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When do predatory mites (Phytoseiidae) attack? Understanding their diel and seasonal predation patterns

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Abstract

Predatory mites of the Phytoseiidae family are considered one of the most important groups of natural enemies used in biological control. The behavioral patterns of arthropods can differ greatly daily and seasonally; however, there is a lack of literature related to Phytoseiidae diel and seasonal predation patterns. The predatory activity of three phytoseiid species (two Tetranychidae-specialists, *Phytoseiulus persimilis* and *Neoseiulus californicus*, and one omnivore, *Euseius stipulatus*) that occur naturally in Spanish citrus orchards was observed under laboratory conditions in winter and summer. The temperature and photoperiod of the climatic chamber where the mites were reared did not change during the experiment. Our study demonstrates that phytoseiids can exhibit diel and seasonal predatory patterns when feeding on *Tetranychus urticae* (Acari: Tetranychidae). *Neoseiulus californicus* was revealed to be a nocturnal predator in summer but diurnal in winter. In contrast, *P. persimilis* activity was maximal during the daytime, and *E. stipulatus* showed no clear daily predation patterns. The predatory patterns described in this study should be taken into account when designing laboratory studies and also in field samplings, especially when applying molecular techniques to unveil trophic relationships.

Key words molecular techniques; photoperiod; phytoseiids; predation behavior; *Tetranychus urticae*

Introduction

Arthropod species are subject to environmental rhythms, and their behavioral patterns can differ greatly daily and seasonally (Weber *et al.*, 2008; Saunders, 2010). Circadian rhythms of activity can allow the coexistence of species sharing the same habitat (Schoener, 1974), and in a biological control scenario, they may be key in shaping communities of pests and their natural enemies. Therefore, the identification of these patterns can allow the full exploitation of ecosystem services such as biological control. Indeed, it is known that predators in a given system exhibit different predatory activity based on spatial (microhabitat) and temporal (season, time of day) traits (Straub *et al.*, 2008; Snyder, 2009). Searching rhythms in many arthropods seem to have evolved in response to resource competition or predation (Bell, 1990). Conclusive evidence of predation can be obtained only by direct observation of prey consumption by a predator, identification of feces or prey remains with orthodox methods such as dissection and subsequent visual gut-content identification, or the use of molecular tools specially designed to identify both prey and predator (Furlong, 2015; Pérez-Sayas *et al.*, 2015; González-Chang *et al.*, 2016). In all these cases, laboratory experiments or samplings do not separately evaluate day and night time activities. Consequently, the contribution of nocturnal predation may be underrepresented due to the cryptic feeding activity patterns of nocturnal predators, which may actually play a greater role than has been recognized (Vickerman & Sunderland, 1975; Pfannenstiel, 2008; Durham *et*

al., 2009). In other words, experiments not distinctly addressing day and night periods may not show a detailed picture of predator-prey relationships.

An increasing number of studies promotes the adjustment of the field sampling time with the diel activity pattern of predators to obtain accurate estimations of their actual density and diversity. For instance, among Chelicerata, different types of diel activity patterns have been described (Jackson & Poulsen, 1990; Kazak *et al.*, 2004; Pekár *et al.*, 2005). Salticidae are almost exclusively diurnal, while other predatory spider families such as Araneidae, Agelenidae, Clubionidae, Corinnidae, Lycosidae and Miturgidae are nocturnal or exhibit periods of nocturnal activity (Costello & Daane, 2005; Woltz & Landis, 2014). In cursorial spiders, which are important predators of moth eggs in cotton, soybean, and corn, predation occurs mainly after dark (Pfannenstiel & Yeargan, 2002; Pfannenstiel, 2008; Durham *et al.*, 2009). Among the Acari, several studies have focused on the diel activity pattern and distribution on the plant of predatory mites (Phytoseiidae) (for example, Fleschner *et al.*, 1955; Garcia-Mari *et al.*, 1985; Magalhães *et al.*, 2002; Onzo *et al.*, 2003, 2010; Villanueva & Childers, 2005). However, there is a lack of literature related to daily and seasonal predation patterns.

Predatory mites of the Phytoseiidae family are considered one of the most important groups of natural enemies used in biological control worldwide (Helle & Sabelis, 1985). They can regulate densities of different pest species, such as phytophagous mites, thrips, and whiteflies (Helle & Sabelis, 1985; Gerson *et al.*, 2003; Zhang, 2003). In Spanish citrus orchards, there is a complex acarofauna population comprising two main herbivores, the Tetranychidae *Tetranychus urticae* Koch and *Panonychus citri* (McGregor), and several

predatory mites belonging to the Phytoseiidae family (Abad-Moyano *et al.*, 2009a; Aguilar-Fenollosa *et al.*, 2011a, 2011b; Jaques *et al.*, 2015). Recently, our research group developed a multiplex PCR method for disentangling trophic relationships among these mites (Pérez-Sayas *et al.*, 2015). With this molecular tool, we were able to identify the two tetranychids and the six most abundant phytoseiid species in Spanish clementine orchards (Aguilar-Fenollosa *et al.*, 2011a, 2011b; Jaques *et al.*, 2015) and to detect both tetranychid mite species in the predators' gut content. However, prey detection changed over time and depended on the predator-prey combination (Pérez-Sayas *et al.*, 2015). Furthermore, corrected predation values, used to quantify the relative importance of each predator (Greenstone *et al.*, 2014), highlighted the higher importance of the omnivorous *Euseius stipulatus* (Athias-Henriot) when compared with the *T. urticae* specialist *Phytoseiulus persimilis* Athias-Henriot (Pérez-Sayas *et al.*, 2015). However, this importance was accredited due to its higher abundance in the field (7 times higher than that of *P. persimilis*) given that *E. stipulatus* was far less effective than expected as it preferred to feed on other food sources whereas *P. persimilis* specimens mostly preyed on tetranychids. Accordingly, *P. persimilis* was the most effective biological control agent in the system (Pérez-Sayas *et al.*, 2015). However, the tetranychid specialist *Neoseiulus californicus* (McGregor), which is usually present in this system at densities above those of *P. persimilis* (Aguilar-Fenollosa *et al.*, 2011b), was barely found during these field samplings, which took place mainly during the day in summer (Pérez-Sayas *et al.*, 2015). Moreover, previous semi-field trials where *N. californicus* was released showed that this species was prone to seek refuge in citrus branches and trunks (Pina *et al.*, 2013). The differences in prey detection over time between phytoseiid species, which is limited by the duration of the digestive process, the irregular phytoseiid

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captures during diurnal field samplings, and the existence of nocturnal predatory species among the Chelicerata led us to hypothesize that phytoseiids could exhibit different diel predation patterns that could also change with season. If these patterns were confirmed, a tuning of the samplings would be required to reveal the actual contribution of the different phytoseiid species. Therefore, the objective of this study was to determine whether phytoseiids exhibit different predation patterns based on the day and season.

Materials and methods

Mites

Tetranychus urticae individuals used as prey were originally collected in 2001 from clementine orchards located in La Plana region (Castelló, Spain). Colonies were maintained on bean plants (*Phaseolus vulgaris* L. [Fabaceae] cv. Chico) in a climatic chamber at 22 ± 3 °C, 60%–80% of relative humidity (RH) and a photoperiod of 16 : 8 light : dark (L : D) h. Bean plants were grown in a pesticide-free greenhouse.

Euseius stipulatus individuals used in the experiment were collected from clementine orchards in Montcada (Valencia, Spain). *Neoseiulus californicus* and *P. persimilis* individuals came from a laboratory colony initiated from specimens purchased from Koppert Biological Systems S.L. (Spical and Spidex, respectively). *Neoseiulus californicus* and *P. persimilis* were reared as described by Overmeer (1985a). *Tetranychus urticae*-infested bean leaves were added as food. The *E. stipulatus* rearing unit was slightly modified due to their different feeding requirements. This phytoseiid was reared on upside-down bean leaves, and

Carpobrotus edulis (L.) (Aizoaceae) pollen was added twice a week as a food source, as well as all stages of *T. urticae*. Phytoseiid colonies were kept in climatic chambers at 25 ± 1 °C and $80\% \pm 5\%$ RH. The photoperiod was set as described below.

Experimental conditions

Experiments were replicated in winter and summer to coincide with minimum and maximum day length for each season. At our location (Montcada: 39°35'18.62"N, 0°23'41.68"W), this coincides with a scotophase of 9 h and a photophase of 15 h in summer and vice versa in winter. The photoperiod in the climatic chamber was set at 15 L: 9 D h to mimic summer light conditions. Lights were switched on and off to coincide with summer solstice sunrise and sunset times, respectively. These conditions were maintained throughout the experiment and throughout the year to unveil any effect of the season on predation patterns. Temperature and RH conditions were the same as for the culture stocks (25 ± 1 °C and $80\% \pm 5\%$ RH).

Behavioral observations at different time points were performed. These were set at midnight (00:00 h solar time, meaning sundial time), midday (12:00 h), winter and summer sunrise and sunset times, and one hour before and after each of the aforementioned time points. The behavioral observations were carried out in the laboratory and after dark, red LED lamps were used to minimize disturbance.

Experimental design

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At least 20 young gravid females were tested per phytoseiid species, time point and season. Gravid females were isolated in modified Huffaker cells (Abad-Moyano *et al.*, 2009b) and kept with no food but with a water supply for 48 h in the rearing climatic chamber conditions. After the starvation period, each adult female was transferred to a new Huffaker cell provided with one *T. urticae* adult female. This prey stage was selected to avoid any prey-stage species-specific preference, as *P. persimilis* and *Euseius* spp. preferentially prey on eggs and larvae, respectively, and *N. californicus* exhibits no prey-stage preference (Blackwood *et al.*, 2001). Each individual was tested only once.

The predatory activity of the phytoseiid was observed under a binocular microscope for up to one hour, and the observation was discontinued as soon as the predator attacked the prey. We considered an attack to be the event where the predator contacted the prey, followed by feeding (independent of the fate of the prey). The time that elapsed until the attack was measured and taken as indicative of both the antipredator behavior of the prey and the foraging success of the predator.

Data analysis

The number of mites which attacked the prey was analyzed by means of logistic regression (attacking versus not-attacking individuals), with as factors the season and time of day during which the attack was observed and their interaction (software STATGRAPHICS Centurion XVI Version 16.1.18; StatPoint Technologies, Inc., 1982-2012). When the interaction was significant, we further analyzed results using circular statistics (Fisher, 1993; Zar, 2010) with

Oriana Software version 4 (Kovach, 2011). Circular distributions were subjected to the Rayleigh uniformity test to check whether predation activity was uniformly distributed throughout the day. In case it was not, the mean vector and its 95% confidence interval (CI) were calculated to allow comparison of temporal heterogeneity between species. For each species, the time elapsed until attack was subjected to two-way ANOVA considering season and time of day as factors.

Results

A total of 1788 phytoseiid mites were observed throughout the experiment: 595 *P. persimilis* (282 in winter and 313 in summer), 551 *N. californicus* (251 in winter and 300 in summer) and 642 *E. stipulatus* (314 in winter and 328 in summer). Predation activity of *P. persimilis* and *N. californicus* varied significantly with the interaction between time of day and season (logistic regression, Table 1). Both species showed clear distinctive diel predation patterns in summer but not in winter (circular statistics, Table 2, Fig. 1), as the mean time vector 95% CI during this season overlapped (Table 2 and Fig. 1). In the case of *E. stipulatus*, the interaction between time of day and season was not significant, time of the day was bordering significance ($P = 0.052$) and season was ($P = 0.002$) (Table 1). A higher number of mites engaged in attacking activities was observed during the summer.

No significant differences were observed for the time elapsed until attack between time of day, season and the interaction of these factors for *E. stipulatus* and *P. persimilis* ($P > 0.05$ in all cases). However, for *N. californicus*, the factor time of the day was significant ($P =$

0.034), and a decrease in the time elapsed until attack was observed at around midnight (10.00 ± 3.79 min versus 39.42 ± 2.02 min for midnight and the rest of the time points, respectively). The average time elapsed until attack was 35.39 ± 2.01 min for *E. stipulatus* ($n = 88$), 37.71 ± 2.04 min for *N. californicus* ($n = 86$) and 34.21 ± 1.34 min for *P. persimilis* ($n = 200$).

Discussion

Among the factors playing a role in shaping predator-prey interactions, diel (circadian) and seasonal patterns are relevant (Cloudsley-Thompson, 1970; Vickerman & Sunderland, 1975; Penteriani *et al.*, 2013; Ximenez-Embun *et al.*, 2014). However, until recently, studies focusing on the reproductive biology or predatory activity of predatory mites had considered the influence of light intensity (quality) or day length (quantity, photoperiod) under simulated conditions in a particular season (Smith & Newsom, 1970; Maeda *et al.*, 2000; Kazak *et al.*, 2004; Zilahi-Balogh *et al.*, 2007). Only recently has the effect of seasonal climatic variations on the efficacy of predatory mites for thrips control in greenhouses been addressed (Hewitt *et al.*, 2015). Our study shows that the behavioral pattern of predation changed daily and seasonally, although photoperiod and temperature in the rearing chambers remained the same during the whole experimental period, and these changes were species-specific. Thus, the pattern of predation of the *Tetranychus* specialist *P. persimilis* was affected by season, time of day and the interaction of these factors; that of the tetranychid specialist *N. californicus* was also affected by season and its interaction with time of day; and that of the omnivorous *E. stipulatus* by season but not by time of day. These differences should be taken into account

when performing laboratory studies on phytoseiid species and/or when establishing the sampling time to obtain a real snapshot of mite trophic relationships, as in the case of the gut content DNA analysis (Pérez-Sayas *et al.*, 2015). Experiments ignoring these differences may lead to biased, species-specific estimations of predation.

Among the seasonal patterns described in arthropods, including mites, one of the most studied is diapause (Morewood, 1993; Castagnoli *et al.*, 1996; Danks, 2003). This seasonal rest is a genetically determined trait and is manifested as suppressed development or reproduction (Danks, 1987; Kroon & Veenendaal, 1998). Primary cues for diapause induction are photoperiod and temperature (Hairston and Kearns, 1995). These factors were not modified in the rearing chambers during our study. However, the two specialist species showed a reduction in predatory activity in winter despite constant climatic conditions in the rearing chamber (Table 2). *Phytoseiulus persimilis* is thought to be incapable of entering diapause (Overmeer, 1985b; Morewood, 1993), as are most of the strains of *N. californicus* (Castagnoli *et al.*, 1996) and *E. stipulatus* (Ferragut *et al.*, 1988). Their main tetranychid prey, *T. urticae*, is described as exhibiting diapause in temperate regions (Takafuji, 1994; Hoy, 2011) and in some regions of the Mediterranean basin (Koveos *et al.*, 1993). In northeastern Spain, females are active throughout the year (Martínez-Ferrer *et al.*, 2006), but nevertheless we cannot discount a reduction in the activity of this poikilothermic species. The strict feeding habits of *P. persimilis* and the strong preference of *N. californicus* for *T. urticae* (McMurtry & Croft, 1997; McMurtry *et al.*, 2013) could have induced predator-prey adaptations in their feeding behavior and physiology to improve survival when prey is scarce (Ragusa & Tsolakis, 2000). In other words, specialist predators could reduce their predation

and oviposition rates in periods when prey is not abundant or less active (Veerman, 1992; Belozarov, 2009). These facts lead us to consider that phytoseiids could have developed an internal biological clock in sync with the periods of reduced activity of their prey. In fact, photoperiodic responses are considered to be an adaptation to seasonal changes due to their direct implication in survival and reproductive success (de Wilde, 1962; Johnsson, 2008; Belozarov, 2009; Ikeno *et al.*, 2010).

There is either evidence for the involvement of the circadian clock in mediating photoperiodic responses in organisms ranging from fungi to mammals (Goto, 2013). In fact, *T. urticae* shows a mixture of circadian and apparently noncircadian features (Saunders, 2010) that could explain different daily rhythms in predators that have coevolved with this phytophagous mite. We found that predation by specialist predators was concentrated at different times depending on the species and the season, but that most avoided feeding during midday hours in summer. In predator-prey systems, light or dark preferences could influence feeding or reproduction, as these are frequently associated with the spatial distribution of food sources (e.g., adaxial or abaxial side of the leaf) (Villanueva & Childers, 2005; Sudo & Osakabe, 2011). *Tetranychus urticae* and *P. citri* distributions, mostly on lower and upper leaf surfaces, respectively (Tachi & Osakabe, 2012), have been associated with their different sensitivity to UV radiation (Ohtsuka & Osakabe, 2009; Fukaya *et al.*, 2013; Suzuki *et al.*, 2013; Ghazy *et al.*, 2016). Therefore, differences in the time of day at which predation occurred could be related to the position of the preferred prey and/or their different UV sensitivity, such as *P. persimilis* being less UV sensitive than *N. californicus* (Tachi & Osakabe, 2012, 2014; Ghazy *et al.*, 2016). Accordingly, our results show that the latter

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preferentially preyed early in the morning and late in the evening in summer and winter, respectively (Table 2). The lack of effect of time of day in the predation pattern observed for *E. stipulatus* may be related to a higher tolerance to UV radiation than that of the other two phytoseiids studied. However, Garcia-Marí *et al.* (1985) found *E. stipulatus* preferentially on the abaxial side of the citrus leaf during the daytime. This behavior could be related to an avoidance of the lethal effects of UVB irradiation, as suggested for *Amblydromalus* (= *Typhlodromalus*) *manihoti* (Moraes) and *Euseius fustis* (Pritchard and Baker) on cassava plants (Onzo *et al.*, 2010).

The time elapsed until the predator attacks and feeds on the prey can be taken as evidence of both the antipredator behavior of the prey and the foraging success of the predator. On the one hand, antipredator behavior is elicited when the prey perceives cues associated with predators and normally includes the avoidance of areas with predators, seeking refuge and escaping; it implies carrying costs such as a reduction in fitness and delayed development (see revision in Dias *et al.*, 2016). On the other hand, foraging behavior comprises a group of processes by which the organisms obtain energy and nutrients and in the case of predators comprises searching, assessment, pursuit and handling (actual prey consumption) (Kramer, 2001). Despite the fact that both behaviors could change with season and time of day, the time elapsed until attack changed only for *N. californicus*.

To sum up, our study reveals that phytoseiids exhibit diel and seasonal predatory patterns. Importantly, these patterns can affect laboratory and field studies. Consequently, different testing and sampling times should be taken into account to obtain comprehensive information, especially when different species are compared. In our study, *N. californicus*

was revealed to be a nocturnal predator in summer but diurnal in winter. In contrast, *P. persimilis* activity was maximal during the daytime (around midday in winter and at approximately 17:00 h in summer), and *E. stipulatus* showed no clear daily predation patterns. If these differences are neglected when planning and executing experiments, the results could lead to biased estimations of predator performance. This is especially important when designing field-sampling plans through multiple PCR approach for gut DNA detection, as prey DNA degrades rapidly in the predator gut (Pérez-Sayas *et al.*, 2015).

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Disclosure

The authors report no conflicts of interest to be declared.

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Table 1 Statistical parameters of the logistic regression analysis of the attacks performed within an hour, considering season and time of day as factors. Logistic regressions fitted for all species were significant ($P < 0.05$).

	<i>Phytoseiulus persimilis</i>			<i>Neoseiulus californicus</i>			<i>Euseius stipulatus</i> [†]		
	χ^2	df	<i>P</i>	χ^2	df	<i>P</i>	χ^2	df	<i>P</i>
Season	6.574	1	0.010	4.980	1	0.026	0.315	1	0.574
Time of day	46.182	15	< 0.001	13.311	15	0.578	18.501	15	0.237
Interaction	47.594	15	< 0.001	34.985	15	0.003	17.222	15	0.306

†As the “season × time of day” interaction was not significant, the model was further depurated excluding this interaction. New *P*-values are presented in the text.

Table 2 Diel predation pattern of attacks (# attacking individuals) by phytoseiid species and season. Mean time vector in a 24-hour time, with *P*-value, according to Rayleigh’s test (the null hypothesis considers the uniformity of attacks by time of day).

Species	Season	# attacking individuals (total # individuals)	Mean time vector (h:min)	95% confidence interval (Mean time vector)	<i>P</i>
<i>Phytoseiulus persimilis</i>	Winter	58 (282)	11:21	10:25–12:16	< 0.001
	Summer	142 (313)	17:29	15:29–19:30	< 0.001
<i>Neoseiulus californicus</i>	Winter	32 (251)	09:50	08:12–11:28	< 0.001
	Summer	54 (300)	22:46	21:15–00:18	< 0.001
<i>Euseius stipulatus</i>	Winter	31 (314)	N/A [†]	N/A	N/A
	Summer	59 (328)	N/A	N/A	N/A

† N/A: not applicable

Figure caption

Fig. 1 Diel pattern of *Tetranychus urticae* predation by phytoseiid species (*Phytoseiulus persimilis* [A] and *Neoseiulus californicus* [B]) presented in columns according to season (winter and summer). Bars in the circular graphs represent the percentage of attacks by time of day (time point), with 00:00 h (solar time) as midnight. The line running from the center of the diagram to the outer edge and the arc represent the mean time vector and 95% confidence interval, respectively.

