



Predator-associated cues modify prey behaviour, but not short-term predator–prey encounter dynamics in *Tetranychus urticae* (Acari: Tetranychidae)

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ABSTRACT

Predator-associated cues can induce behavioural and life-history changes in prey without direct predator–prey interactions, yet whether these responses reduce subsequent predation risk remains poorly understood. We investigated how predator-associated cues from the phytoseiid mites *Amblyseius swirskii* and *Neoseiulus californicus* influenced spatial organization and reproductive potential in the two-spotted spider mite, *Tetranychus urticae*. Adult female *T. urticae* were exposed to experimentally manipulated levels of predator-associated cues representing increasing perceived predation risk, and spatial organization and oviposition were subsequently measured. Two additional, independent experiments used experimentally imposed aggregated and dispersed prey distributions to test predator–prey encounter frequency and predator patch choice. Predator-associated cues induced trait-specific and predator-dependent responses. Exposure to *A. swirskii* cues caused a transient increase in prey dispersion after 3 h, whereas *N. californicus* cues had no significant effect on spatial organization. Cues from both predator species significantly reduced oviposition, with stronger reductions occurring under higher perceived risk. In the independent encounter experiment, experimentally imposed prey spatial distribution did not significantly affect encounter frequency for either predator species. In the independent patch-choice experiment, *A. swirskii* preferentially selected aggregated prey patches and initiated attacks there, whereas *N. californicus* showed no significant patch preference. These findings indicate that predator-associated cues can modify prey behaviour and reproduction, but the ecological consequences of prey spatial distribution depend on the stage of predator foraging and predator identity.

KEYWORDS

Foraging behaviour, herbivorous mites, phytoseiid mites, predator recognition, risk perception, trait-mediated interactions

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INTRODUCTION

Behaviour is one of the primary mechanisms through which organisms respond to environmental change. Many animals adjust their behaviour before direct interactions with environmental challenges occur by using information obtained from their surroundings. Among the most important sources of such information are predator-associated cues, which enable prey to detect potential threats and modify movement, habitat use, aggregation, foraging, and reproductive decisions before encountering predators.



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Although these responses may reduce predation risk, they often involve trade-offs because defensive behaviours can limit access to food, mates, or suitable habitats (Lima and Dill 1990; Kats and Dill 1998; Dall et al. 2005; Ferrari et al. 2009).

Predators therefore influence prey populations not only through direct consumption but also through non-consumptive effects mediated by prey perception of risk. These effects occur when prey respond to predator-associated information such as chemical residues, faeces, eggs, or damaged conspecifics, resulting in changes in behaviour, physiology, or life-history traits even in the absence of direct predation. Such responses can alter prey fitness and generate trait-mediated effects that extend beyond the directly exposed individuals, potentially influencing population and community processes (Dicke and Grostal 2001; Schmitz et al. 2004; Lima 1998). In arthropod systems, chemical information from natural enemies is particularly important because prey can detect predator-derived cues before direct contact and adjust their behaviour or reproduction accordingly (Dicke and Grostal 2001).

Spider mites (Acari: Tetranychidae) and their phytoseiid predators provide an excellent model for studying information-mediated predator–prey interactions. The two-spotted spider mite, *Tetranychus urticae* Koch, is one of the most economically important herbivorous mites worldwide and has been extensively used in studies of antipredator behaviour. Previous research has shown that *T. urticae* can detect predator-associated cues and modify movement, dispersal, web production, habitat selection, and reproductive output in response to perceived predation risk (Grostal and Dicke 1999; Dicke and Grostal 2001; Škaloudová et al. 2007; Dias et al. 2016). However, most previous studies have focused on prey responses themselves rather than on how predators subsequently respond to prey spatial organization.

Spatial organization is an important component of antipredator behaviour because prey arrangement determines the spatial environment encountered by searching predators. Changes in aggregation or dispersion may alter local prey density and affect predator searching efficiency, encounter rates, or patch-selection decisions. Whether such effects occur may depend on whether predators are forced to search within a patch or are allowed to select among alternative prey patches.

Although predator-associated cues are known to alter spider mite behaviour, the present study did not directly test whether the spatial distribution induced by predator cues subsequently changes predator encounters. The predator-cue experiment and the two prey-distribution experiments were therefore treated as separate experimental components. The latter experiments tested the effects of experimentally imposed prey distributions on predator encounter frequency and patch choice rather than providing a direct causal test of the consequences of cue-induced spatial changes.

In this study, we investigated how predator-associated cues from *A. swirskii* and *N. californicus* affect the spatial organization and reproductive potential of *T. urticae* under different levels of perceived predation risk. In two separate experiments, we then examined how experimentally imposed aggregated and dispersed prey distributions affected predator–prey encounter frequency and predator patch choice. We hypothesized that predator-associated cues would induce behavioural and reproductive responses in *T. urticae* and that these responses would depend on predator identity and cue intensity. For the independent prey-distribution experiments, we predicted that predator responses would differ between aggregated and dispersed prey arrangements and between predator species.

MATERIAL AND METHODS

Host plant

Red bean, *Phaseolus vulgaris* L. var. Alamuti (Fabaceae), was used as the host plant for maintaining *T. urticae* colonies and for behavioural experiments. Seeds were soaked in distilled water for 24 h, germinated in moist cotton, and transplanted individually into plastic pots (12 cm diameter × 12 cm height) containing pure perlite. Plants were irrigated every two days with complete nutrient solution (NPK 20:20:20). Unless otherwise stated, plants and mites were maintained at 25 ± 1 °C, $65 \pm 10\%$ RH, and a 16:8 h light: dark photoperiod. Plants were used at the five-leaf stage, and new plants were produced every two weeks to provide healthy leaf material.

Rearing of *T. urticae*

The initial colony of *T. urticae* was obtained from the Acarology Laboratory, Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Iran. Colonies were established by transferring adult males and females onto healthy bean plants. Spider mite colonies were maintained under the environmental conditions described above. Fresh bean plants were introduced every 2–3 days to maintain colony development, while deteriorated plants were removed and replaced regularly.

Rearing of predatory mites

Colonies of *N. californicus* and *A. swirskii* were originally obtained from Koppert Biological Systems (The Netherlands) and subsequently maintained in the Acarology Laboratory, University of Tehran. Predatory mites were reared on bean leaves infested with *T. urticae* using an island-type rearing system under controlled conditions (25 ± 1 °C, $70 \pm 5\%$ RH, 16:8 h light:dark photoperiod). The rearing system consisted of a water-saturated sponge inside a plastic container partially filled with water, with a plastic sheet on the sponge serving as the rearing platform. Tissue paper extending into the water reservoir maintained moisture and prevented mite escape. Predators were provided continuously with all developmental stages of *T. urticae* and supplemented with maize pollen. Fresh infested leaves were added every 3–4 days and deteriorated leaves were removed regularly. Predator colonies were maintained for five generations before experimentation.

Preparation of experimental arenas

Behavioural experiments were conducted using modified double-Petri-dish arenas. Each arena consisted of two connected Petri dishes (9 cm diameter) supplied continuously with water through a tissue-paper wick. A ventilation opening covered with fine mesh in the lid allowed air exchange while preventing mite escape. Bean leaf discs (3 cm diameter) were excised from healthy leaves and placed abaxial side upward on moist filter paper. The margins of each leaf disc were surrounded with moist tissue paper and cotton to maintain humidity and prevent mite escape throughout the experiments.

Synchronization of *T. urticae* females

Approximately 20 adult female *T. urticae* were transferred onto bean leaf sections and allowed to oviposit for 24 h. Females were then removed, and the resulting cohort was maintained under laboratory conditions until adult emergence. Newly emerged adult females (approximately 11 days after oviposition) were used in all experiments, thereby standardizing the adult age class across treatments.

Predator-associated cue experiment

This experiment examined whether predator-associated cues influence the spatial organization and reproductive potential of *T. urticae* females. Four levels of perceived predation risk were established according to Dittmann and Schausberger (2017): control (no predator-associated cues), low risk (predator-associated traces without predator eggs), intermediate risk (predator-associated traces with two predator eggs), and high risk (predator-associated traces with five predator eggs).

1. Control: leaf discs without predator-associated cues.
2. Low risk: predator-associated traces without predator eggs.
3. Intermediate risk: predator-associated traces with two predator eggs.
4. High risk: predator-associated traces with five predator eggs.

Predator-associated cues were generated by allowing a single well-fed adult female predator to remain on each leaf disc for 10 h in the absence of prey. The 10-h exposure period was used to allow sufficient deposition of naturally occurring predator-associated traces and, where applicable, predator eggs before prey introduction. For the low-risk treatment, predators deposited walking traces and faecal material but no eggs were retained. For the intermediate- and high-risk treatments, two or five predator eggs, respectively, were left on the leaf disc together with naturally deposited traces after predator removal. Predators were then removed and each arena was inspected to confirm complete removal. Five same-aged adult female *T. urticae* were transferred onto each leaf disc. Female positions were recorded

every 30 min for 2.5 h (six observations), followed by one additional observation 24 h later. Spatial organization was evaluated at 3 and 27 h after prey introduction. These time points were selected to capture an early behavioural response and its persistence into the following day; one-hour and 24-hour sampling intervals were not tested. Oviposition was determined by counting eggs laid by the five females during the 24 h following the final spatial observation. Each treatment consisted of 20 independent replicates.

Effect of experimentally imposed prey spatial distribution on predator encounter dynamics

This independent experiment examined whether experimentally imposed prey spatial distribution influences predator–prey encounter frequency. Bean leaf discs (3 cm diameter) were divided into four equal sectors. Four same-aged adult female *T. urticae* were arranged in one of two spatial configurations: dispersed distribution, with one female in each sector; or aggregated distribution, with all four females placed within a single sector approximately 1 mm apart.

- Dispersed distribution: one female was placed in each sector.
- Aggregated distribution: all four females were placed within a single sector approximately 1 mm apart.

To maintain standardized prey positions during observations, females were gently fixed to the leaf surface using minimal quantities of cold wax. This manipulation was necessary to maintain the predefined artificial spatial distributions but may reduce the ecological realism of the encounter assay; this limitation is considered in the Discussion. One starved adult female predator (*A. swirskii* or *N. californicus*) was introduced into the center of each arena after 5 h of starvation. Predator behaviour was observed for 120 min, with observations every 5 min during the first hour and every 20 min during the second hour. The total number of predator–prey encounters was recorded. An encounter was defined as the first physical contact between the predator's anterior legs and a spider mite. Each predator species was tested using 20 independent replicates for each prey distribution.

Effect of experimentally imposed prey spatial distribution on predator patch choice

This experiment examined whether prey spatial distribution influences predator patch selection. A two-choice arena was constructed using two bean leaf discs (3 cm diameter) connected by a narrow parafilm bridge (approximately 1 cm long and 0.5 cm wide). Four synchronized adult female *T. urticae* were used on each leaf disc to maintain identical prey density while allowing direct comparison between aggregated and dispersed prey distributions. One leaf disc contained the four females arranged in an aggregated distribution, whereas the second contained the four females arranged in a dispersed distribution. The position of the two prey distributions (left or right) was randomized among replicates. Spider mite females were gently fixed to the leaf surface using minimal quantities of cold wax to maintain their spatial arrangement throughout the experiment. One starved adult female predator (*Amblyseius swirskii* or *Neoseiulus californicus*) was released at the midpoint of the bridge after 5 h of starvation. Predator behaviour was observed continuously for 60 min. The following variables were recorded:

- first patch entered;
- location of the first successful attack.

Each predator species was tested using 20 independent replicates under controlled laboratory conditions (25 ± 1 °C, $65 \pm 10\%$ RH, 16:8 h light: dark photoperiod). The overall experimental design and the independence of the three experimental components are summarized in Figure 1.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). Normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene tests prior to parametric analyses. The effects of predator-associated cues on mean inter-individual distance and oviposition were analyzed separately for each predator species using one-way analysis of variance (ANOVA). When significant treatment effects were detected, differences among means were

determined using Tukey's honestly significant difference (HSD) test. Differences in predator-prey encounter frequency between aggregated and dispersed prey distributions were analyzed separately for each predator species using independent-samples *t*-tests. When assumptions of normality or homogeneity of variance were not satisfied, Mann-Whitney *U* tests were used. Predator patch-choice data (first patch entered and location of the first successful attack) were analyzed separately for each predator species using two-sided exact binomial tests, assuming an expected probability of 0.5 for selection of either prey distribution. Statistical significance was accepted at $P < 0.05$.

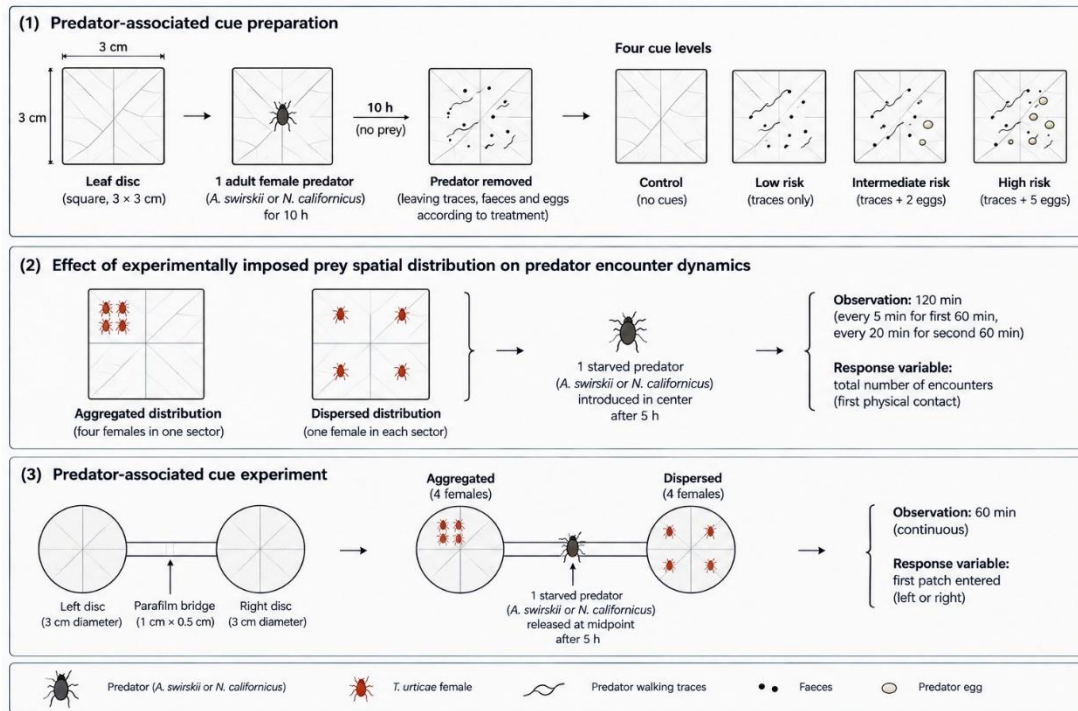


Figure 1. Schematic representation of the experimental design for assessing the effects of predator-associated cues and experimentally imposed prey spatial distribution on *Tetranychus urticae* behaviour and predator responses. **1.** Predator-associated cue preparation using four risk levels (control, low, intermediate, and high risk), followed by assessment of prey spatial organization and oviposition. **2.** Predator-prey encounter dynamics under aggregated and dispersed prey distributions during a 120-min observation period. **3.** Predator patch-choice assay comparing aggregated and dispersed prey patches during 60 min of continuous observation.

RESULTS

Responses of *T. urticae* to predator-associated cues

Spatial organization

Exposure to *A. swirskii*-associated cues significantly affected mean inter-individual distance among adult females after 3 h of exposure (one-way ANOVA: $F = 3.27$, $df = 3$, $P = 0.026$). Females exposed to the low-risk treatment exhibited a greater mean inter-individual distance (2.83 ± 0.18 cm) than control females (2.03 ± 0.17 cm), and this comparison was significant according to the original Tukey's HSD analysis ($P < 0.05$). Although the mean distance was numerically higher under intermediate- and high-risk conditions, these additional pairwise comparisons were not statistically significant in the original analysis. After 27 h of exposure, *A. swirskii*-associated cues no longer significantly affected female spatial organization (one-way ANOVA: $F = 2.09$, $df = 3$, $P = 0.108$). For *N. californicus*, mean inter-individual distance at 3 h was 2.62 ± 0.15 cm in the control, 3.07 ± 0.22 cm under low risk, 2.55 ± 0.22 cm under intermediate risk, and 2.63 ± 0.22 cm under high risk. At 27 h, the corresponding means were 2.27 ± 0.14 , 2.73 ± 0.18 , 2.27 ± 0.22 , and 2.27 ± 0.23 cm, respectively. These differences were not statistically significant (one-way ANOVA, $P > 0.05$).

Table 1. Mean inter-individual distance (cm $\pm$ SE) among adult female *Tetranychus urticae* exposed to predator-associated cues from *Amblyseius swirskii* and *Neoseiulus californicus*.

Predator species	Treatment	3 h (Mean $\pm$ SE, cm)	27 h (Mean $\pm$ SE, cm)
<i>A. swirskii</i>	Control	2.03 $\pm$ 0.17	2.15 $\pm$ 0.19
	Low risk	2.83 $\pm$ 0.18	3.23 $\pm$ 0.17
	Intermediate risk	3.24 $\pm$ 0.18	3.02 $\pm$ 0.21
	High risk	3.49 $\pm$ 0.18	2.84 $\pm$ 0.18
<i>N. californicus</i>	Control	2.62 $\pm$ 0.15	2.27 $\pm$ 0.14
	Low risk	3.07 $\pm$ 0.22	2.73 $\pm$ 0.18
	Intermediate risk	2.55 $\pm$ 0.22	2.27 $\pm$ 0.22
	High risk	2.63 $\pm$ 0.22	2.27 $\pm$ 0.23

Values are means $\pm$ SE (n = 20). For *A. swirskii*, the overall treatment effect at 3 h was significant (one-way ANOVA, $F = 3.27$, $df = 3$, $P = 0.026$), whereas no significant treatment effect was detected at 27 h ($F = 2.09$, $df = 3$, $P = 0.108$). According to the original post-hoc analysis, the control and low-risk treatments differed significantly at 3 h (Tukey's HSD, $P < 0.05$). No significant treatment effect was detected for *N. californicus*.

Oviposition response

Predator-associated cues significantly affected oviposition by *T. urticae* females, although responses differed according to predator identity (Table 2). Exposure to *A. swirskii*-associated cues significantly reduced egg production (one-way ANOVA: $F = 8.86$, $df = 3$, $P < 0.001$). Females exposed to the high-risk treatment produced the lowest number of eggs (14.05 ± 1.68 eggs female⁻¹), representing approximately a 37% reduction compared with control females (22.25 ± 1.06 eggs female⁻¹). Egg production in the low-risk treatment (21.70 ± 1.46 eggs female⁻¹) did not differ from the control treatment, whereas females exposed to the intermediate-risk treatment produced significantly fewer eggs (16.10 ± 1.16 eggs female⁻¹).

Similarly, *N. californicus*-associated cues significantly affected oviposition (one-way ANOVA: $F = 9.82$, $df = 3$, $P < 0.001$). Control females produced significantly more eggs (22.25 ± 1.18 eggs female⁻¹) than females exposed to low-, intermediate-, and high-risk treatments (17.40 ± 1.87 , 18.35 ± 2.36 , and 13.90 ± 1.75 eggs female⁻¹, respectively). However, egg production did not differ significantly among the three predator-associated risk treatments.

Table 2. Effects of predator-associated cues on oviposition by *Tetranychus urticae*.

Predator species	Treatment	Eggs laid by five females during 24 h (mean $\pm$ SE)
<i>A. swirskii</i>	Control	22.25 $\pm$ 1.06 a
	Low	21.70 $\pm$ 1.46 a
	Intermediate	16.10 $\pm$ 1.16 b
	High	14.05 $\pm$ 1.68 b
ANOVA		$F = 8.86$, $P < 0.001$
<i>N. californicus</i>	Control	22.25 $\pm$ 1.18 a
	Low	17.40 $\pm$ 1.87 b
	Intermediate	18.35 $\pm$ 2.36 b
	High	13.90 $\pm$ 1.75 b
ANOVA		$F = 9.82$, $P < 0.001$

Values are means $\pm$ SE (n = 20). Within each predator species, means followed by different lowercase letters differ significantly (Tukey's HSD, $P < 0.05$).

Effect of experimentally imposed prey spatial distribution on predator encounter dynamics

Experimentally imposed prey spatial arrangement did not significantly influence predator-prey encounter frequency for either predatory mite species (Table 3). For *A. swirskii*, the mean number of predator-prey encounters during the 120-min. observation period was 1.30 ± 0.33 in dispersed prey

patches and 1.20 ± 0.53 in aggregated prey patches (t-test, $P = 0.066$). For *N. californicus*, mean encounter numbers were 0.70 ± 0.26 and 1.30 ± 0.21 in dispersed and aggregated prey distributions, respectively (t-test, $P = 0.370$). Analysis of encounter occurrence at individual observation times using Mann-Whitney U tests also revealed no significant differences between prey distributions for either predator species throughout the observation period (all $P > 0.05$). Thus, under the forced-search conditions of this independent assay, prey spatial arrangement alone did not alter short-term predator encounter frequency.

Predator patch choice

Predator responses to prey spatial organization differed between the two phytoseiid species (Table 4). For *A. swirskii*, predators showed a significant preference for aggregated prey patches. Sixteen of the 20 predators (80%) first entered the aggregated patch, whereas only four (20%) initially entered the dispersed patch (two-sided exact binomial test, $P = 0.013$). Likewise, the first successful attack occurred predominantly in the aggregated patch, with 18 predators (90%) initiating predation there compared with only two (10%) in the dispersed patch (two-sided exact binomial test, $P < 0.001$).

Table 3. Effect of prey spatial distribution on predator-prey encounter frequency.

Predator species	Dispersed prey	Aggregated prey	Statistical test	<i>P</i>
<i>Amblyseius swirskii</i>	1.30 ± 0.33	1.20 ± 0.53	Independent Sample t-test	0.066
<i>Neoseiulus californicus</i>	0.70 ± 0.26	1.30 ± 0.21	Independent sample t-test	0.370

Values are means $\pm$ SE of predator-prey encounters recorded during the 120-min observation period.

Table 4. Initial patch choice and location of the first successful attack by predatory mites in relation to prey spatial distribution.

Predator species	First patch entered (Aggregated)	First patch entered (Dispersed)	Exact <i>P</i>	First attack (Aggregated)	First attack (Dispersed)	Exact <i>P</i>
<i>Amblyseius swirskii</i>	16	4	0.013	18	2	<0.001
<i>Neoseiulus californicus</i>	14	6	0.115	11	9	0.824

Exact two-sided binomial tests were performed assuming an expected probability of 0.5 for selection of either prey patch ($n = 20$ independent predators per species).

In contrast, *N. californicus* showed no significant preference for either prey distribution. Fourteen predators (70%) first entered the aggregated patch and six (30%) entered the dispersed patch, but this difference was not statistically significant (two-sided exact binomial test, $P = 0.115$). Similarly, the location of the first successful attack did not differ significantly between prey distributions, with 11 predators initiating their first attack in the aggregated patch and nine in the dispersed patch (two-sided exact binomial test, $P = 0.824$).

Overall, these findings indicate that *A. swirskii* preferentially selected and initiated attacks within aggregated prey patches, whereas *N. californicus* exhibited no significant preference for either prey distribution.

DISCUSSION

Predator-associated cues induced multiple non-consumptive responses in *T. urticae*, but these responses depended on predator identity and differed among traits. Exposure to *A. swirskii* cues temporarily increased prey dispersion, whereas *N. californicus* cues had no detectable effect on spatial organization. Cues from both predator species reduced oviposition, indicating that reproductive potential was more broadly responsive to perceived predation risk than spatial behaviour. Importantly, the present study did not directly test whether the spatial distribution induced by predator cues subsequently changed predator behaviour. Instead, two separate experiments used experimentally imposed aggregated and dispersed prey distributions to evaluate encounter frequency and patch choice.

The transient increase in prey dispersion following exposure to *A. swirskii* cues suggests a flexible adjustment to immediate perceived risk. The response disappeared by 27 h, consistent with a short-lived behavioural adjustment. Such reversibility may reduce the costs of maintaining antipredator behaviour when cues become less reliable. In contrast, reduced oviposition occurred in response to cues from both predator species, indicating that reproductive potential may respond more consistently to perceived risk than spatial organization. Spider mites are known to detect chemical residues deposited by phytoseiid predators, including walking traces and faecal deposits, and use these cues to modify behaviour before direct encounters occur (Grostal and Dicke 1999; Dicke and Grostal 2001). Our results extend these observations by demonstrating that not all predator-associated cues elicit equivalent responses. The absence of a significant behavioural response to *N. californicus* suggests that prey may discriminate among predator species, presumably because predator cues differ in their reliability or because the two predators represent different levels of perceived risk. Such selective responses are consistent with theoretical expectations that prey should balance the benefits of predator avoidance against the costs of unnecessary defensive behaviour (Ferrari *et al.* 2009).

The findings of the independent prey-distribution experiments provide a more complex picture of the relationship between prey spatial organization and predator responses. In the predator-encounter experiment, experimentally imposed aggregation or dispersion of *T. urticae* did not significantly affect the number of predator-prey encounters during the 120-min. observation period for either predator species. For *A. swirskii*, mean encounter frequency was similar between dispersed and aggregated prey distributions (1.30 ± 0.33 and 1.20 ± 0.53 encounters, respectively; $P = 0.066$). Likewise, for *N. californicus*, encounter frequency did not differ significantly between dispersed and aggregated prey distributions (0.70 ± 0.26 and 1.30 ± 0.21 encounters, respectively; $P = 0.370$). These results contrast with previous findings suggesting that prey aggregation can reduce or delay predator encounters and attacks (Janssen *et al.* 1998; Dittmann and Schausberger 2017). One possible explanation is that the present encounter assay imposed a relatively constrained searching environment and used experimentally fixed prey positions, potentially limiting the extent to which predators could respond to prey spatial organization. In addition, the relatively short observation period may have been insufficient to detect differences in cumulative encounter frequency, particularly when encounter numbers were low.

In contrast, prey spatial distribution strongly influenced the initial patch choice of *A. swirskii*. Sixteen of 20 predators first entered the aggregated prey patch, compared with four that first entered the dispersed patch ($P = 0.013$). The preference was even stronger for the location of the first successful attack, with 18 of 20 predators initiating their first attack in the aggregated patch ($P < 0.001$). *Neoseiulus californicus*, however, showed no statistically significant preference: 14 predators first entered the aggregated patch and six entered the dispersed patch ($P = 0.115$), while the first successful attack occurred in the aggregated and dispersed patches in 11 and nine cases, respectively ($P = 0.824$). Thus, the two predator species differed not only in their responses to predator-associated cues but also in their responses to alternative prey distributions. Differences of this kind can arise when predators use spatial information differently during initial searching and subsequent prey exploitation (Sabelis and Dicke 1985; Stephens and Krebs 1986).

At first sight, the absence of an effect of prey distribution on encounter frequency but the strong effect on patch choice may appear contradictory. However, these measurements capture different components of predator behaviour. Encounter frequency quantified the total number of physical predator-prey contacts during a forced-search assay, whereas patch choice measured the predator's initial decision between two alternative prey patches. A predator may therefore show a strong preference for entering an aggregated patch without producing a corresponding increase in the total number of encounters over a subsequent observation period. In particular, the strong effect on first patch entry and first attack by *A. swirskii* suggests that prey aggregation influenced the predator's initial spatial decision, whereas it did not necessarily translate into a higher cumulative encounter rate once the predator was searching within the arena. This distinction is consistent with the broader view that predator responses to prey depend on the searching context and the stage of the foraging process (Sabelis and Dicke 1985; McMurtry *et al.* 2013).

Nevertheless, the magnitude and direction of these findings warrant careful consideration. Because the encounter-dynamics and patch-choice experiments were independent experimental components, the results should not be interpreted as a direct causal sequence in which cue-induced dispersion was subsequently tested for its effect on predator encounters or patch selection. Furthermore, the apparent discrepancy raises the possibility of an experimental, data-entry, or analytical error that cannot be excluded from the present results. In particular, the raw observations, coding of aggregated versus dispersed patches, definition of an encounter, timing of observations, and statistical calculations should be rechecked to ensure that the two experiments were scored consistently and that no reversal or transcription error occurred. This verification is especially important because the encounter assay and patch-choice assay used different experimental arrangements and response variables. If the reported results are confirmed, the discrepancy would suggest that prey spatial distribution affects different stages of predator foraging behaviour in different ways rather than producing a uniform effect on predator-prey interactions.

The contrasting responses of *A. swirskii* and *N. californicus* may further reflect species-specific foraging strategies and differences in prey-detection and prey-use behaviour (Janssen *et al.* 1998; McMurtry *et al.* 2013). *Amblyseius swirskii* showed a clear tendency to select and attack aggregated prey patches, despite the absence of a significant difference in total encounter frequency. This pattern suggests that aggregation may provide a detectable spatial target for this predator during the initial search phase. By contrast, *N. californicus* did not show a significant preference for either prey arrangement, consistent with its lack of response to predator-associated cues in the prey spatial-organization experiment. Together, these findings indicate that prey spatial organization does not necessarily have a single predictable effect on predator-prey encounter dynamics. Instead, its consequences may depend on predator identity, the behavioural stage being measured, and the spatial constraints imposed by the experimental arena.

Overall, the present results suggest that predator-associated cues can induce short-lived changes in prey dispersion, but such changes should not automatically be interpreted as reducing subsequent predation risk. The independent prey-distribution experiments show that spatial arrangement may influence predator decision-making at the patch-selection stage without necessarily altering the cumulative number of predator-prey encounters. This distinction is important when linking non-consumptive effects of predators on prey behaviour to their potential consequences for subsequent predator foraging.

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Generative AI statement: Generative AI (ChatGPT) was used solely for language editing and improvement of English

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نشانه‌های مرتبط با شکارگر رفتار طعمه را تغییر می‌دهند، اما بر پویایی مواجهه شکارگر-طعمه در هرنا‌ی تارتن دولکه‌ای (*Tetranychus urticae* (Acari: Tetranychidae) تأثیری ندارند

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چکیده

نشانه‌های مرتبط با شکارگر می‌توانند بدون وقوع برهم‌کنش مستقیم شکارگر-شکار، موجب تغییرات رفتاری و ویژگی‌های زیستی در شکار شوند؛ با این حال، اینکه آیا این پاسخ‌ها خطر شکارگری را کاهش می‌دهند، هنوز مشخص نیست. در این پژوهش، اثر نشانه‌های مرتبط با شکارگر در دو هرنا‌ی شکارگر فیتوزئید، *Amblyseius swirskii* و *Neoseiulus californicus*، بر توزیع فضایی و رفتار تولیدمثلی هرنا‌ی تارتن دولکه‌ای، *Tetranychus urticae*، بررسی شده و مشخص می‌شود که آیا تغییرات القاشده توسط شکارگر در پراکنش شکار بر مدل مواجهه بعدی شکارگر-شکار و انتخاب پیچ توسط شکارگر اثر می‌گذارد یا خیر. ماده‌های بالغ *T. urticae* در معرض سطوح آزمایشی دستکاری‌شده‌ای از نشانه‌های مرتبط با شکارگر، متناظر با افزایش خطر شکارگری، قرار گرفتند. سپس توزیع فضایی، با در نظر گرفتن میانگین فاصله بین افراد، و میزان تخم‌گذاری بررسی شد. در آزمایش‌های تکمیلی، فراوانی مواجهه شکارگر-شکار در آرایش‌های تجمعی و پراکنده شکار و همچنین انتخاب پیچ توسط شکارگر بین آرایش‌های مختلف شکار بررسی شد. نشانه‌های مرتبط با شکارگر پاسخ‌هایی وابسته به صفت و وابسته به گونه شکارگر ایجاد کردند. مواجهه با نشانه‌های *A. swirskii* موجب افزایش موقتی پراکندگی طعمه پس از ۳ ساعت شد، در حالی که نشانه‌های *N. californicus* تأثیر معنی‌داری بر توزیع فضایی شکار نداشتند. در مقابل، نشانه‌های هر دو گونه شکارگر به‌طور معنی‌داری موجب کاهش میزان تخم‌گذاری شدند و این کاهش در سطوح بالاتر درک خطر شدیدتر بود. با وجود این تغییرات رفتاری، توزیع فضایی شکار تحت شرایط آزمایشی این پژوهش بر فراوانی مواجهه شکارگر-شکار برای هیچ‌یک از دو گونه شکارگر تأثیر نداشت. با این حال، *A. swirskii* به‌طور ترجیحی پیچ‌های دارای شکار تجمعی را انتخاب کرد و حمله‌های خود را نیز در این پیچ‌ها آغاز کرد، در حالی که *N. californicus* هیچ ترجیح معنی‌داری برای پیچ‌های شکار نشان نداد. این یافته‌ها نشان می‌دهند که انعطاف‌پذیری رفتاری القاشده توسط شکارگر لزوماً به کاهش میزان مواجهه با شکارگر منجر نمی‌شود؛ بلکه پیامدهای بوم‌شناختی آن به برهم‌کنش میان پاسخ‌های شکار و راهبردهای جستجوگری وابسته به گونه شکارگر بستگی دارد.

واژگان کلیدی: رفتار جستجوگری، هرنا‌های گیاه‌خوار، هرنا‌های فیتوزئید، شناسایی شکارگر، ادراک خطر، برهم‌کنش‌های وابسته به صفت

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