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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20 Running title: Role of *Anaphothrips obscurus* in the biological control of *Tetranychus*

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Abstract

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

Keywords

Festuca arundinacea · Phytoseiidae · Functional responses · Prey preference · Apparent competition

Key message

- The implementation of a *Festuca arundinacea* cover in clementine orchards, 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)

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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[®]). *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 ($17 \times 12.5 \times 7.5$ 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, $70 \pm 5\%$ 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 ($6 \times 3 \times 1.5$ 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® 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 ± 1 ºC, $70 \pm 10\%$ 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 $$N_e = N_0 \frac{N_d - N_c}{N_0 - N_c} \qquad (1)$$

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

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

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 χ^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 ± 0.339 days^{-1}) than those of *N. californicus* and *E.*

333   *stipulatus* (0.542 ± 0.151 and 0.711 ± 0.175 days^{-1}, 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 ± 0.003 days) than

337   *N. californicus* (0.169 ± 0.019 days), and the latter spent less time handling than *E.*

338   *stipulatus* (0.264 ± 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 ± 0.04) and deutonymphs (0.47 ± 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 χ^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* (Rodriguez-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

15

505   1995), even in clementines (Abad-Moyano et al. 2010a, b). Interestingly, *N.*

506   *californicus,* which could predate effectively on *A. obscurus* and probably increase its

507   populations feeding on this thrips species, can also outcompete *P. persimilis* (Abad-

508   Moyano et al. 2010a, b). Therefore, when using a *F. arundinacea* cover the

509   disappearance of *P. persimilis* from the system would be anticipated. However, Guzmán

510   et al. (2016) pointed at the presence of a shared resource as a key factor to reduce, or

511   even prevent, intra-guild predation in the phytoseiids, which may not be as common as

512   previously thought within this family. Consequently, the presence of large amounts of

513   *A. obscurus* in the *F. arundinacea* cover during the whole season could diminish

514   intraguild predation in the system and result in better biological control of the target

515   pest (*T. urticae*). Indeed, field results showing that *P. persimilis* is consistently present

516   in clementine orchards grown in association with *F. arundinacea* (Aguilar-Fenollosa et

517   al. 2011b) may be partly due to the presence of this alternative food source for *E.*

518   *stipulatus*, *N. californicus* and *N. barkeri* in the cover. These results note the importance

519   of the type of the alternative food source for the success of the biological control of a

520   shared pest prey. Contrary to *A. obscurus*, high quality pollen allows the explosion of *E.*

521   *stipulatus* populations (Pina et al. 2012). As this type of pollen is available during the

522   whole year when clementine trees are grown in association with a resident (not sown)

523   cover, pollen availability both in the cover and in the canopy allows the populations of

524   *E. stipulatus* to outcompete the specialist *P. persimilis*. However, the low quality of

525   pollen produced by *F. arundinacea* only once in spring does not allow for such an

526   explosion of *E. stipulatus*. This fact, together with the provision of *A. obscurus* during

527   the whole season in the cover only, is probably key for the success of the

528   implementation of a *F. arundinacea* cover in clementine orchards as a means to control

529   *T. urticae*. Now we can answer our initial question and respond that *A. obscurus* is

530   actually a key alternative host, which allows better regulation of the citrus key pest *T.*

531   *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.

552

Compliance with ethical standards

554

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

Ethical approval All applicable international, national, and/or institutional guidelines
for the care and use of animals were followed.

558

References

560

Abad-Moyano R, Pina T, Ferragut F, Urbaneja A (2009) Comparative life-history traits
        of three phytoseiid mites associated with *Tetranychus urticae* (Acari:
        Tetranychidae) colonies in clementine orchards in Eastern Spain: implications for
        biological control. Exp Appl Acarol 47:121–132

Abad-Moyano R, Urbaneja A, Hoffmann D, Schausberger P (2010a) Effects of *Euseius
        stipulatus* on establishment and efficacy in spider mite suppression of *Neoseiulus
        californicus* and *Phytoseiulus persimilis* in clementine. Exp Appl Acarol 50:329–
        341

Abad-Moyano R, Urbaneja A, Schausberger P (2010b) Intraguild interactions between
        Euseius stipulatus and the candidate biocontrol agents of *Tetranychus urticae* in
        Spanish clementine orchards: *Phytoseiulus persimilis* and *Neoseiulus californicus*.
        Exp Appl Acarol 50:23–34

573 Aguilar-Fenollosa E, Ibáñez-Gual V, Pascual-Ruiz S, Hurtado MA, Jacas JA (2011a)
574   Effect of ground-cover management on spider mites and their phytoseiid natural
575   enemies in clementine mandarin orchards (I): bottom-up regulation mechanisms.
576   Biol Control 59:158–170

577 Aguilar-Fenollosa E, Ibáñez-Gual V, Pascual-Ruiz S, Hurtado MA, Jacas JA (2011b)
578   Effect of ground-cover management on spider mites and their phytoseiid natural
579   enemies in clementine mandarin orchards (II): top-down regulation mechanisms.
580   Biol Control 59:171–179

581 Aguilar-Fenollosa E, Pina T, Gómez-Martínez MA, Hurtado MA, Jacas JA (2012) Does
582   host adaptation of *Tetranychus urticae* populations in clementine orchards with a
583   *Festuca arundinacea* cover contribute to a better natural regulation of this pest
584   mite? Entomol Exp Appl 144:181–190

585 Aguilar-Fenollosa E, Jacas JA (2013) Effect of ground cover management on
586   Thysanoptera (thrips) in clementine mandarin orchards. J Pest Sci 86:469–481

587 Aguilar-Fenollosa E, Rey-Caballero J, Blasco JM, Segarra-Moragues JG, Hurtado MA,
588   Jaques JA (2016) Patterns of ambulatory dispersal in *Tetranychus urticae* can be
589   associated with host plant specialization. Exp Appl Acarol 68:1–20

590 Aucejo S, Foó M, Gimeno E, Gómez-Cadenas A, Monfort R, Obiol F, Prades E, Ramis
591   M, Ripollés JL, Tirado V, Zaragozà L, Jacas JA, Martínez-Ferrer MT (2003)
592   Management of *Tetranychus urticae* in citrus in Spain: acarofauna associated to
593   weeds. IOBC/WPRS Bull 26: 213–220

594 Boller EF, Häni F, Poehling HM (2004) Ecological Infraestructures: Ideabook on
595   functional biodiversity at the farm level temperate zones of Europe. IOBC wprs.
596   LBL, Lindau

597 Bonde J (1989) Biological studies including population growth parameters of the
598   predatory mite *Amblyseius barkeri* (Acarina: Phytoseiidae) at 25 ºC in the
599   laboratory. Entomophaga 34:275–287

600 Brohmer P, Ehrmann P, Ulmer G (1966) Die Tierwelt Mitteleuropas. Insekten 1: Teil.
601   Thysanoptera. Lief. 2 (Heft IX). Quelle & Meyer, Leipzig

602 Chailleux A, Mohl EK, Teixeira-Alves M, Messelinkg GJ, Desneux N (2014) Natural
603   enemy-mediated indirect interactions among prey species: potential for enhancing
604   biocontrol services in agroecosystems. Pest Manag Sci 70:1769–1779

605 El-Kholy MY, El-Sayed MAK El-Saiedy (2009) Biological control of *Thrips tabaci*

606 (Lind.) and *Aphis gossypii* (Glover) using different predatory phytoseiid mites and the

607 biocide vertimec on eggplant at Behaira Governorate. Egypt Acad J Biol Sci 2:13–22

608 EU, 2009. Directive 2009/128/EC of the European Parliament of 21 October 2009

609 establishing a framework for Community action to achieve sustainable use of

610 pesticides. OJEU L 309, 71e86

611 Eubanks MD, Denno RF (2000) Health food versus fast food: the effects of prey quality

612 and mobility on prey selection by a generalist predator and indirect interactions

613 among prey species. Ecol Entomol 25:140–146

614 Fan YQ, Petitt FL (1994) Functional response of *Neoseiulus barkeri* Hughes on two-

615 spotted spider mite (Acari: Tetranychidae). Exp Appl Acarol 18:613–621

616 Fantinou AA, Baxevani A, Drizou F, Labropoulos P, Perdikis D, Papadoulis G (2012)

617 Consumption rate, functional response and preference of the predaceous mite

618 *Iphiseius degenerans* to *Tetranychus urticae* and *Eutetranychus orientalis*. Exp

619 Appl Acarol 58:133–44

620 Ferragut F, García-Marí F, Costa-Comeiles J, Laborda R (1987) Influence of food and

621 temperature on development and oviposition of *Euseius stipulatus* (Athias-Henriot)

622 and *Typhlodromus phialatus* Athias-Henriot (Acari: Phytoseiidae). Exp Appl Acarol

623 3:317–329

624 Gómez-Marco F, Tena A, Jaques JA (2016a). Early arrival of predators controls *Aphis

625 spiraecola* colonies in citrus clementines. J Pest Sci 89:69–79

626 Gómez-Marco F, Urbaneja A, Tena A (2016b) A sown grass cover enriched with wild

627 forb plants improves the biological control of aphids in citrus. Basic Appl Ecol

628 17:210–219

629 Gómez-Martínez MA, Aguilar-Fenollosa E, Jaques JA, Pina T (2017) Eco-biology of

630 *Anaphothrips obscurus*, a new dweller of citrus orchards brought in by more

631 sustainable pest management practices. Agric. For. Entomol. doi:

632 10.1111/afe.12233

633 Gutierrez J (1985) Mounting techniques. In: Helle W, Sabelis M (eds) Spider mites.

634 Their biology, natural enemies and control. World Crop Pests Vol 1A. Elsevier

635 Science Publishers BV, Amsterdam, pp 351–353

636 Guzmán C, Sahún RM, Monserrat M (2016) Intraguild predation between phytoseiid

637 mite species might not be so common. Exp Appl Acarol 68:441–453

638 Hanna R, Wilson LT, Zalom FG, Flaherty DL (1997) Effects of predation and
639   competition on the population dynamics of *Tetranychus pacificus* on grapevines. J
640   Appl Ecol 34:878–888

641 Hansen LS (1988) Control of *Thrips tabaci* (Thysanoptera) on glasshouse cucumber
642   using large introductions of predatory mites *Amblyseius barkeri* (Phytoseiidae).
643   Entomophaga 33:33–42

644 Harmon J, Andow DA (2004) Indirect effects between shared prey: Predictions for.
645   BioControl 49:605–626

646 Hassell MP (1978) Functional responses. In: The dynamics of arthropod predator-prey
647   Systems. University Press, New Jersey, pp 28–49

648 Holling CS (1959) The components of predation as revealed by a study of small
649   mammal predation of European pine sawfly. Can Entomol 91:293–329

650 Holt RD (1977) Predation, apparent competition, and structure of prey communities.
651   Theor Popul Biol 12:197–229

652 Holt RA, Lawton JH (1994) The ecological consequences of shared natural enemies.
653   Ann Rev Ecol Syst 25:495-520

654 Jalali MA, Tirry L, De Clercq P (2010) Effect of temperature on the functional response
655   of *Adalia bipunctata* to *Myzus persicae*. BioControl 55:261–269

656 Jeschke JM, Kopp M, Tollrian R (2002) Predator functional responses: discriminating
657   between handling and digesting prey. Ecol Monogr 72:95–112

658 Jonsson M, Wratten SD, Landis DA, Gurr GM (2008) Recent advances in conservation
659   biological control of arthropods by arthropods. Biol Control 45:172–175

660 Juliano SA (2001) Nonlinear curve fitting. Predation and functional response curves. In:
661   Scheiner SM, Gurevitch J (eds) Desing and Analysis of Ecological Experiments.
662   Oxford University Press, New York, pp 178–196

663 Kabicek J (1995) Development and predation by *Amblyseius barkeri* (Acarina:
664   Phytoseiidae) on the mite *Phytoseiulus persimilis* (Acarina: Phytoseiidae). Ochrana
665   Rostlin-UVTIZ 31:57–62

666 Karban R, Hougen-Eitzmann D, English-Loeb G (1994) Predator-mediated apparent
667   competition between two herbivores that feed on grapevines. Oecologia 97:508–
668   4511

669 Karg W, Mack S, Baier A (1987) Advantages of oligophagous predatory mites for
670   biological control. Bull SROP/ WPRS 10:66–73

671   Landis DA, Wratten SD, Gurr GM (2000) Habitat management to conserve natural
672   enemies of arthropod pests in agriculture. Ann Rev Entomol 45:175–201

673   LiangW, Huang M (1994) Influence of citrus orchards ground cover plants on arthropod
674   communities in China: A review. Agr Ecosyst Environ 50:29–37

675   Liu CZ, Yan L, Li HR, Wang G (2006) Effects of predator-mediated apparent
676   competition on the population dynamics of *Tetranychus urticae* on apples
677   BioControl 51:453–463

678   Madadi H, Enkegaard A, Brodsgaard HF, Kharrazi-Pakdel A, Mohaghegh J, Ashouri A
679   (2007) Host plant effects on the functional response of *Neoseiulus cucumeris* to
680   onion thrips larvae. J Appl Entomol 131:728–733

681   McMurtry JA, Croft BA (1997) Life-styles of phytoseiid mites and their roles in
682   biological control. Ann Rev Entomol 42:291–321

683   McMurtry JA, De Moraes GJ, Sourassou NF (2013) Revision of the lifestyles of
684   phytoseiid mites (Acari: Phytoseiidae) and implications for biological control
685   strategies. Syst Appl Acarol 18:297–320

686   Messelink GJ, Van Maanen R, Van Steenpaal SEF, Janseen A (2008) Biological control
687   of thrips and whiteflies by a shared predator: Two pests are better than one. Biol
688   Control 44:372–379

689   Momen FM (2010) Intra- and interspecific predation by *Neoseiulus barkeri* and
690   *Typhlodromus negevi* (Acari: Phytoseiidae) on different life stages: Predation rates
691   and effects on reproduction and juvenile development. Acarina 18:81–88

692   Monzó C, Mollá O, Vanaclocha P, Montón H, Melic A, Castañera P, Urbaneja A (2011)
693   Citrus-orchard ground harbours a diverse, well-established and abundant ground-
694   dwelling spider fauna. Spa J Agric Res 9:606–616

695   Morris RJ, Lewis OT, Godfray HCJ (2005) Apparent competition and insect community
696   structure: towards a spatial perspective. Ann Zool Fenn :449–462

697   Muller CA, Godfray HCJ (1997) Apparent competition between two aphid species. J
698   Anim Ecol 57–64

699   Murdoch WW (1969) Switching in general predators: experiments on predator
700   specificity and stability of prey populations. Ecol Monogr 39:335–354

701   Murdoch WW, Oaten A (1975) Predation and population stability. Adv Ecol Res 9:1–
702   131

703 Overmeer WPJ (1985) Rearing and handling. Spider mites. Their biology, natural
704   enemies and control. Vol. 1B. In Helle W, Sabelis MW (eds) World Crop Pests.
705   Elsevier Science Publishers BV, Amsterdam, pp 161–170

706 Pérez-Sayas C, Pina T, Gómez-Martinez MA, Camañes G, Ibáñez-Gual MV, Jaques JA,
707   Hurtado MA (2015) Disentangling field mite predator-prey relationships by
708   multiplex PCR. Mol Ecol Resour 15:1330–1345

709 Pina T, Argolo PS, Urbaneja A, Jacas JA (2012) Effect of pollen quality on the efficacy
710   of two different life-style predatory mites against *Tetranychus urticae* in citrus.
711   Biol Control 61:176–183

712 Polis GA, Holt RD (1992) Intraguild predation: The dynamics of complex trophic
713   interactions. Trends Ecol Evol 7:151–154

714 Polis GA, Myers CA, Holt RD (1989) The ecology and evolution of intraguild
715   predation: potential competitors that eat each other. Ann Rev Ecol Syst 20:297–
716   330

717 R Core Team (2016). R: A language and environment for statistical computing. R
718   Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-
719   project.org/

720 Ramakers PMM, Van Lieburg MJ (1982) Start of commercial production and
721   introduction of *Amblyseius mckenziei* Sch. & Pr. (Acarina: Phytoseiidae) for the
722   control of *Thrips tabaci* Lind. (Thysanoptera: Thripidae) in glasshouses. Meded Fac
723   Landbouww Rijksuniv Gent 47:541–545

724 Rodríguez-Reina JM, García-Marí F, Ferragut F (1992) Actividad depredadora de
725   varios ácaros fitoseidos sobre distintos estados de desarrollo del trips de las flores
726   *Frankliniella occidentalis.* Bol San Veg Plagas 18:253-263

727 Rogers D (1972) Random search and insect population models. J Anim Ecol 41:369–
728   383

729 Rosenheim JA (1998) Higher-order predators and the regulation of insect herbivore
730   populations. Ann Rev Entomol 43:421–447

731 Sabelis MW, van Rijn PCJ (2006) When does alternative food promote biological pest
732   control? IOBC/WPRS Bull 29:195–200

733 Schausberger P (1997) Inter- and intraspecific predation on immatures by adult females
734   in *Euseius finlandicus*, *Typhlodromus pyri* and *Kampimodromus aberrans* (Acari,
735   Phytoseiidae). Exp Appl Acarol 21:131–150

736   Schausberger P, Hoffman D (2008) Maternal manipulation of hatching asynchrony

737   limits sibling cannibalism in the predatory mite *Phytoseiulus persimilis*. J Anim

738   Ecol 77:1109–1114

739   Solomon ME (1949) The natural control of animal populations. J Anim Ecol 18:1–35

740   Stannard LJ (1968) The thrips or Thysanoptera of Illinois. Bull Ill Nat Hist Surv

741   29:215–552

742   STATGRAPHICS Centurion XVI Version 16.1.18 for Windows. StatPoint

743   Technologies Inc., USA

744   Statgraphics Centurion, XVI. User Manual, 2009. StatPoint Technologies

745   van Baal E, Houten YV, Hooger Brugge H, Bolckmans K (2007) Side effects on thrips

746   of the spider mite predator *Neoseiulus californicus*. Proc Neth Entomol Soc Meet

747   18:37–42

748   Walzer A, Paulus HF, Schausberger P (2004) Ontogenetic shifts in intraguild predation

749   on thrips by phytoseiid mites: the relevance of body size and diet specialization.

750   Bull Entomol Res 94:577–584

751   Xia JY, Rabbinge R, Van Der Werf W (2003) Multistage Functional Responses in a

752   Ladybeetle-aphid system: scaling up from the laboratory to the field. Environ

753   Entomol 32:151-162

754   Yao H, Zheng W, Tariq K, Zhang H (2014) Functional and numerical responses of three

755   species of predatory phytoseiid mites (Acari: Phytoseiidae) to *Thrips favidulus*

756   (Thysanoptera:Thripidae). Neotrop Entomol 43:437–444

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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 ± 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 ± 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)

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