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Article

Innate and acquired response of *Neoseiulus barkeri* and *N. californicus* (Acari: Phytoseiidae) to spider mite induced plant volatiles

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ABSTRACT

Predators may adjust their foraging behavior according to herbivore-induced plant volatiles innately or through learning. We investigated whether the phytoseiid predatory mites, *Neoseiulus barkeri* and *N. californicus*, exhibited an innate ability to respond to odors of *Tetranychus urticae*-infested bean leaves. Naïve female mites were reared on washed *T. urticae* as prey on filter paper with no prior exposure to spider mite-induced plant volatiles (SIPV). The olfactory experiments showed that significantly more *N. barkeri* and *N. californicus* moved towards *T. urticae*-infested bean leaves compared to clean air. *Neoseiulus californicus* significant preference of *T. urticae*-infested bean leaves rather than clean tomato (as unfamiliar environment) confirmed the innate response in this species, while *N. barkeri* could not differentiate the latter odor sources, suggesting a weak innate response. In a separate set of experiments, naïve *N. californicus* females with no preference towards HIPV were experienced by receiving odors related to *T. urticae*-infested bean leaves. The changed response (significant preference) of the experienced individuals showed the predators ability for acquired response. As the predator paired the odor with food absence/presence, associative learning was suggested as the mechanism. No acquired response was recorded for *N. barkeri*.

KEY WORDS: Innate response; learning; olfactory response; Phytoseiidae; sensitization.

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INTRODUCTION

Prey are often difficult to be detected visually, and predators can use information from the prey environment to locate them (De Boer *et al.* 2005). Predatory mites are known to use herbivore-induced plant volatiles (HIPVs) to help locate their prey (third trophic level). HIPVs are reliable signals for predatory mites, especially at high densities of herbivores (War *et al.* 2012). Some plants under herbivore attack emit HIPVs from both the infested and non-infested plant tissue, which serves to send a larger volatile signal to predators making prey more detectable (Dicke *et al.* 2009; War *et al.* 2011). These volatiles, which often consist of blends of molecules, are known to vary as a function of host plant species, plant development stage and the species of herbivore attacking the plant (De Moraes *et al.* 1998; Dicke *et al.* 1998; van Wijk *et al.* 2008; War *et al.* 2011). Thus predators are confronted with a wide variety of information about potential prey, which is expected to be counterpoised by a combination of an experience-free response (innate response) and learned/induced response.

Learning, a change in behavior following experience (Christiansen *et al.* 2016), plays an important role in predatory mite locating prey (Drukker *et al.* 2000). Egas and Sabelis (2001) documented learning by *Tetranychus urticae* Koch, whereby a higher oviposition rate occurred on its cucumber host over tomato. Similarly, Drukker *et al.* (2000) demonstrated the ability of *Phytoseiulus persimilis* (Athias-Henriot) (Acari: Phytoseiidae) to associate the presence of spider mites with HIPVs. They reported strong evidence of an acquired response of *P. persimilis* to HIPVs when paired with food as a positive stimulus, whereas naïve predators could not discriminate clean air from HIPVs. Seiter and Schausberger (2015) showed that *P. persimilis* offspring whose mother had experienced intraguild predation risk by *Amblyseius andersoni* Chant (Acari: Phytoseiidae), moved less compared to those emerged from unstressed mothers. Perlata Quesada and Schausberger (2012) also showed that prenatal chemosensory experiences in *Neoseiulus californicus* (Mc Gregor) (Acari: Phytoseiidae) influenced juvenile foraging preferences. Dicke *et al.* (1990) documented sensitization, the gradual increase in response to a stimulus with repeated exposure to that stimulus, in *P. persimilis*. They found that when *P. persimilis* was reared on *T. urticae*-infested lima bean plants and then transferred to cucumber plants infested by the same prey, a gradual preference (moving towards) occurred for the latter host. Drukker *et al.* (2000) reported the ability of acquired response in *P. persimilis* due to their changed response (from non-preference to a strong preference towards infested bean leaves) after experiencing odors from spider-mite-infested bean leaves. They reported the underlying mechanism of the acquired response as associative learning rather than sensitization due to the change in response that was recorded after experiencing a new context.

Rahmani *et al.* (2009) suggested that *P. persimilis* could be an excellent model for studying the evolution of learning and information processing. Here, we studied the innate and acquired response of the predatory mites, *Neoseiulus barkeri* Hughes and *N. californicus*, to herbivore-induced plant volatiles. The mechanism of the acquired response is discussed.

MATERIALS AND METHODS

Plants and mites

We grew common bean plants (*Phaseolus vulgaris* L. var. Red Alamouti) (Fabaceae) and tomato plants (*Solanum lycopersicum* L. var. Buffalo) (Solanaceae) in plastic pots in a greenhouse (soil: perlite; 50: 50%) under controlled conditions ($25 \pm 5^\circ\text{C}$, 16L:8D hour photoperiod, $65 \pm 5\%$ RH) at the Department of Plant Protection, Faculty of Agriculture, University of Tehran, Karaj, Iran. Plants were watered daily with tap water and fertilizer solution of NPK (20-20-20, every other day). Spider mites, *T. urticae* (green form) were reared on bean plants as a food source for the predatory mites. We transferred infested bean plants to fresh 10 day old bean plants at controlled conditions ($25 \pm 5^\circ\text{C}$, full time photoperiod and $65 \pm 10\%$ RH) were added to the rearing system regularly. Dead old plants were removed and replaced with fresh ones to continue renewing the colony. Colonies of predatory mites, *N. barkeri* and *N. californicus*, were reared separately on masses of excised bean leaves, infested with *T. urticae*. Infested leaves were placed upside down (abaxial surface up) on a sheet of plastic that was itself placed on top of a water-saturated sponge. The plastic sheet was surrounded by Kleenex tapes, which were put into the water from another side, so that the predatory mites could acquire water. Fresh *T. urticae*-infested leaves were added to the rearing system every days, and the old predator-free leaves removed. The cultures were kept in separate growth chambers under controlled conditions ($25 \pm 1^\circ\text{C}$, 16L:8D hour photoperiod, $65 \pm 5\%$ RH) in Acarology laboratory at Jalal Afshar Zoological Museum, Department of Plant Protection, Faculty of Agriculture, University of Tehran, Karaj, Iran.

Olfactory experiments

We used an olfactometer set to test whether the olfactory responses of female predatory mites

would change in response to receiving various odor sources. The olfactometer consisted of a Y-shaped glass tube (4 cm in diameter, 13 cm in total length) with a Y-shaped metal wire in the middle of the tube, positioned parallel to the tube walls (Sabelis and van de Baan 1983). Odors from the source containers were introduced using plastic tubing through the lateral arms of the olfactometer and flowed towards the base of the Y-tube. Charcoal purified air was blown by a fan into the odor source boxes. Hotwire air flow meters were positioned before the lateral arms of the olfactometer, provided a fixed airflow of 0.5 m/s into each arm. Based on Drukker *et al.* (2000), the odor sources consisted of (1) *T. urticae*-infested bean plants (infested with 50 *T. urticae* 2 days prior to use in the assay system) as the standard environment on which the predators were reared, (2) uninfested tomato plants as an unfamiliar environment, and (3) clean air as control.

Assessment of innate response

To assess the innate response of predatory mites we produced naïve predators that had not previously been exposed to spider mite-induced plant volatiles from bean plants. We prepared a diet of mixed-aged *T. urticae* that were rinsed (with distilled water) in order to loose odors related to host plant (HIPVs). We transferred the eggs of each predator species from the main culture to a separate arena consisting of detached bean leaf ($2.5 \times 2.5 \text{ cm}^2$) placed upside down on same-sized water saturated sponge in a Petri dish (9 cm in diameter). The predators fed on washed spider mites (dead) during their development. The emerged adult females were considered as naïve predators. The same-aged predators were released on the wire in the Y-tube at the downwind end of the olfactometer and we assessed their response to the following two treatment combinations: (1) *T. urticae*-infested bean plants (familiar environment) vs. clean air (control), (2) *T. urticae*-infested bean plants vs. clean tomato (unfamiliar environment) according to Drukker *et al.* (2000). The same experiment was performed with the same-aged experienced predators that had been reared on *T. urticae*-infested bean plants and were familiar with the HIPVs resulting from a spider mite infestation. We compared the olfactory responses of naïve and experienced predators of both species to determine their probable innate response towards spider mite induced plant volatiles.

Assessment of acquired response

Naïve predators were tested for their olfactory response towards *T. urticae*-infested bean leaves vs. clean air. The same predators were then transferred singly to Petri dishes (9 cm diameter) containing a detached inverted bean leaf ($2.5 \times 2.5 \text{ cm}^2$) infested with 15 *T. urticae* active stages on a water saturated sponge for 16 hours (spider mites transferred on to the leaf disk 2 hours prior to introducing the predators). In this way, the naïve predators got experienced with HIPV. Then the experienced predators were tested for their olfactory response with the same odor sources. In this way, we tested whether predators' behavioral response changed after they became experienced with volatiles that were combined with a positive stimulus (food) (Drukker *et al.* 2000). As the predator individuals needed to be accessible for a longer experimental time (first olfactory experiment, getting experienced and second olfactory experiment), the predator keeping arena was changed to $2.5 \times 2.5 \text{ cm}^2$ Petri dishes and maize pollen was added to the rearing system. Thus, the results of our first olfactory experiment set could not be comparable with that in the innate response set.

Since the acquired response may result from either sensitization to chemical cues or associative learning (Drukker *et al.* 2000), we performed additional complementary experiments. To assess the role of sensitization, the gradual increase in response should be independent of the paired stimulus. We therefore had to prepare a situation of food absence for the predators. Naïve predators were tested for their olfactory response towards *T. urticae*-infested bean plants vs. clean tomato leaves. The same predators were transferred into a plastic tube that was covered at the both ends with gauze. *Tetranychus urticae*-infested bean leaves (ca. 5 *T. urticae* per cm^2 leaf) were placed at one end of the tube as an odor source. Air was passed over the odor source at a rate of 0.5 m/s towards the naïve

predators, which were maintained as such with no food for 16h. In this way predators received the odors related to the food, but they had no food access. The predators then were tested for the same olfactory response. For the next step the predators were put in the plastic tubes with the same conditions mentioned above, but in the presence of food (washed *T. urticae* eggs). The airflow consisted of odors related to *T. urticae*-infested bean leaves. The olfactory response of the predators was tested towards *T. urticae*-infested bean plants vs. clean tomato plants.

Under the null hypothesis, we expected 50% of the predators to enter each of the two arms of the Y-tube and we tested for significant deviations from this expectation. Statistical analysis was performed using a replicated G-test in Excel (Microsoft Corporation, Seattle Washington), which includes a test for heterogeneity among replicate experiments (Sokal and Rohlf 1995). The change in response due to experiences was analyzed with Pearson Chi Square in SPSS 19.

RESULTS

Innate response

Experienced *N. californicus* females that had previously fed on spider mites on bean leaves showed a significant preference towards volatiles emitted from *T. urticae*-infested bean leaves representing a familiar environment over both the clean air (control) and odors from uninfested tomato representing an unfamiliar environment ($P < 0.01$, Table 1). Naïve *N. californicus* females that fed on water-rinsed spider mites on filter paper also showed a significant preference towards the *T. urticae*-infested bean leaves ($P < 0.05$, Table 1). Experienced *N. barkeri* females that previously fed on spider mites on bean leaves showed a significant preference towards volatiles emitted from *T. urticae*-infested bean leaves representing a familiar environment over the both clean air (control) and odors from uninfested tomato representing an unfamiliar environment ($P < 0.01$, Table 1). Naïve *N. barkeri* females that fed on water-rinsed spider mites on filter paper showed no significant preference towards either arm of the olfactometer ($P > 0.05$, Table 1).

Table 1. Results of replicated G-test for the response of *N. californicus* (not bold values) and *N. barkeri* (values in bold) to odors related to *T. urticae*-infested bean leaves (TUB), clean air (CA) and clean tomato (CT). G_p , G_h and G_t indicate the significance of the pooled result, heterogeneity among replicate experiments and overall results respectively.

| Odor source | state | G_p | P-value | G_h | P-value | G_t | P-value |
|-------------|--------------------|--------------|--------------------------|-------------|--------------------------|--------------|--------------------|
| CA vs. TUB | experienced | 7.308 | < 0.01** | 0.33 | 0.08 ^{ns} | 7.64 | < 0.02** |
| | naïve | 5.008 | < 0.05* | 3.26 | 0.19 ^{ns} | 8.26 | < 0.05* |
| | experienced | 10.45 | < 0.01** | 1.28 | 0.52^{ns} | 11.73 | < 0.01** |
| | naïve | 5.59 | < 0.05* | 3.97 | 0.13^{ns} | 9.56 | < 0.01** |
| CT vs. TUB | experienced | 8.59 | < 0.01** | 4.79 | 0.09 ^{ns} | 13.38 | < 0.01** |
| | naïve | 3.97 | < 0.05* | 5.59 | > 0.05 ^{ns} | 9.56 | < 0.01** |
| | experienced | 9.47 | < 0.01* | 1.59 | 0.45^{ns} | 11.06 | < 0.01** |
| | naïve | 2.47 | 0.11^{ns} | 3.67 | 0.01^{ns} | 7.64 | < 0.05* |

Acquired response

Naïve *N. californicus* females that fed on water-rinsed spider mites and maize pollen on filter paper showed no significant preference towards the odor sources ($P > 0.05$) (Table 2a & b). When the same individuals were transferred to *T. urticae*-infested bean leaves for 16 h and exposed to HIPVs (getting experienced with HIPV), 34 out of 45 predators moved towards one of the arms, 73% of which preferred the odors related to *T. urticae*-infested bean leaves ($P < 0.05$) (Table 2a). We interpret this change as an evidence of acquired response to *T. urticae*-induced plant volatiles when paired with food (prey) presence.

Table 2. Results of replicate experiments of olfactometer tests (a) and replicated G-test (b) for the response of naïve (a) and experienced (b) *N. californicus* females to odors from *T. urticae*-infested leaves (+) and clean air (-).

a) Replicated experiments of olfactometer test

| Independent replicate | n (+) | | n (-) | | n (0) | | n (total) |
|-----------------------|-------|---|-------|---|-------|---|-----------|
| | A | B | A | B | A | B | |
| 1 | 4 | 8 | 5 | 3 | 6 | 4 | 15 |
| 2 | 6 | 8 | 4 | 3 | 5 | 4 | 15 |
| 3 | 4 | 9 | 4 | 3 | 6 | 3 | 15 |

b) Replicated G-test

| | df | G-statistics | | P-value | |
|-------|----|--------------|-------|---------|------|
| | | A | B | A | B |
| G_p | 2 | 0.013 | 4.349 | 0.9 | 0.03 |
| G_h | 1 | 1.195 | 0.02 | 0.5 | 0.09 |
| G_i | 3 | 1.173 | 4.370 | 0.5 | 0.1 |

Naïve *N. barkeri* females that fed on water-rinsed spider mites and maize pollen on filter paper showed no significant preference towards the odor sources ($P > 0.05$; Table 3a & b). When the same individuals were transferred to *T. urticae* bean leaves for 16 hours and exposed to HIPVs, 22 out of 45 predators moved towards one of the arms, 62% of which preferred the odors related to *T. urticae*-infested bean leaves, although this difference was not statistically significant ($P > 0.05$; Table 3a). The rate of predator females that preferred the HIPVs increased from 56% to 62% after 16 hours of experience, which was not significant (Pearson Chi-Square: 0.286, df: 1, P-value > 0.05).

Table 3. Results of replicate experiments of olfactometer tests (a) and replicated G-test (b) for the response of naïve (a) and experienced (b) *N. barkeri* females to odors from *T. urticae*-infested leaves (+) and clean air (-).

a) Replicated experiments of olfactometer test

| Independent replicate | n (+) | | n (-) | | n (0) | | n (total) |
|-----------------------|-------|---|-------|---|-------|---|-----------|
| | A | B | A | B | A | B | |
| 1 | 5 | 7 | 3 | 5 | 7 | 3 | 15 |
| 2 | 5 | 8 | 4 | 3 | 6 | 4 | 15 |
| 3 | 4 | 7 | 4 | 5 | 7 | 3 | 15 |

b) Replicated G-test

| | df | G-statistics | | P-value | |
|-------|----|--------------|-------|---------|-------|
| | | A | B | A | B |
| G_p | 2 | 0.0006 | 1.493 | 0.98 | 0.22 |
| G_h | 1 | 0.255 | 0.601 | 0.88 | 0.740 |
| G_i | 3 | 0.256 | 2.094 | 0.87 | 0.350 |

Sensitization or associative learning

An acquired response may result from either sensitization (a gradual increase in attraction irrespective of the presence of food) or associative learning (the dependent response of a predator changes according to the presence or absence of food). To investigate which mechanism is responsible for *N. californicus* acquired response, we recorded the olfactory response of the predator females to odors related to clean tomato plants representing an unfamiliar environment vs. *T. urticae*-infested bean plants representing a familiar environment. Olfactometer experiments revealed that 21 out of the 45 predators tested moved towards one of the arms, 47% of which moved toward the familiar environment, although this was not a statistically significant difference ($P > 0.05$; Table 4a).

& b). The same individuals were then kept on filter paper in the absence of prey for 16 h and received odors from *T. urticae*-infested bean leaves and their olfactory response was checked again. Experiments determined that 19 out of 45 predators moved towards one of the arms, 36% of which moved towards *T. urticae* induced bean odors ($P > 0.05$; Table 3a & b). The percentage of *N. californicus* females that preferred the familiar environment decreased from 47 to 36% after 16 hours of exposure (Pearson Chi-Square: 41.486, df: 4, P-value < 0.01), a gradual switch to the opposite response (Drukker *et al.* 2000).

Table 4. Results of replicate experiments of olfactometer tests (a) and replicated G-test (b) for the response of naïve *N. californicus* provided with washed prey (a) and *N. californicus* receiving HIPV for 16 h without prey (b) to odors from *T. urticae*-infested leaves (+) and clean tomato (-).

a) Replicated experiments of olfactometer test

| Independent replicate | n (+) | | n (-) | | n (0) | | n (total) |
|-----------------------|-------|---|-------|---|-------|---|-----------|
| | A | B | A | B | A | B | |
| 1 | 4 | 2 | 4 | 4 | 7 | 9 | 15 |
| 2 | 3 | 2 | 3 | 4 | 9 | 9 | 15 |
| 3 | 3 | 3 | 4 | 4 | 8 | 8 | 15 |

b) Replicated G-test

| | df | G-statistics | | P-value | |
|-------|------|--------------|-------|---------|------|
| | | A | B | A | B |
| G_p | 2 | 0.629 | 1.493 | 0.042 | 0.83 |
| G_h | 1 | 0.095 | 0.601 | 0.340 | 0.84 |
| G_t | 3 | 0.725 | 2.094 | 0.383 | 0.82 |

Table 5. Results of replicate experiments of olfactometer tests (a) and replicated G-test (b) for the response of naïve *N. californicus* provided with HIPV and washed prey (a) and *N. californicus* provided with clean tomato odors and washed prey (b) to odors from *T. urticae*-infested leaves (+) and clean tomato (-).

a) Replicated experiments of olfactometer test

| Independent replicate | n (+) | | n (-) | | n (0) | | n (total) |
|-----------------------|-------|---|-------|---|-------|---|-----------|
| | A | B | A | B | A | B | |
| 1 | 8 | 2 | 1 | 7 | 2 | 2 | 15 |
| 2 | 8 | 3 | 2 | 6 | 1 | 2 | 15 |
| 3 | 6 | 3 | 2 | 7 | 3 | 1 | 15 |

b) Replicated G-test

| | df | G-statistics | | P-value | |
|-------|------|--------------|-------|---------|-------|
| | | A | B | A | B |
| G_p | 2 | 0.590 | 8.769 | 0.005 | 0.003 |
| G_h | 1 | 7.7 | 0.293 | 0.744 | 0.863 |
| G_t | 3 | 8.29 | 9.063 | 0.015 | 0.01 |

To determine if *N. californicus* are capable of associative learning, the same individual predators used above, were exposed to odors related to *T. urticae*-induced bean leaves for 16 hours in the presence of prey. After 16 hours, they were tested for their olfactory response towards odors related to *T. urticae*-infested bean plants vs. clean tomato plants. Olfactory experiments determined 27 out of 45 predators moved towards one of the arms, 81% of which preferred infested bean plants ($P < 0.01$) (Table 5 a & b). In another experiment, the predators received odors related to clean tomato plants for 16 h in the presence of *T. urticae*. They were then tested for their olfactory response towards

odors related to *T. urticae*-infested bean plants vs. clean tomato plants. Observations determined that 28 out of 45 predators moved towards one of the arms, 71% of which preferred uninfested tomato plants ($P < 0.01$) (Table 5 a & b).

DISCUSSION

Alba *et al.* (2012) explained that there are two kinds of naivety in arthropods: (1) those who have never experienced a prey patch on their host plant (true naivety), and (2) those whose prey patch has been exterminated recently, which are not truly naïve. They reported that over 50% of truly naïve arthropods were able to respond to herbivore induced plant volatiles. Our results showed that experienced *N. californicus* and *N. barkeri* females (reared on *T. urticae*-infested bean plants) showed a significant preference towards *T. urticae*-induced plant volatiles over clean air and clean tomato leaves. The naïve *N. californicus* females (reared on water-rinsed spider mites on filter paper- also showed a significant preference towards *T. urticae*) induced plant volatiles despite the reduction rate of predators moved towards infested bean plants. This could be due to the strong innate response of *N. californicus* towards HIPV. The naïve *N. barkeri* females (reared on washed spider mites on filter paper) preferred *T. urticae* bean plants rather than clean air, while the number of predators moved towards *T. urticae*-infested bean leaves was not significantly more than those preferred clean tomato leaves. This shows that the innate response in *N. barkeri*, if present, is weak. Drukker *et al.* (2000) also reported a weak innate response to the same odor sources for *P. persimilis*. They discussed that the predator non-preference behavior could not be due to the inferior quality of plant volatiles as the experienced individuals had shown a strong preference towards the same odor sources. Sznajder *et al.* (2010) demonstrated the innate preference of *P. persimilis* to the volatile blends of *T. urticae*-infested bean leaves and noted that this response could be due to the predator's innate preference to a special individual compound (Methyl Salicylate) that is often shared in most blends of many plant species. Walzer and Schausberger (2011) documented innate response of *P. persimilis* in the presence of *Amblyseius andersoni* as high-risk intraguild predator. Grostal and Dicke (2000) reported that *T. urticae* were able to detect *P. persimilis*, which resulted in an increase in escape attempts and decreased rates of oviposition. Our findings about innate response in *N. californicus* contrasts with Papaj *et al.* (1994) and Drukker *et al.* (2000) who demonstrated that naïve predators showed no preference for volatiles prior to their association with a rewarding experience (presence of prey). To our knowledge, this is the first study that has documented the comparative innate response of two phytoseiid species in addition to *P. persimilis*.

Naïve *N. californicus* females (reared on washed spider mites and pollen) tested for their olfactory response to *T. urticae*-infested bean plants vs. clean air, showed no significant preference towards each of the odor sources. After a 16 hours experience with *T. urticae* bean leaves, the same individual olfactory responses were checked and a significant preference towards HIPVs was recorded. This is interpreted as a strong evidence of acquired response in *N. californicus*. The behavior was also reported by Drukker *et al.* (2000), who tested *P. persimilis* female's olfactory response. Our results are similar to Egas *et al.* (2003) who reported that spider mites were able to learn to distinguish the food quality within one host plant. Glinwood *et al.* (2011) also showed that *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) was able to not only associate the odor of aphid-infested plants with the presence of prey, but also to discriminate the variability between cultivars of the same plant. Here, the fact that *N. californicus* was able to perceive the relationship between two coincident events (odor and prey presence/absence), suggests that these predators are engaging in associative learning and not sensitization. Only a few studies have demonstrated the capacity for associative learning in arthropod predators (De Boer and Dicke 2006). Santos *et al.* (2013) also documented associative learning in the Neotropical harvestman, *Discocyrtus invalidus* Piza (Arachnida, Opilions) for the first time. They showed that *D. invalidus* was able to associate

shelter with the chemical stimulus. Santer and Hebets (2009) reported about the ability of *Phrynus marginemaculatus* C. L. Koch (Arachnida, Amblypygi) to learn tactile cues and associate them with a refuge. Learned avoidance of aposematic prey has been documented in mantids, spiders and dragonflies (Berenbaum and Miliczky 1984, Kauppinen and Mappes 2003; Skow and Jakob 2005). Here, we have not investigated the fitness consequences (i.e. oviposition rate and survival) of host preference learning in *N. californicus*, so that we could not link this behavior to adaptive learning like Egas *et al.* (2003), who demonstrated a higher performance result of *T. urticae* on undamaged host plants.

Neoseiulus barkeri and *N. californicus* belong to type II life style of phytoseiids (i.e. selective predators of tetranychid mites) and type III (i.e. generalist phytoseiids from soil/litter habitats), respectively (McMurtry *et al.* 2013). In our research, both predator colonies were reared on spider mite infested bean leaves, but *N. californicus* has reported to show adaptations for living in spider mite colonies with dense webbing while this is not reported for *N. barkeri* (McMurtry *et al.* 2013). This could provide the basis for the species difference in learning behavior observed as part of our current study. For associative learning, a simple link should be mediated between two or more events like HIPV and food presence (Giurfa 2013), which its framework has probably been less provided in *N. barkeri* associations with *T. urticae* in our stock culture.

Dukas (2008) demonstrated that the threshold of nervous system required for learning is low. They rejected the belief that small arthropods (i.e. insects) exhibit little learning owing to their small brain and short lifespan. The prevalence of learning in predatory mites and the ecological factors affect it are still unknown. Further investigations are needed to evaluate why the learning types are different in closely related species.

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پاسخ ذاتی و اکتسابی *Neoseiulus barkeri* و *N. californicus* (Acari: Phytoseiidae) به مواد فرار بویایی گیاه آلوده به کنه تارتن

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چکیده

شکارگرها رفتار کاوشگری‌شان را در برابر دریافت مواد فرار بویایی ناشی از تغذیه گیاهخوارها، به صورت ذاتی یا در قالب یادگیری تنظیم می‌کنند. در این پژوهش، پاسخ ذاتی کنه‌های شکارگر *Neoseiulus barkeri* و *N. californicus* نسبت به مواد فرار بویایی برگ‌های آلوده به *Tetranychus urticae* بررسی شد. کنه‌های ماده بی‌تجربه روی *T. urticae* شسته شده، بر روی کاغذ صافی و بدون قرار گرفتن در معرض مواد فرار بویایی ناشی از گیاه آلوده به کنه تارتن پرورش یافتند. آزمون‌های بوسنجی نشان داد که *N. californicus* و *N. barkeri*، بیشتر به سمت بازوی منتهی به برگ‌های لوبیای آلوده به *T. urticae* (نسبت به هوای پاک) حرکت کردند. گرایش معنی‌دار *N. californicus* به سمت برگ‌های لوبیای آلوده به کنه تارتن در مقایسه با گیاه گوجه‌فرنگی سالم (به عنوان محیط ناآشنا) توانایی پاسخ ذاتی در این گونه را تأیید می‌کند. این درحالی است که *N. barkeri* توانایی تفکیک این دو منبع مواد فرار را نداشته و در نتیجه، امکان بروز پاسخ ذاتی در این گونه، ضعیف است. در مجموعه جداگانه‌ای از آزمون‌ها، کنه‌های ماده بی‌تجربه *N. californicus* که پیش‌تر هیچ گرایشی به سمت HIPV نشان نداده بودند، با دریافت مواد فرار بویایی ناشی از برگ‌های لوبیای آلوده به کنه تارتن، تجربه کسب کردند. تغییر پاسخ افراد باتجربه (به صورت گرایش معنی‌دار)، نمایان‌گر توانایی این شکارگر در بروز پاسخ اکتسابی است. از آنجایی که این گونه شکارگر توانست وجود ماده فرار بویایی را با وجود یا عدم وجود غذا جفت کند، سازوکار مربوطه از نوع یادگیری ارتباطی تشخیص داده شد. در مورد *N. barkeri* شواهدی مبنی بر یادگیری اکتسابی ثبت نشد.

واژگان کلیدی: پاسخ ذاتی؛ یادگیری؛ پاسخ بویایی؛ Phytoseiidae؛ حساس‌سازی.

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