

1 **When the ground cover brings guests: is *Anaphothrips obscurus* a friend or a foe**  
2 **for the biological control of *Tetranychus urticae* in clementines?**

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21 *urticae*

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32

33 **Abstract**

34

35 Biological control of *Tetranychus urticae* Koch (Acari: Tetranychidae), a key pest of  
36 clementines, can be improved in this crop with the establishment of a ground cover of  
37 *Festuca arundinacea* Schreber (Poaceae). This cover houses an abundant and diverse  
38 community of predatory Phytoseiidae mites including *Euseius stipulatus* (Athias-  
39 Henriot), *Neoseiulus barkeri* (Hughes), *Neoseiulus californicus* (McGregor) and  
40 *Phytoseiulus persimilis* Athias-Henriot, and a dense population of the grass thrips  
41 *Anaphothrips obscurus* Müller (Thysanoptera: Thripidae) throughout the year. The aim  
42 of this study was to determine whether the presence of this thrips species could be  
43 related to the improvement of the biological control of *T. urticae*. Therefore, the  
44 capacity of the abovementioned phytoseiids to feed and reproduce on *A. obscurus* and  
45 their feeding preferences when *T. urticae* and *A. obscurus* were simultaneously offered,  
46 were analyzed. The results show that *E. stipulatus*, *N. barkeri* and *N. californicus* have a  
47 type II functional response when offered *A. obscurus* nymphs, whereas *P. persimilis*  
48 barely feeds on this thrips species. Furthermore, *N. barkeri* and *N. californicus* can  
49 reproduce feeding only on thrips. Regarding prey preference, the *Tetranychus* spp.  
50 specialist *P. persimilis* preferably preyed on *T. urticae*, the generalists *N. barkeri* and *E.*  
51 *stipulatus* preferred *A. obscurus*, and the selective predator of tetranychid mites *N.*  
52 *californicus* showed no preference. Therefore, we hypothesize that the enhanced  
53 biological control of *T. urticae* observed could be related to *A. obscurus* becoming an  
54 alternative prey for non-specialist phytoseiids, without altering the control exerted by  
55 the *T. urticae*-specialist *P. persimilis* and likely reducing intraguild predation.

56

57 **Keywords**

58

59 *Festuca arundinacea* · Phytoseiidae · Functional responses · Prey preference · Apparent  
60 competition

61

62 **Key message**

63

- 64 • The implementation of a *Festuca arundinacea* cover in clementine orchards,  
65 which results in the enhanced biological control of *Tetranychus urticae*,

66 increases *Anaphothrips obscurus* abundance. The relationship between these two  
67 phytophagous insects remains unclear.

- 68 • Functional responses of predatory mites feeding on *A. obscurus* and their prey  
69 preferences when *T. urticae* is offered are species-specific.
- 70 • *Festuca arundinacea* cover may benefit *T. urticae* biological control by  
71 providing a shared prey (*A. obscurus*) for predatory mites, probably through  
72 apparent competition and reducing intraguild predation.

73

## 74 **Introduction**

75 Conservation biological control (CBC) has increased in importance as agricultural  
76 systems become more intensively managed and pesticide use becomes more restrictive  
77 (EU 2009). CBC practices usually provide shelter, refuge or alternative food to natural  
78 enemies resulting in enhanced biological control (Boller et al. 2004; Liang and Huang  
79 1994; Landis et al. 2000; Jonsson et al. 2008). In Spanish clementine orchards, *Festuca*  
80 *arundinacea* Schreber (Poaceae) is used as ground cover to successfully manage some  
81 citrus key pests including the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann)  
82 (Diptera: Tephritidae), aphids and the two-spotted spider mite *Tetranychus urticae*  
83 Koch (Acari: Tetranychidae). In the case of *C. capitata*, this cover increases the  
84 abundance of soil-dwelling predators (Monzó et al. 2011). For aphids, grassy covers  
85 promote the early arrival of aphid natural enemies (Gómez-Marco et al. 2016a, b). In  
86 the case of the clementine key pest *T. urticae*, the use of this cover improves its control  
87 by reducing the abundance of this mite in the tree canopies (Aguilar-Fenollosa et al.  
88 2011a; 2012) and by enhancing the diversity and abundance of effective predatory  
89 species belonging to the Phytoseiidae family (Acari), both in the canopy and in the  
90 cover (Aguilar-Fenollosa et al. 2011b). *Festuca arundinacea* also provides alternative  
91 food (pollen, honeydew, different microarthropods as mites and thrips) to phytoseiids  
92 (Aguilar-Fenollosa et al. 2011a, b; Pina et al. 2012; Aguilar-Fenollosa and Jacas 2013;  
93 Gómez-Martínez et al. 2017). According to their feeding preferences, phytoseiids can be  
94 grouped from diet specialists, selective predators of tetranychids, to extreme diet  
95 generalists, omnivores feeding on both animal and plant-derived food (McMurtry and  
96 Croft 1997; McMurtry et al. 2013). The ability of some phytoseiids to exploit different  
97 food sources allows them to persist even when the prey they regulate is scarce or absent.  
98 Therefore, the maintenance of non-crop plants providing these alternative food sources  
99 may be key to enhancing biological control.

100 One abundant prey group brought into clementine orchards with the implementation of  
101 a *F. arundinacea* ground cover is thrips. These minute insects are scarcely present when  
102 growing clementines on bare soil but become highly abundant in the cover when  
103 growing clementines in association with *F. arundinacea* (Aguilar-Fenollosa and Jacas  
104 2013). Different species of Thysanoptera have been described as prey, either preferred  
105 or alternative, for the most abundant predatory mites associated with *T. urticae*  
106 (Rodríguez Reina et al. 1992; van Baal 2007; El-Kholy and El-Sayed 2009).  
107 *Anaphothrips obscurus* (Müller) (Thysanoptera: Thripidae) stands out among the most  
108 frequent and abundant thrips when *F. arundinacea* is used as a ground cover (Aguilar-  
109 Fenollosa and Jacas 2013). This thrips species feeds mainly on grasses (Poaceae)  
110 (Brohmer et al. 1966; Stannard 1968). In *F. arundinacea*, *A. obscurus* exhibits a high  
111 intrinsic rate of increase and short generation time, which could allow competition with  
112 *T. urticae* (Gómez-Martínez et al. 2017). Furthermore, Gómez-Martínez et al. (2017)  
113 demonstrated that in clementine orchards grown in association with *F. arundinacea*, *T.*  
114 *urticae* populations decreased while those of *A. obscurus* and phytoseiids as a whole  
115 increased in the cover. Because *A. obscurus* was unable to feed on spider mite eggs and  
116 clementine leaves (Gómez-Martínez et al. 2017), zoophytophagy could be discarded as  
117 the cause of the dynamics observed.

118 Previous laboratory experiments demonstrated that some phytoseiids present in  
119 clementine orchards and feeding on *T. urticae* can attack *A. obscurus* as well (Gómez-  
120 Martínez et al. 2017). Two prey species sharing a natural enemy, even if they are  
121 separated in time or space, are related by indirect interactions such as apparent  
122 competition (Chailleaux et al. 2014). Holt (1977) called this indirect ecological  
123 interaction “apparent competition” because the dynamics it generated could resemble, to  
124 an observer unaware of the shared predator, that of direct competition where a decline  
125 in one species coincides with an increase in the other. Apparent competition may have a  
126 number of consequences in biological control as prey species may affect each other. In  
127 the case of a shared predator, the presence or absence of an alternative prey species can  
128 affect the predator’s ability to control the target prey and therefore can modify trophic  
129 interactions, population dynamics and community structures (Muller and Godfray 1997;  
130 Harmon and Andow 2004; Morris et al. 2005). For instance, in California grapevines,  
131 significant reductions of the serious pest *Tetranychus pacificus* McGregor  
132 (*Tetranychidae*) occurred when both the apparent competitor *Eotetranychus villamettei*  
133 (McGregor) (*Tetranychidae*) and the predator *Metaseiulus occidentalis* (Nesbitt)

134 (Phytoseiidae) were artificially released (Karban et al. 1994; Hanna et al. 1997).  
135 However, when the two greenhouse pests, the thrips *Frankliniella occidentalis*  
136 (Pergande) (Thripidae) and the whitefly *Trialeurodes vaporariorum* (Westwood)  
137 (Hemiptera) shared the predators *Amblyseius swirskii* (Athias-Henriot) and *Euseius*  
138 *ovalis* (Evans) (Phytoseiidae), the presence of whiteflies did not affect thrips density.  
139 On the contrary, thrips presence dramatically reduced whitefly density (Messelink et al.  
140 2008). In our context, phytoseiids in the *F. arundinacea* ground cover could be  
141 exploiting this thrips species as an additional food source and, as a result, apparent  
142 competition between *T. urticae* and *A. obscurus* would appear. However, the effect of  
143 this thrips species on the regulation of *T. urticae* by phytoseiid mites remains unknown.  
144 The suitability of a prey species for a specific predator may be unveiled by studying its  
145 functional response. This predator-prey specific response describes the relationship  
146 between individual prey consumption with food density (Solomon 1949; Holling 1959;  
147 Jeschke et al. 2002). Functional responses of predatory mites feeding on different target  
148 pest species have been thoroughly studied (Fan and Petit 1994; Jalali et al. 2010;  
149 Fantinou et al. 2012; Yao et al. 2014). However, studies focusing on alternative non-  
150 pest prey species are rare. The impact of a generalist predator on a prey species will  
151 depend not only on the abundance and susceptibility of that prey species but also on  
152 those of the other species that share the predator and its prey preference (Eubanks and  
153 Denno 2000). Thus, knowledge about prey preference of a given natural enemy may  
154 help to predict its success or failure in a defined ecosystem.

155 Therefore, the objectives of this study have been to examine i) the ability of different  
156 life-style phytoseiids present in clementine orchards to feed on *A. obscurus* through  
157 functional response analysis, and ii) their feeding preferences when offered a choice of  
158 *T. urticae* and *A. obscurus*.

159

## 160 **Materials and Methods**

161

### 162 **Mites and thrips colonies**

163

164 Predatory mites feeding on *T. urticae* in clementines grown in association with *F.*  
165 *arundinacea* exhibit different life-styles (McMurtry et al. 2013). In Spanish clementine  
166 orchards, these styles range from *Tetranychus* spp. specialization in *Phytoseiulus*  
167 *persimilis* Athias-Henriot to omnivory in *Euseius stipulatus* (Athias-Henriot) and

168 include species with intermediate feeding habits such as the selective predator of  
169 tetranychids *Neoseiulus californicus* (McGregor), and the generalist *Neoseiulus barkeri*  
170 Hughes (Aguilar-Fenollosa et al. 2011a). These four species as well as *T. urticae* were  
171 used in our assays. These species were initially collected in different citrus orchards  
172 near Castelló de la Plana (UTM: 30N, 753344.973 m E, 4430087.389 m N). The only  
173 exception was *N. californicus*, which was obtained from a commercial producer  
174 (Koppert Biological Systems; SPICAL<sup>®</sup>). *Euseius stipulatus* and *P. persimilis* were  
175 obtained from orange and clementine trees, respectively, whereas *N. barkeri* was  
176 collected from *F. arundinacea* plants. The stock colony of *T. urticae* was obtained from  
177 clementine trees in the same area and maintained on lemons (Aucejo et al. 2003).  
178 Phytoseiids were reared following the method described by Overmeer (1985). In the  
179 case of *E. stipulatus*, the plastic tile used in the rearing unit was substituted by an  
180 upside-down bean leaf. Pollen of *Carpobrotus edulis* (L.) N. E. Br (Aizoaceae) and a  
181 mixture of different stages of *T. urticae* obtained from a rearing maintained on lemon  
182 [*Citrus limon* (L.) Burm f. (Rutaceae)] were regularly added to the rearing as a food  
183 source.

184 *Anaphothrips obscurus* individuals were originally collected from *F. arundinacea*  
185 plants grown in experimental plots at Universitat Jaume I (Castelló de la Plana, Spain).  
186 They were later maintained on the same type of plants (*F. arundinacea* 'Fórmula  
187 frutales y cítricos', Semillas Fitó S.A., Barcelona, Spain) grown in a pesticide-free  
188 greenhouse in the Institut Valencià d'Investigacions Agràries (IVIA) (Montcada,  
189 Valencia, Spain). The *A. obscurus* rearing unit consisted of detached *F. arundinacea*  
190 leaves set adaxially on a water-saturated sponge covered by filter paper in a plastic  
191 container (17 × 12.5 × 7.5 cm). Both leaf ends were fixed with wet cotton strips, which  
192 prevented the escape of the thrips.

193 All stock colonies were maintained in a climatic chamber at 25 ± 1 °C, 70 ± 5% RH and  
194 a 16:8 h (L:D) photoperiod.

195

## 196 **Experimental set-up**

197

198 Functional response was assessed in Petri dishes (9 cm Ø) with a hole in the lid (6 cm  
199 Ø) covered with anti-thrips mesh (14 × 95 µm). Petri dishes were filled with water and  
200 provided with a plastic tile (6 × 3 × 1.5 cm) fixed to the center of the Petri dish with  
201 glue. A 6 cm long fragment of a *F. arundinacea* leaf was placed adaxially on the top of

202 the plastic tile and both leaf ends were fixed with wet cotton strips. Tile borders were  
203 covered with wet paper to supply water to thrips and prevent them from escaping from  
204 the experimental arena. Dishes were sealed with PARAFILM<sup>®</sup> M.  
205 The experimental units used for the predation assays with *P. persimilis* immature stages  
206 consisted of a PVC plate (80 × 35 × 3 mm) containing two 15 mm in diameter  
207 chambers. The bottom of these chambers was covered by a fine mesh glued to the plate  
208 and closed on the upper side by a microscope slide held in place by two rubber bands  
209 (Schausberger 1997).  
210 The choice experiment was performed using the T-shaped cages described by  
211 Schausberger and Hoffmann (2008). They consisted of a PVC plate (same dimensions  
212 as before) containing three circular chambers connected through a T-shaped excavation  
213 of 2 mm wide and 10 and 5 mm long for the horizontal and vertical bars, respectively.  
214 The two chambers located at the extremes of the horizontal bar were 15 mm in diameter  
215 and the one located at the end of the vertical bar was 5 mm. The cage was closed as  
216 before.

217

## 218 **Experimental design**

219

### 220 *Functional response*

221

222 The functional response of each predator species when offered *A. obscurus* nymphs was  
223 investigated in different assays. First instar nymphs (N1) are the most vulnerable thrips  
224 stage (Madadi et al. 2007) and this was the prey stage chosen for these experiments. To  
225 obtain N1, cohorts of eggs less than 24 h old were established in the rearing unit  
226 described above. Newly hatched N1 were transferred to the experimental arena with a  
227 fine camel hair brush and the following densities were considered: 1, 3, 5, 10 and 20  
228 N1. The preliminary results suggested a superior predatory activity for *N. californicus*  
229 and *N. barkeri*. Therefore, two extra densities of 30 and 40 N1 were considered for *N.*  
230 *californicus* and *N. barkeri*, respectively. Once *A. obscurus* nymphs stopped moving,  
231 one gravid phytoseiid female at its maximum peak of oviposition rate (2-3 day old) was  
232 introduced into each arena. These females were obtained from less than 24 h old egg  
233 cohorts. To standardize the response, all phytoseiid females were individually starved  
234 for 24 h before the onset of the assay in the same type of PVC plates as those used for  
235 the *P. persimilis* predation assays. During this period, they had access to water only,

236 which was supplied twice per day through the mesh using a wet brush. Up to 34  
237 replicates per prey density and phytoseiid species were considered. Furthermore, up to  
238 28 arenas without predator for each prey density were used as control. Thrips killed  
239 during the experiment were not replaced (prey depletion method). After 24 h, the  
240 numbers of N1 alive, killed by predation and dead by other undetermined reasons were  
241 recorded. As phytoseiids suck out the body fluids of their prey, collapsed N1 corpses  
242 were taken as evidence of predation. Additionally, the number of eggs laid by each  
243 predator and at each prey density offered was counted. Eventually, phytoseiids were  
244 slide mounted in Hoyer's medium (Gutiérrez 1985) to confirm their identity. These  
245 assays took place in a climatic chamber at  $25 \pm 1$  °C,  $70 \pm 10\%$  RH and 16:8 h (L: D)  
246 photoperiod.

247

#### 248 *Phytoseiulus persimilis* predation experiment

249

250 Due to largely absent predation on thrips by adult *P. persimilis* in the functional  
251 response experiment (see results), additional experiments were conducted with  
252 immature stages of *P. persimilis* only. To determine the ability of *P. persimilis* to  
253 exploit *A. obscurus*, protonymphs and deutonymphs starved for 24 h were individually  
254 transferred to the arena where they were offered 10 N1 thrips. As in the previous  
255 experiments, the numbers of N1 alive, killed by predation and dead by other  
256 undetermined reasons were recorded the following day. Environmental conditions were  
257 the same as above.

258

#### 259 *Prey preference*

260

261 One *T. urticae* deutonymph and one *A. obscurus* N1 were set at the center of each large  
262 15 mm circular chamber and simultaneously offered to the phytoseiid, which was  
263 released in the center of the small 5 mm circular chamber. Both preys had been  
264 previously killed (5 min at -80 °C) to avoid any movement between chambers during  
265 the experiment. Adult females of *N. californicus*, *N. barkeri* and *E. stipulatus* and  
266 deutonymphs of *P. persimilis* were used in these experiments. Phytoseiid activity was  
267 continuously monitored using a binocular microscope. The position of the predator, the  
268 first and successive feeding events and the time spent feeding on each prey were  
269 continuously observed under a dissecting binocular microscope for a total of 120 min at



270 room conditions. The initial position of each prey was consistently interchanged among  
 271 replicates to avoid any inadvertent positional effect. Each specimen was used only once  
 272 and then discarded. Cages were cleaned with 70% ethanol before use. All predators  
 273 came from cohorts established on *F. arundinacea* rearing units with *C. edulis* pollen and  
 274 a similar proportion of *A. obscurus* and *T. urticae* as food supply. Because *E. stipulatus*  
 275 was unable to complete its development in this rearing system, newly emerged adults  
 276 from a cohort fed as the stock colonies were maintained in this new system for 5 days  
 277 prior to the experiment.

278

### 279 **Data analysis**

280

281 Predation was corrected for control mortality using the formula proposed by Xia et al.  
 282 (2003):

$$283 \quad N_e = N_0 \frac{N_d - N_c}{N_0 - N_c} \quad (1)$$

284 where  $N_e$  represents the number of prey killed,  $N_0$  the initial number of prey,  $N_d$  the  
 285 number of prey eaten and dead in the treatment and  $N_c$  the number of prey dead in the  
 286 control. Functional response of each predator species was then analyzed in two steps: i)  
 287 determination of functional response type, and ii) estimation of the parameters of the  
 288 fitted curve. A cubic logistic regression of the relative proportion of N1 preyed was  
 289 performed to evaluate the shape of the functional response curve that best fit the data for  
 290 each phytoseiid species (Juliano 2001):

$$291 \quad \frac{N_e}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)} \quad (2)$$

292 where  $N_e$  represents the number of prey eaten;  $N_0$  represents the initial number of prey;  
 293 and  $P_0$ ,  $P_1$ ,  $P_2$  and  $P_3$  represent the estimated intercept, linear, quadratic and cubic  
 294 coefficients, respectively. A linear coefficient not significantly different from 0  
 295 indicates a type I functional response; a significant negative linear coefficient indicates  
 296 a type II response, while a significant positive linear term indicates a type III response.  
 297 Once the functional response type was determined, average data were further fitted by  
 298 iteration to Rogers random predator equation (Rogers 1972), which takes into account  
 299 predator handling time and prey depletion over time.

$$300 \quad N_e = N_0 \left\{ 1 - \exp \left[ a' (T_h N_e - T) \right] \right\} \quad (3)$$

301 where, as before,  $N_e$  represents the number of prey eaten,  $N_0$  represents the initial  
302 density of prey,  $T$  represents total time available for attack and the estimated parameters  
303  $a'$  and  $T_h$  represent the attack constant and handling time, respectively. The attack  
304 constant relates the predator-prey encounter rate with prey density and the handling time  
305 includes all the time the predator spends with the prey being unable to attack another  
306 prey (Juliano 2001). The 95% confidence intervals of the estimated parameters of the  
307 functional response ( $a'$  and  $T_h$ ) were used to evaluate differences between phytoseiid  
308 species (Juliano 2001). The maximum predation rate was estimated from  $T/T_h$  (Hassell  
309 1978). The value of  $a'/T_h$  indicates the effectiveness of predation. Data were analyzed  
310 using R 3.3.1 (R Core Team 2016).

311 The average number of eggs laid during the first 24 h by the three phytoseiids was  
312 linearly regressed against the number of nymphs offered. Analyses were performed  
313 using STATGRAPHICS Centurion XVI ver. 16.1.18.

314 The effect of prey position on prey choice and that of the identity of the first prey  
315 species attacked on the probability of a second attack and the identity of this second  
316 prey (either the same species or not) were analyzed by Pearson's  $\chi^2$  test with Yates'  
317 continuity correction. Prey preference was analyzed by a one-sample proportion test  
318 with continuity correction, and the time feeding on each prey was analyzed by Welch's  
319 two sample  $t$ -test. These data were analyzed using R 3.3.1 (R Core Team 2016).

320

## 321 **Results**

322

### 323 *Functional response*

324

325 *Neoseiulus californicus*, *N. barkeri* and *E. stipulatus* showed a type II functional  
326 response, as determined by a negative and significant estimated linear coefficient  $P_1$   
327 (Table 1). The number of prey eaten by these predators increased with increasing prey  
328 density (Fig. 1). Rogers random predation equation (3) fit the observed data for all  
329 phytoseiid species with determination coefficients approximately 0.98 (Table 2).

330 Estimates of the attack constant and handling time and the 95% CI for each predator are  
331 shown in table 2. As 95% CI did not overlap, the attack constant of *N. barkeri* was  
332 significantly higher ( $1.766 \pm 0.339$  days<sup>-1</sup>) than those of *N. californicus* and *E.*  
333 *stipulatus* ( $0.542 \pm 0.151$  and  $0.711 \pm 0.175$  days<sup>-1</sup>, respectively). The attack constants of  
334 these two species were not significantly different from each other. On the contrary,

335 handling time estimates exhibited significant differences between the three species.  
336 *Neoseiulus barkeri* spent less time handling *A. obscurus* N1 ( $0.080 \pm 0.003$  days) than  
337 *N. californicus* ( $0.169 \pm 0.019$  days), and the latter spent less time handling than *E.*  
338 *stipulatus* ( $0.264 \pm 0.021$  days). The estimated maximum number of N1 preyed on by *N.*  
339 *barkeri* was 11.33, and this figure is 1.9 and 2.9 times higher than *N. californicus* and *E.*  
340 *stipulatus*, respectively (Table 2). At low prey densities, which may be taken as  
341 indicative of prey searching efficiency in a worst case scenario, *N. barkeri* always  
342 consumed all prey offered at a density of 1 and an average of 2.60 prey at a density of 3.  
343 Conversely, the average of N1 preyed by *N. californicus* and *E. stipulatus* was 0.68 and  
344 1.62 at densities of 1 and 3, respectively.  
345 The functional response could not be described for *P. persimilis* females due to the low  
346 number of N1 preyed independently of the N1 density. Predation by *P. persimilis* on *A.*  
347 *obscurus* N1 was positive only in 3 out of 33 replicates and only one prey was  
348 consumed. Furthermore, 36% of *P. persimilis* females died during the experiment, and  
349 the surviving females did not lay any eggs.  
350 The number of eggs laid per day increased linearly as a function of the nymphs offered,  
351 with a positive and highly significant correlation in *N. californicus* ( $R^2 = 0.704$ ,  $P =$   
352  $0.023$ ) and *N. barkeri* ( $R^2 = 0.647$ ,  $P = 0.033$ ) (Fig. 2). The number of eggs laid by *E.*  
353 *stipulatus* was independent of the nymph density ( $P = 0.974$ ).

354

#### 355 *Phytoseiulus persimilis* predation experiment

356

357 *Phytoseiulus persimilis* protonymphs and deutonymphs were more aggressive than adult  
358 females, as prey attacks occurred more frequently. All protonymphs ( $n = 14$ ) and  
359 deutonymphs ( $n = 9$ ) preyed on *A. obscurus* N1. Mean predation rates for protonymphs  
360 ( $0.43 \pm 0.04$ ) and deutonymphs ( $0.47 \pm 0.04$ ) were not significantly different (Mann-  
361 Whitney  $U$  test,  $P > 0.05$ ). Mortality during the assay was null for protonymphs and  
362 18% for deutonymphs.

363

#### 364 *Prey preference*

365

366 Feeding behavior was different for each predator tested. More than one half of the *E.*  
367 *stipulatus* (35 out of 61) and almost half of the *P. persimilis* specimens tested (18 out of  
368 35) did not feed on either prey species. This percentage dropped to 10 and 14% for *N.*

369 *barkeri* (7 out of 66) and *N. californicus* (6 out of 44), respectively. Prey location within  
370 the arena did not affect first prey choice (Pearson's  $\chi^2$  test with Yates' continuity  
371 correction,  $P > 0.05$  in all predators).  
372 All predator species, except *N. californicus*, preferentially fed on one prey species.  
373 *Phytoseiulus persimilis* showed a strong preference for *T. urticae* ( $\chi^2 = 7.563$ ,  $P =$   
374  $0.006$ ), whereas *N. barkeri* and *E. stipulatus* preferred *A. obscurus* ( $\chi^2 = 6.780$ ,  $P =$   
375  $0.009$ ;  $\chi^2 = 4.654$ ,  $P = 0.031$ , respectively) (Fig. 3). These preferences were also  
376 reflected in the time spent feeding on each prey species (Table 3). A second feeding  
377 event was observed in 68.4% of *N. californicus*, 56.3% of *P. persimilis*, 35.6% of *N.*  
378 *barkeri* and 30.8% of *E. stipulatus*. Only for *N. barkeri* did the identity of the first prey  
379 species chosen determine the second feeding event ( $\chi^2 = 7.599$ ,  $P = 0.006$ ). The highest  
380 number of second feeding events was observed when *T. urticae* was the first prey ( $\chi^2 =$   
381  $15.429$ ,  $P < 0.001$ ). Furthermore, prey change during the second feeding event was  
382 observed for *N. barkeri* and *N. californicus* ( $\chi^2 = 0.805$ ,  $P = 0.045$ ;  $\chi^2 = 7.583$ ,  $P =$   
383  $0.006$ , respectively) whereas *P. persimilis* always fed on the same prey (*T. urticae*) even  
384 when this prey had been previously handled and partially or totally consumed. *Euseius*  
385 *stipulatus* usually fed on the same prey species in the second feeding event even though  
386 differences were not significant ( $\chi^2 = 2.133$ ,  $P = 0.144$ ).

387

## 388 **Discussion**

389

390 Understanding the interactions between pests and their natural enemies is essential for a  
391 successful pest management program. Our work demonstrates that most phytoseiid  
392 species exploiting *T. urticae* can also exploit *A. obscurus*. We suggest that in the field,  
393 *T. urticae* and *A. obscurus* likely interact via apparent competition, despite the artificial  
394 nature of the arenas and short measurement periods used in our assays. Below, we will  
395 discuss the relationship between each phytoseiid species, *A. obscurus* and *T. urticae*,  
396 and their potential implications on the biological control of this pest mite in clementine  
397 orchards with a *F. arundinacea* ground cover.

398

### 399 *Phytoseiulus persimilis*

400 *Phytoseiulus persimilis* is considered a *Tetranychus* spp. specialist predator (McMurtry  
401 et al. 2013). It can also feed on thrips as *Frankliniella occidentalis* (Pergande)  
402 (Thysanoptera: Thripidae), a food resource allowing full immature development

403 (Walzer et al. 2004). Although in our assays *P. persimilis* immature stages fed on *A.*  
404 *obscurus*, deutonymphs exhibited a strong preference for *T. urticae*. Indeed, in case of  
405 *T. urticae* depletion, they still preferred to revisit *T. urticae* corpses rather than changing  
406 to *A. obscurus*. This tetranychid specialization was even stronger for adult females,  
407 which rarely fed on the thrips (only in 3 out of 33 replicates). Indeed, one third of them  
408 died without feeding on it. These results are in agreement with observations in Walzer  
409 et al. (2004) that *P. persimilis* diet specialization changes with development. Therefore,  
410 the availability of *A. obscurus* as an alternative prey should not negatively affect the  
411 natural regulation of *T. urticae* by *P. persimilis*. Rather, the presence of this thrips in the  
412 cover might result in enhanced biological control of *T. urticae* due to a reduction of *P.*  
413 *persimilis* immature mortality as a consequence of preying, even in low numbers, on  
414 this non-preferred prey. This reduction in immature mortality could result in high adult  
415 phytoseiid populations that entail a potential reduction of the target prey density  
416 (Sabelis and van Rijn 2006). As a successful dispersal of phytoseiids from ground cover  
417 to the tree canopy has been observed in this system (Aguilar-Fenollosa et al. 2016), we  
418 could expect a better regulation of *T. urticae* both in the canopy and in the ground  
419 cover.

420

#### 421 *Neoseiulus californicus*

422 *Neoseiulus californicus* is considered a selective predator of tetranychid mites  
423 (McMurtry and Croft 1997; McMurtry et al. 2013). Moreover, it has been described as a  
424 candidate biological control agent of some pestiferous thrips species (van Baal et al.  
425 2007; Walzer et al. 2004). Herein we have demonstrated that *N. californicus* benefits  
426 from feeding on *A. obscurus* by increasing prey consumption (type II functional  
427 response) and oviposition with increasing thrips densities. Determining the suitability of  
428 a given prey for predator reproduction by starving the predators for one day and  
429 subsequently feeding them for another day on the target prey could be a priori  
430 inadequate or insufficient, as predators do not immediately convert the ingested food  
431 into eggs. However, taking into account that all individuals had the same feeding status  
432 at the onset of the assay (i.e., ad libitum feeding and standard 24 h of starvation), the  
433 increase observed in oviposition with increasing thrips density suggests that this is not  
434 an artifact. These aptitudes (prey consumption and oviposition) should allow the  
435 maintenance and augmentation of *N. californicus* populations solely feeding on *A.*  
436 *obscurus*, and assuming that they can complete juvenile development with this prey.

437 Furthermore, as *N. californicus* did not show any preference for the two herbivores, a  
438 prey switch would be expected in response to the relative availability of *A. obscurus* and  
439 *T. urticae*, as it often happens in non-specific entomophagous species (Murdoch 1969;  
440 Murdoch and Oaten 1975; Holt 1977, Holt and Lawton 1994). As *T. urticae* is present  
441 in both the *F. arundinacea* cover and the clementine canopy (Aguilar-Fenollosa et al.  
442 2011a) whereas *A. obscurus* is mostly found in the cover (Aguilar-Fenollosa and Jacas  
443 2013), *N. californicus* would be expected to prey randomly on both prey species in the  
444 cover and mostly on *T. urticae* in the tree canopies. Therefore, the presence of *A.*  
445 *obscurus* in clementine orchards could result in higher *N. californicus* densities and  
446 stronger predation pressure on both herbivores, thus benefiting *T. urticae* biological  
447 control by apparent competition. If this was the case, it would be similar to that reported  
448 by Liu et al. (2006) in apples, where *T. urticae* populations were reduced by the  
449 addition of the apparent competitor *Eotetranychus pruni* Oudemans with *Euseius*  
450 *finlandicus* (Oudemans) as a shared predator.

451

#### 452 *Neoseiulus barkeri*

453 *Neoseiulus barkeri* is a generalist predator from soil/litter habitats (McMurtry et al.  
454 2013) that has been reported as a biological control agent of *T. urticae* (Karg et al. 1987,  
455 Bonde 1989, Fan and Petit 1994) and used for the biological control of thrips (Ramakers  
456 and van Lieburg 1982; Hansen 1988). In our study, *N. barkeri* presented a type II  
457 functional response, which is in agreement with the results of Fan and Petit (1994) when  
458 this species fed on *T. urticae*. Among the species considered in this study, *N. barkeri*  
459 was the most effective predator as it exhibited the highest attack constant and the lowest  
460 handling time. Furthermore, an increment in oviposition was observed with increasing  
461 prey densities. In the prey preference assays, *N. barkeri* preferred *A. obscurus* as a first  
462 prey and attacked the other species in the second attack. When a shared predator prefers  
463 the non-pest prey species, the potential of negative indirect interactions (i.e., apparent  
464 competition) to enhance the biological control of the pest are reduced (Chailleux et al.  
465 2014). However, as *A. obscurus* and *N. barkeri* are rare in the clementine canopy,  
466 especially when the trees are grown in association with *F. arundinacea* (Aguilar-  
467 Fenollosa et al. 2011a, b), these negative interactions may not be relevant for the  
468 biological control of *T. urticae*.

469

#### 470 *Euseius stipulatus*

471 *Euseius stipulatus* is a pollen-feeding generalist predator able to feed on  
472 microarthropods and vegetal or animal exudates (McMurtry et al. 2013). In clementine  
473 orchards, this species can feed on *T. urticae* and *P. citri* (Pérez-Sayas et al. 2015).  
474 Moreover, the populations of this omnivore can explode when pollen is available (Pina  
475 et al. 2012) and outcompete more efficient *T. urticae* specialist phytoseiids (*P.*  
476 *persimilis* and *N. californicus*) (Abad-Moyano et al. 2010a, b). For this reason, wild  
477 cover crops, producing an abundant pollen supply throughout the year, are not  
478 considered adequate for the management of *T. urticae* in citrus orchards (Aguilar-  
479 Fenollosa et al. 2011b). *Euseius stipulatus* has also been described feeding on thrips  
480 species as *F. occidentalis* (Rodríguez-Reina et al. 1992). In our study, this phytoseiid  
481 preferred to feed on *A. obscurus* and increased prey consumption as thrips density  
482 increased (type II functional response). Despite the fact that *E. stipulatus* laid some eggs  
483 when feeding on *A. obscurus*, oviposition could not be related to prey density, same as  
484 when *T. urticae* was the prey (Ferragut et al. 1987; Abad-Moyano et al. 2009).  
485 Therefore, both prey species alone are unsuitable for increasing *E. stipulatus*  
486 populations. This might preclude the occurrence of apparent competition and,  
487 importantly, the buildup of high populations of this predator, which is usually  
488 accompanied by a reduction and even the disappearance of the most efficient *T. urticae*  
489 predators from clementine orchards (Aguilar-Fenollosa et al. 2011b).

490

#### 491 *Intraguild predation*

492 Up until now, we have discussed the effects of the presence of *A. obscurus* in the cover  
493 on *T. urticae* regulation in clementine orchards at a predator species-specific level.  
494 However, we have not considered how this presence could affect interactions within the  
495 mite predatory guild. At the third trophic level, competition and intraguild predation  
496 may alter the species composition and therefore affect herbivore suppression (Polis et  
497 al. 1989, Polis and Holt 1992, Rosenheim 1998). Additional prey may change the  
498 outcome of competition and intraguild predation by promoting one species over the  
499 others (Sabelis and van Rijn 2006). Superior intraguild predators in Spanish clementine  
500 orchards are mainly *E. stipulatus* (Abad-Moyano et al. 2010a, b) and *N. barkeri*  
501 (Momen 2010). The former occurs in the canopy and the cover, whereas the latter is  
502 mostly found in the cover (Aguilar-Fenollosa et al. 2011b). In our study, both species  
503 showed a marked preference for *A. obscurus*. They have also been described to  
504 competitively displace the *Tetranychus* spp. specialist predator *P. persimilis* (Kabicek

1995), even in clementines (Abad-Moyano et al. 2010a, b). Interestingly, *N. californicus*, which could predate effectively on *A. obscurus* and probably increase its populations feeding on this thrips species, can also outcompete *P. persimilis* (Abad-Moyano et al. 2010a, b). Therefore, when using a *F. arundinacea* cover the disappearance of *P. persimilis* from the system would be anticipated. However, Guzmán et al. (2016) pointed at the presence of a shared resource as a key factor to reduce, or even prevent, intra-guild predation in the phytoseiid, which may not be as common as previously thought within this family. Consequently, the presence of large amounts of *A. obscurus* in the *F. arundinacea* cover during the whole season could diminish intraguild predation in the system and result in better biological control of the target pest (*T. urticae*). Indeed, field results showing that *P. persimilis* is consistently present in clementine orchards grown in association with *F. arundinacea* (Aguilar-Fenollosa et al. 2011b) may be partly due to the presence of this alternative food source for *E. stipulatus*, *N. californicus* and *N. barkeri* in the cover. These results note the importance of the type of the alternative food source for the success of the biological control of a shared pest prey. Contrary to *A. obscurus*, high quality pollen allows the explosion of *E. stipulatus* populations (Pina et al. 2012). As this type of pollen is available during the whole year when clementine trees are grown in association with a resident (not sown) cover, pollen availability both in the cover and in the canopy allows the populations of *E. stipulatus* to outcompete the specialist *P. persimilis*. However, the low quality of pollen produced by *F. arundinacea* only once in spring does not allow for such an explosion of *E. stipulatus*. This fact, together with the provision of *A. obscurus* during the whole season in the cover only, is probably key for the success of the implementation of a *F. arundinacea* cover in clementine orchards as a means to control *T. urticae*. Now we can answer our initial question and respond that *A. obscurus* is actually a [key alternative host](#), which allows better regulation of the citrus key pest *T. urticae*.

532

### 533 **Author contribution statement**

534

535 JJ, MAGM and TP were involved in the design and discussion of the assays. MAGM  
536 and TP performed all the experiments. MAGM, MVIG and TP statistically analyzed the  
537 results, which were discussed by all authors. All authors contributed to the writing of  
538 the manuscript.



539

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541

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552

553 **Compliance with ethical standards**

554

555 **Conflict of interest** The authors declare that they have no conflict of interest.

556 **Ethical approval** All applicable international, national, and/or institutional guidelines  
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558

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758

759 **Fig. 1** Functional response of *Neoseiulus californicus*, *N. barkeri* and *Euseius stipulatus*  
760 to different densities of *Anaphothrips obscurus* first instar nymphs during 24 h.

761 Symbols represent the observed mean  $\pm$  SE. The lines represent the functional response  
762 curves predicted from the model (random predation equation with prey depletion)

763

764 **Fig. 2** Oviposition by *Neoseiulus californicus*, *N. barkeri* and *Euseius stipulatus* when  
765 offered different densities of *Anaphothrips obscurus* N1 during 24 h. Symbols represent

766 the observed number of eggs (mean  $\pm$  SE). The dotted line represents the regression line  
767 predicted for *N. barkeri* ( $y = 0.532 + 0.035x$ ), and the continuous line represents the

768 regression line for *N. californicus* ( $y = 0.143 + 0.015x$ )

769

770 **Fig. 3** Percentages of each phytoseiid species that have chosen *Tetranychus urticae* or  
771 *Anaphothrips obscurus* as prey when offered simultaneously. Significant differences are

772 based on a one-sample proportion test with continuity correction. One asterisk (\*)

773 represents  $P < 0.05$ ; two asterisks (\*\*) represent  $P < 0.01$  (predator species are ordered

774 in decreasing order of diet specialization)

775