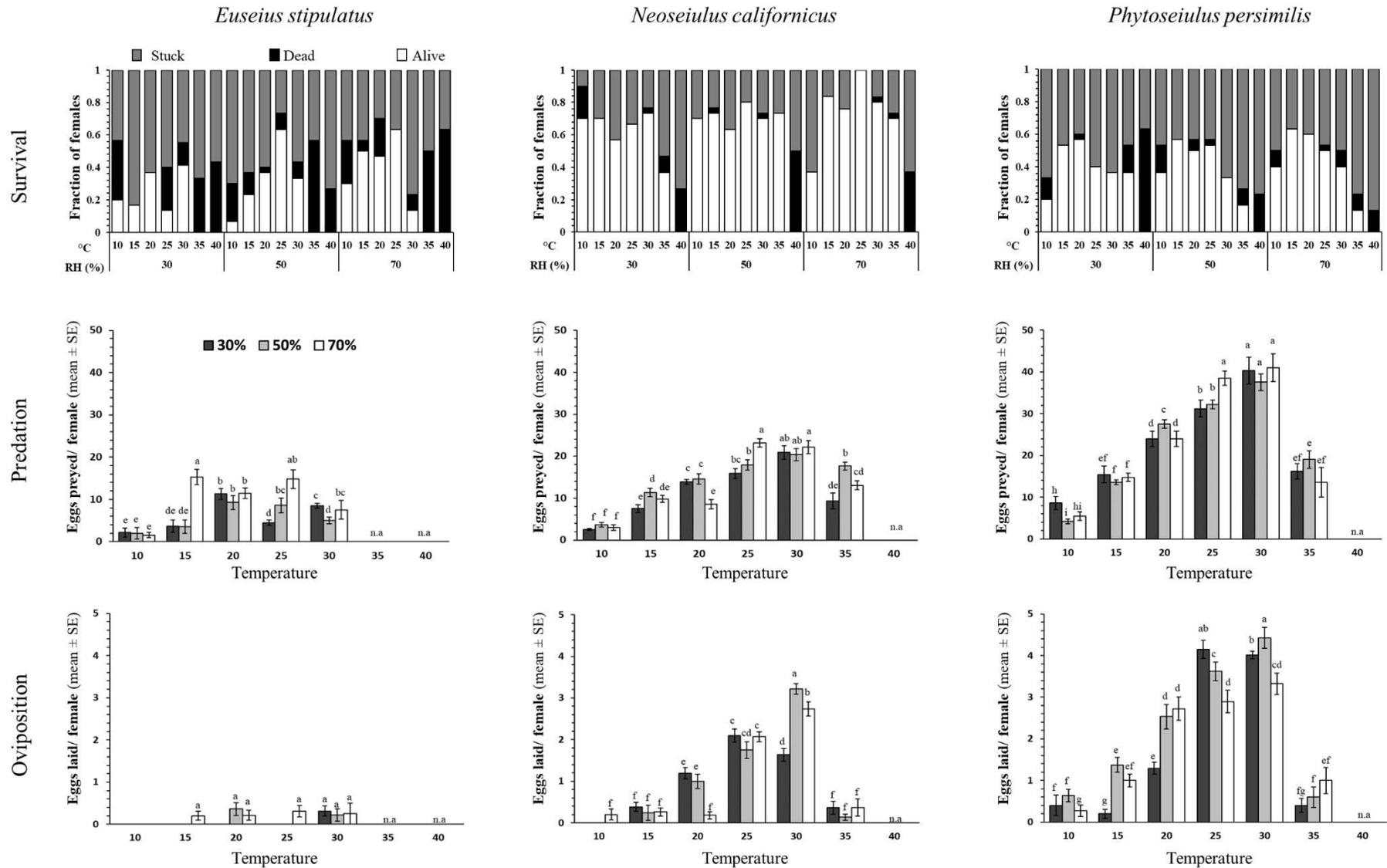
Phytoseiulus persimilis
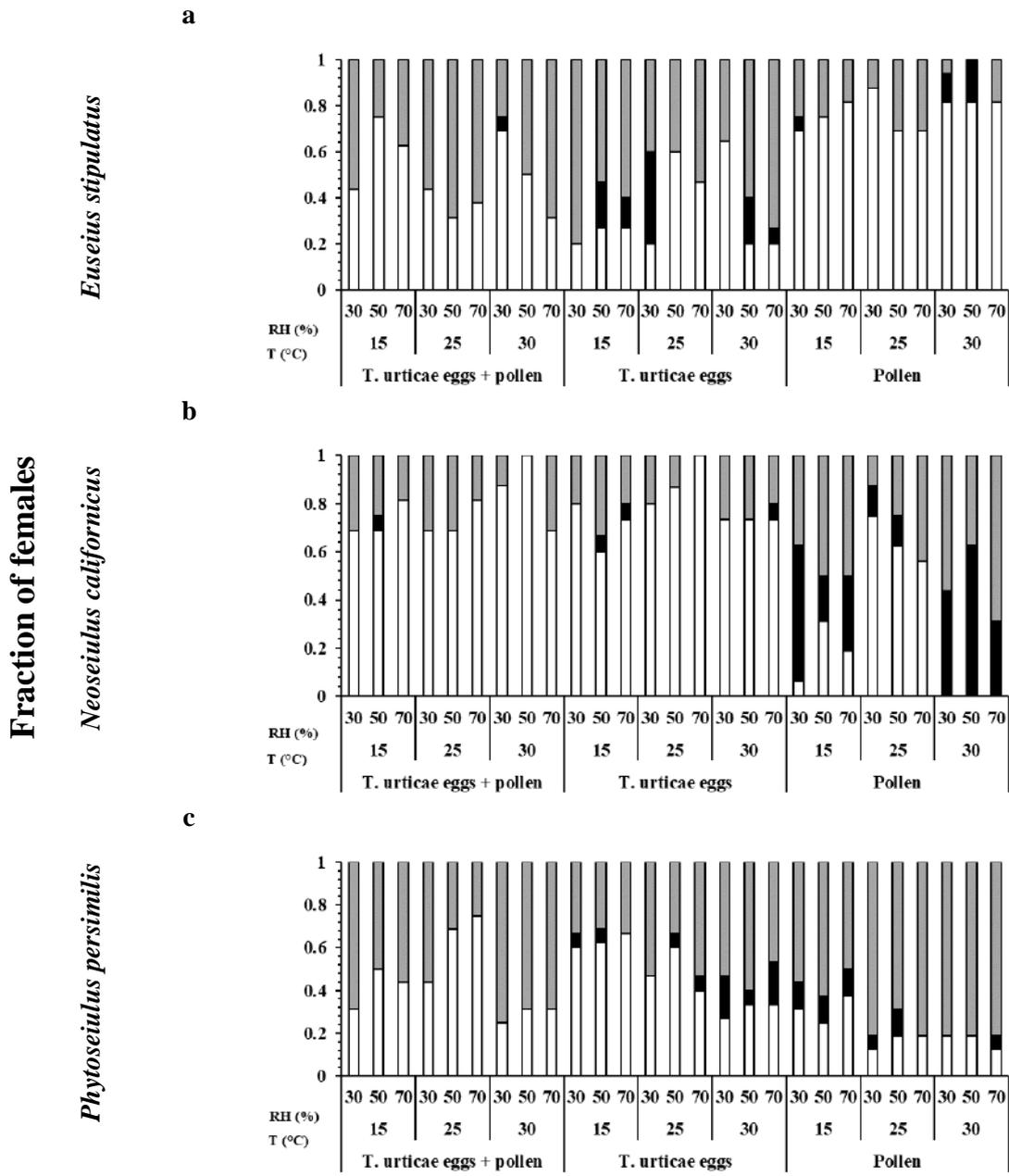
739
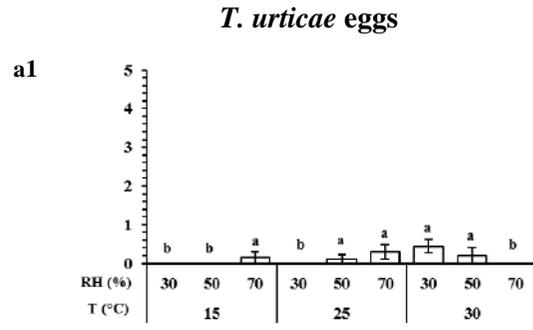740

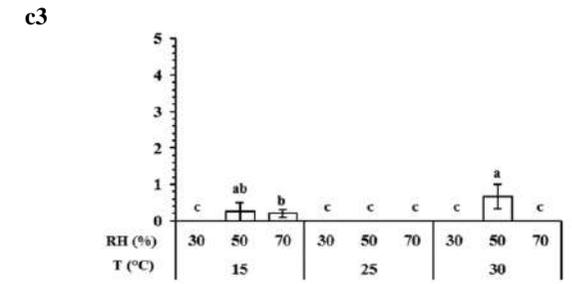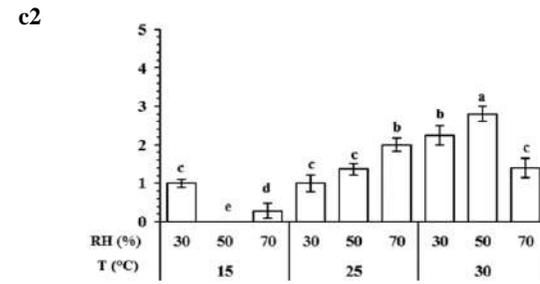741   Figure 3.

742

743

Number of *T. urticae* eggs preyed

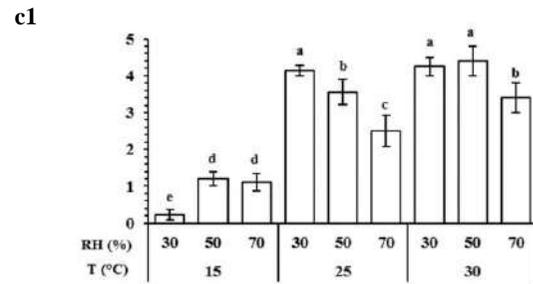*T. urticae* eggs

T. urticae eggs + Pollen

Euseius stipulatus

Neoseiulus californicus

Phytoseiulus persimilis

744

745   Figure 4.

746

Figure 5