

Article

Olfactory responses of the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae) to rose leaves: starvation and previous host plant experience

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Abstract

The effect of host plant experience and different hunger periods on the olfactory response of *Phytoseiulus persimilis* was studied on rose leaves under laboratory conditions. When clean air was considered as the olfactometer fixed arm, *P. persimilis* showed a significant preference towards odours related to the alternative arms *i.e.* clean leaves, *T. urticae*-infested leaves, leaves occupied by *Frankliniella occidentalis* and leaves occupied by conspecifics regardless of starvation time and experience. When we replaced clean air by clean rose leaves, the naïve predators with 10 hours starvation and the experienced ones with both 5 and 10 hours starvation showed a significant preference (towards *T. urticae*-infested leaves) rather than clean leaves. When the predators were offered clean leaves vs. leaves occupied by thrips, neither naïve nor experienced *P. persimilis* with 5 hours starvation made a significant preference between the arms, while the ones kept starved for 10 hours preferred clean air regardless of experience. Receiving odours related to conspecifics vs. clean rose, the naïve *P. persimilis* females with 5 hours starvation and the naïve and experience ones kept starved for 10 hours avoided odours related to conspecifics. The five hour-starved experienced predators and the 10 hour-starved naïve and experienced ones moved towards *T. urticae*-infested leaves when the alternative arm was consisted of leaves occupied by thrips. Receiving odours related to *T. urticae*-infested leaves from one arm and odours related to leaves occupied by conspecifics, most of the predators moved towards the former arm regardless of experience and starvation time. When the predators were offered *T. urticae*-infested leaves vs. leaves occupied by thrips, a significant movement towards spider mites was observed both in experienced and naïve treatments kept starved for 10 hours and in five hour-starved experienced ones. Irrespective of the starvation time, the predatory mite's previous experience did not have a significant effect on the time needed by *P. persimilis* to pass the lateral arms of the olfactometer. Both experience and starvation affected the predator's oviposition rate.

Key words: experience, odour source, foraging behavior, Phytoseiidae, volatile.

Introduction

Experience of predatory mites through their rearing on different host plant species modifies their subsequent foraging behavior. In order to increase the effectiveness of predators in biological control programs, sufficient knowledge about the stimuli and

their functional role is of high importance (Lewis & Martin 1990). These behavioral responses in tritrophic systems are complex processes influenced by many stimuli such as genetic system, the physiological condition of the searching predator, environment, experiences and the interactions among these factors (Dicke *et al.* 1990; Lewis *et al.* 1990; Vet & Groenewold 1990).

Several studies have demonstrated the role of previous experience on the foraging behavior of predators and parasitoids (Lewis & Takasu 1990; Lewis & Tumlinson 1988; Lewis *et al.* 1990; Maeda & Takabayashi 2005; Vet & Groenewold 1990; Zhang & Sanderson 1992). A learning process during antennation on larval host in *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) made them link the plant volatiles to the host, so that parasitoids reared on the host that fed on plant material were more attracted to host plant odours (Lewis & Martin 1990). Zhang & Sanderson (1992), showed that experience made by the predatory *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) on different host plants infested by the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) affects the foraging behavior of the predator in a variable range that depends both on the host plant and predator species. Dicke *et al.* (1990), also have demonstrated the important role of previous experience on the olfactory response of *P. persimilis*. They showed that predators reared on bean leaves for seven days prior to olfactory experiments, preferred volatiles related to infested bean leaves rather than infested cucumber leaves.

The two-spotted spider mite, *T. urticae* is considered as one of the most important pests that attack rose (*Rosa* spp.) plants in greenhouses along with the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). The traditional chemical control of spider mites and thrips seems inefficient because of pest resistance problem and phytotoxicity on ornamentals (Gough 1991; Kumral *et al.* 2010; Nicetic *et al.* 2001). Thus, using biological control agents such as predatory mites, is considered as an alternative method to reduce the infestation level (Holt *et al.* 2006; Maeda *et al.* 2001). The predatory mite, *P. persimilis* has been well studied with respect to its capacity to control spider mites in greenhouses. This predator is commercially mass-reared on bean plants and used as biological control agent on various crop species such as rose and other ornamentals so that considering its behavioral characters both in insectary and after releasing is of high importance.

In the present study, we investigated the olfactory response of *P. persimilis* to rose leaves when they had previously been reared on bean or rose plants. The effect of hunger duration on the predator's response has also been investigated.

Materials and methods

Plants and mites

Plants of the common bean, *Phaseolus vulgaris* L. (Fabaceae), and rose, *Rosa* spp. (Rosaceae) hybrids var. Black Magic, were grown in plastic pots containing mixture of soil and perlite. The potted plants were irrigated every day with tap water and a solution of NPK (20 × 20 × 20) fertilizer, and kept in controlled conditions (23 ± 2 °C, 16:8 h L:D, 60–70% RH) in growth chambers. The two-spotted spider mite, *T. urticae* and the western flower thrips, *F. occidentalis* were reared on bean and cucumber plants, respectively in two separate growth chambers (25 ± 2 °C, 16:8 h L:D, 60–70% RH). Corn pollen was added to thrips colonies once every 3–4 days. Next to the cucumber pots there were soil trays in which thrips pupae could develop into adults. Predatory mites were reared on detached rose leaves infested with *T. urticae* under the

conditions specified above for the climate in the growth chambers. *Tetranychus urticae*-infested bean leaves were used to feed the predatory mites (Fig. 1).

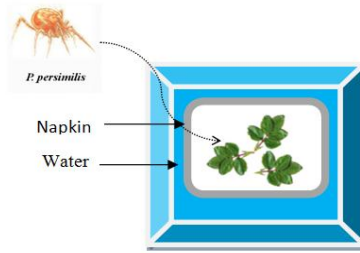


Figure 1. Predatory mite mass rearing system

Olfactory experiments

A Y-tube olfactometer was used for assaying the response of the searching predators to odours associated with rose leaves, *T. urticae*, *F. occidentalis* and conspecific predatory mites. The olfactometer consisted of a Y-shaped glass tube (Y: 4 cm) with a Y-shaped metal wire in the middle of the tube and positioned parallel to the tube walls (Sabelis & van de Baan 1983). The two lateral arms of the olfactometer through which the odours flew towards the searching predator, were connected to the odour source boxes by means of plastic tubes. Clean air blown by a fan, passed through charcoal sacs just before reaching the odour source boxes (Fig. 2).

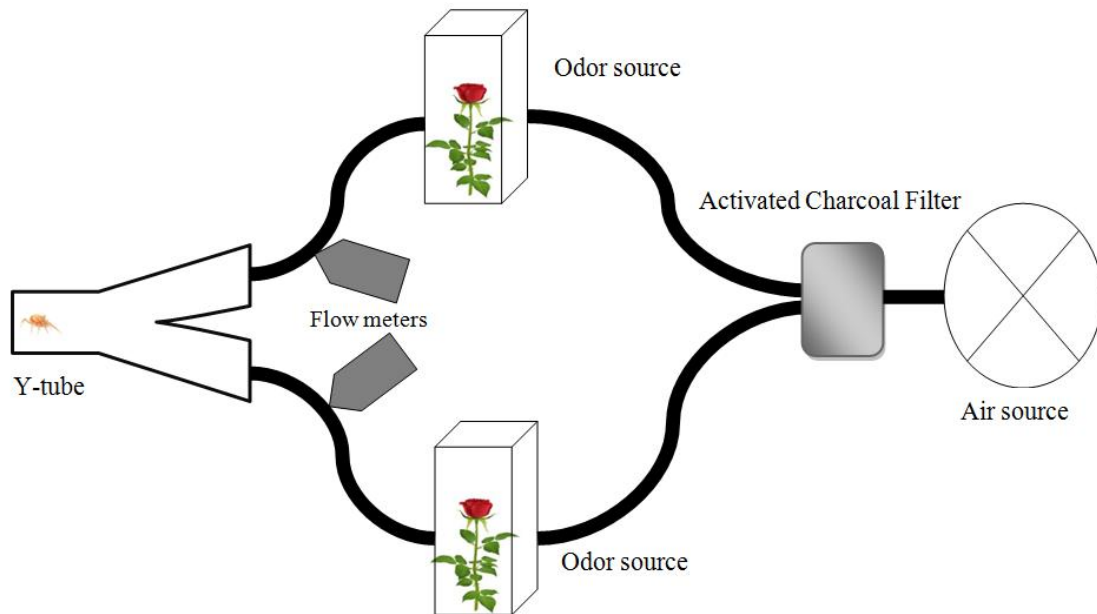


Figure 2. Y-shaped olfactometer.

Olfactory experiments were divided into three treatment groups. For each treatment, tests were performed in three independent replicates, each with 20 predators and a new source of odours. Three rose stems, each bearing five leaves were cut and put in a small water glass as one of the odour sources (clean leaves). For the treatments in which spider mite volatiles or thrips odours were needed, 25 same-aged adult females of *T. urticae* or five same-aged adult females of *F. occidentalis* were introduced into the patches (this experiment was performed both on patches with leaf discs and on patches

without leaf, on Petri dish and water saturated cotton wool), respectively. Five same-aged adult female predatory mites, put on a single patch, were considered as conspecific odour source. All odour sources were prepared 24 hours prior to olfactory experiments.

To analyze the effects of starvation period on the olfactory responses of *P. persimilis*, the searching predators were starved either for 5 or 10 hours before being used in the experiments. To cancel out responses to any unforeseen asymmetry in environmental factors, odour sources were swapped each time when five predators were tested. The olfactory experiments were performed using two types of the predatory mite cultures. One, with predators that had previously been reared on spider mite infested bean plants and transferred on rose leaves just before the experiments; and another, with predators that had a six month history of rearing on *T. urticae*-infested rose plants. So, the experiments were designed as either the 5/10 hour-starved predators that have experienced rose plants before tests; or the 5/10 hour-starved predators without any previous experience on rose plants.

Treatment group No.1 (clean air as the fixed odour source)

This treatment group contained four experiments in which the odour sources (second arm) involved: (1) odours related to clean rose leaves from one arm and clean air from another arm, (2) odours related to spider mites from one arm and clean air from another arm, (3) odours related to thrips from one arm and clean air from another arm and (4) odours related to conspecific predators (without leaf) from one arm and clean air from another arm (Fig. 3, Group 1).

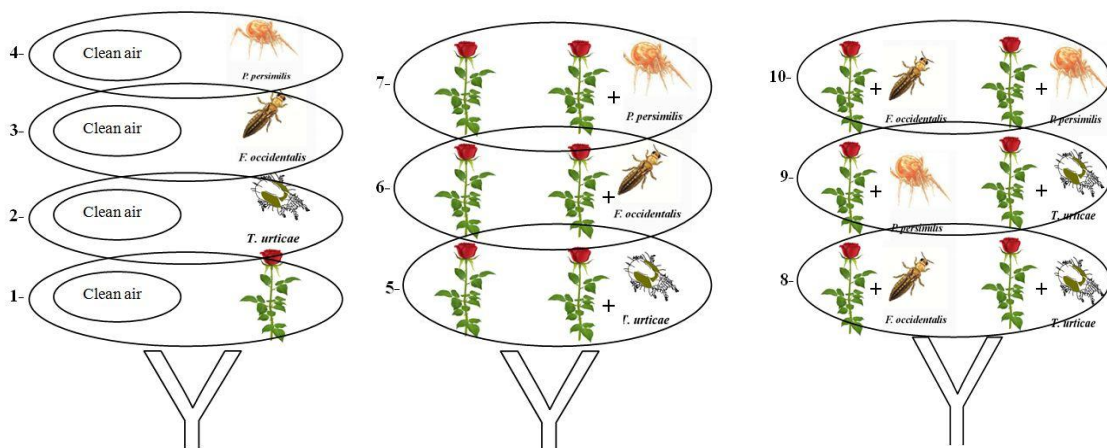


Figure 3. Olfactory treatment groups: Group No. 1 (treatments 1–4) with clean air as the fixed source; Group No. 2 (treatments 5–7) with clean rose leaves as the fixed odour source and Group No. 3 (treatments 8–10) with interactive prey and predators.

Treatment group No. 2 (clean rose leaves as the fixed odour source)

This treatment group contained three experiments in which the alternative odour sources (second arm) involved: (1) odours related to rose leaves occupied by spider mites from one arm and clean rose leaf odours from another arm, (2) odours related to rose leaves occupied by thrips and clean rose leaf odours from another arm, (3) odours related to rose leaves occupied by conspecific predators and clean rose leaf odours from another arm (Fig. 3, Group 2).

Treatment group No. 3

This treatment group contained three experiments as follows: in the first experiment, the predatory mites received odours related to spider mite-infested leaves from one arm and thrips-infested leaves from the other arm; in the second experiment the predatory mites received odours related to spider mite-infested leaves from one arm and leaves occupied by conspecific predators from the other arm; in the third experiment, the predators received odours related to leaves infested by thrips from one arm and odours related to leaves occupied by conspecific predators from the other arm (Fig. 3, Group 3).

The period of time in which the predatory mites reached the ends of the lateral arms of the olfactometer (since directed to each of the lateral arms from the junction point) was recorded for each of the treatment groups mentioned above. These periods were compared with each other with General Liner Model (Univariate, Full Factorial) by SPSS 19.

At last, for investigating the effects of starvation and experience on the olfactory response of the predatory mites, value numbers of G_p related to each olfactory test were compared among each other using the General Liner Model (Univariate, Full Factorial) by SPSS 19. G_p is a test for goodness of fit to the 50:50 hypothesis for the pooled data. This value will be compared to a λ^2 distribution with “a-1” degree of freedom when “a” is defined as number of directions.

Oviposition test

Same-aged rose leaves ($2 \times 3 \text{ cm}^2$) were cut, each fixed on 5% agar solution in a Petri dish (6 cm in diameter, 1.5 cm height). Each leaf patch was infested with 25 same-aged female *T. urticae*. We also prepared same-aged ovipositing *P. persimilis* from the rearing units either with or without previous experience with rose plants. The predatory mites were starved for either 5 or 10 hours so that four treatments, each with 30 replicates (predatory mites) were prepared for the experiment as follows: (1) five hour-starved predatory mites with previous experience with rose leaves, (1) five hour-starved predatory mites without previous experience with rose leaves; (3) 10 hour-starved predatory mites with previous experience with rose leaves; and 10 hour-starved predatory mites without previous experience with rose leaves. Each Petri dish was monitored regularly for 24 hours and the numbers of eggs laid were recorded. The total numbers of eggs in each treatment were compared with each other to evaluate the effects of experience and starvation on oviposition rate of the predator through General Liner Model (Univariate, Full Factorial) by SPSS 19.

Results

Olfactory experiments

Predatory mites without previous experience with rose leaves

Treatment group 1. When the predatory mites received clean air from one arm and odours related either to clean rose leaves, rose leaves infested by *T. urticae*, rose leaves infested by thrips, or rose leaves occupied by conspecific predators from the other arm, they significantly preferred the latter choices both with five and 10 hour-starvation ($P < 0.05$) (Table 1).

Treatment group 2. When *P. persimilis* received odours from clean rose leaves from one arm and odours related to *T. urticae*-infested rose leaves from the other arm, no significant preference was observed for predators starved for five hours ($P = 0.13$),

whereas *P. persimilis* with 10 hour-starvation moved significantly towards the arm with *T. urticae*-infested rose leaves ($P < 0.05$). With clean rose leaves as the fixed odour source, the five hour-starved *P. persimilis* showed non-preference behavior when the alternative arms were connected to thrips-infested rose leaves ($P = 0.06$) or to rose leaves occupied by conspecifics ($P = 0.14$); whereas when the searching *P. persimilis* were starved for 10 hours, they significantly moved towards the clean rose leaves in both treatments ($P < 0.05$).

Table 1. Results of replicated experiments of olfactory tests and replicated G test for the olfactory response of *P. persimilis* without previous experience on rose leaves.

Source of odour	5 h starvation						10 h starvation					
	N ₁	N ₂	G _p	P _{GP}	G _h	P _{Gh}	N ₁	N ₂	GP	P _{GP}	G _h	P _{Gh}
1	18	35	5.55	0.01*	0.01	0.99 ^{ns}	14	35	9.29	0.002**	0.04	0.97 ^{ns}
2	12	35	11.75	0.006**	0.36	0.83 ^{ns}	12	36	12.55	0.000**	1.07	0.58 ^{ns}
3	17	33	5.21	0.02*	0.14	0.93 ^{ns}	14	41	13.84	0.000**	0.24	0.88 ^{ns}
4	14	30	5.95	0.01*	0.16	0.91 ^{ns}	12	35	11.75	0.000**	0.23	0.88 ^{ns}
5	22	33	2.21	0.13 ^{ns}	0.48	0.78 ^{ns}	18	37	6.7	0.009**	0.45	0.79 ^{ns}
6	18	31	3.49	0.06 ^{ns}	0.23	0.88 ^{ns}	30	16	4.32	0.03*	0.06	0.97 ^{ns}
7	34	23	2.13	0.14 ^{ns}	0.64	0.72 ^{ns}	34	13	9.72	0.001**	0.47	0.78 ^{ns}
8	32	20	2.79	0.09 ^{ns}	0.03	0.98 ^{ns}	41	16	11.34	0.001**	2.09	0.36 ^{ns}
9	41	17	10.23	0.001**	0.13	0.93 ^{ns}	49	8	32.7	0.000**	0.30	0.85 ^{ns}
10	32	20	2.79	0.09 ^{ns}	1.13	0.56 ^{ns}	36	19	5.34	0.02*	1.14	0.56 ^{ns}

* means significant at 5% level; ** means significant at 1% level; n.s. means not significant

1. Clean air vs. clean leaves; 2. Clean air vs. *T. urticae*-infested leaves; 3. Clean air vs. leaves occupied by *F. occidentalis*; 4. Clean air vs. leaves occupied by *P. persimilis*; 5. Clean leaves vs. *T. urticae*-infested leaves; 6. Clean leaves vs. leaves occupied by *F. occidentalis*; 7. Clean leaves vs. leaves occupied by *P. persimilis*; 8. *T. urticae*-infested leaves vs. leaves occupied by *F. occidentalis*; 9. *T. urticae*-infested leaves vs. leaves occupied by *P. persimilis*; 10. Leaves occupied by *F. occidentalis* vs. leaves occupied by *P. persimilis*.

Treatment group 3. Receiving odours related to *T. urticae*-infested rose leaves from one arm and odours related to leaves occupied by thrips, five hour-starved *P. persimilis* showed no preference ($P = 0.09$) whereas 10 hour-starved ones significantly preferred *T. urticae*-infested leaves ($P < 0.05$). When thrips was replaced by *P. persimilis* as an odour source, both predators with 5 and 10 hour-starvation, preferentially moved towards odours related to *T. urticae*-infested leaves ($P < 0.01$).

When *P. persimilis* received odours related to rose leaves occupied by thrips from one arm and odours related rose leaves occupied by conspecific predators from the other arm, a non-preference behavior was recorded in the *P. persimilis* with five hour-starvation ($P = 0.09$), while the ones with 10 hour-starvation moved significantly towards thrips-occupied leaves ($P < 0.05$) (Table 1).

Predatory mites with previous rearing experience on rose leaves

Treatment group 1. When the predatory mites received clean air from one arm and odours related either to clean rose leaves, leaves infested by *T. urticae*, leaves infested by thrips, or leaves occupied by conspecific predators from the other arm, they significantly preferred the latter choices, both with five and 10 hour-starvations ($P < 0.05$) (Table 2).

Treatment group 2. When *P. persimilis* received odours from clean rose leaves from one arm and odours related to *T. urticae*-infested rose leaves from the other arm, both the predators with five and 10 hour-starvation preferred *T. urticae*-induced plant volatiles significantly ($P < 0.01$). When one of the arms was connected to clean rose leaves and the other one to thrips-occupied rose leaves, five hour-starved *P. persimilis* showed non-preference behavior ($P = 0.25$) while *P. persimilis* with 10 hour-starvation moved significantly towards clean leaves ($P < 0.05$). Replacing thrips by *P. persimilis*, we recorded a significant number of *P. persimilis* moving towards clean rose leaves for both starvation periods ($P < 0.01$).

Treatment group 3. In the olfactory tests in which *T. urticae*-infested rose leaves were considered as the fixed odour source, both five and 10 hour-starved predators preferred odours related to *T. urticae*-infested arms rather than odours related to leaves occupied by thrips or conspecifics ($P < 0.01$). At last, when *P. persimilis* received odours related to leaves occupied by thrips from one arm and odours related to conspecific predators from the other arm, most *P. persimilis* moved towards thrips occupied leaves ($P < 0.01$) in both starvation periods (Table 2).

Table 2. Results of replicated experiments of olfactory tests and replicated G test for the olfactory response of *P. persimilis* with previous experience on rose leaves.

Source of odour	5 h starvation						10 h starvation					
	N ₁	N ₂	G _p	P _{GP}	G _h	P _{Gh}	N ₁	N ₂	GP	P _{GP}	G _h	P _{Gh}
1	14	36	10.01	0.001**	1.06	0.58 ^{ns}	6	46	34.89	0.001**	0.62	0.83 ^{ns}
2	13	40	14.42	0.000**	0.51	0.77 ^{ns}	7	48	34.31	0.000**	0.29	0.86 ^{ns}
3	17	34	5.77	0.016*	0.41	0.81 ^{ns}	8	49	32.78	0.000**	0.19	0.90 ^{ns}
4	15	29	4.53	0.03*	0.71	0.69 ^{ns}	14	39	12.27	0.000**	0.03	0.98 ^{ns}
5	10	46	25.07	0.000**	1.13	0.56 ^{ns}	4	53	50.05	0.000**	0.403	0.81 ^{ns}
6	21	29	1.28	0.25 ^{ns}	0.07	0.96 ^{ns}	46	9	27.22	0.000**	0.47	0.79 ^{ns}
7	46	13	19.56	0.000**	0.92	0.63 ^{ns}	51	4	47.57	0.000**	0.44	0.80 ^{ns}
8	41	11	18.42	0.000**	0.02	0.98 ^{ns}	48	5	40.25	0.000**	1.34	0.51 ^{ns}
9	42	9	23.16	0.000**	0.89	0.64 ^{ns}	56	3	58.07	0.000**	0.001	0.99 ^{ns}
10	37	17	7.58	0.005**	1.74	0.41 ^{ns}	45	6	33.75	0.000**	1.06	0.58 ^{ns}

* means significant at 5% level; ** means significant at 1% level; n.s. means not significant

1. Clean air vs. clean leaves; 2. Clean air vs. *T. urticae*-infested leaves; 3. Clean air vs. leaves occupied by *F. occidentalis*; 4. Clean air vs. leaves occupied by *P. persimilis*; 5. Clean leaves vs. *T. urticae*-infested leaves; 6. Clean leaves vs. leaves occupied by *F. occidentalis*; 7. Clean leaves vs. leaves occupied by *P. persimilis*; 8. *T. urticae*-infested leaves vs. leaves occupied by *F. occidentalis*; 9. *T. urticae*-infested leaves vs. leaves occupied by *P. persimilis*; 10. Leaves occupied by *F. occidentalis* vs. leaves occupied by *P. persimilis*.

Discussion

Treatment group 1

When *P. persimilis* received clean air from one arm as a fixed odour source, it significantly moved towards the alternative arms both with 5 and 10 hours starvation and regardless of being experienced with the rose plants or not. The predator's attraction towards clean rose leaves is consistent with Maleknia *et al.* (unpublished data) who demonstrated that clean detached rose leaves themselves could be attractive for *P. persimilis* and Zhong *et al.* (2011) who reported that *Neoseiulus cucumeris* (Oudemans) preferred the odours of undamaged eggplant, pepper and tomato rather than clean air. This results contrast, however with the finding by Fadini *et al.* (2010) who reported that *Phytoseiulus macropilis* (Banks) preferred clean air to uninfested strawberry plants. Here, as this was true both for experienced and unexperienced predators, it could be concluded that some odour components might be common to clean rose and bean (their previous host plant) leaves and which allowed the predator to detect the clean rose leaves even without previous experience, and to correlate the leaf odours to a more appropriate patch rather than an empty patch (clean air). Several studies have demonstrated the qualitative and quantitative differences among odours related to herbivore infested heterospecific plants (Shimoda & Dicke 2000) but none of them studied the probable volatile component similarity between non-infested ones, therefore our hypothesis needs more investigations.

In the experiments where *P. persimilis* received odours related to *T. urticae*-infested rose leaves, neither starvation time nor previous experience affected the predator's olfactory response. For *P. persimilis* females with previous experience on rose leaves, attraction towards *T. urticae*-infested leaves was similar to that observed by Drukker *et al.* (2000) who noted that foraging predators could associate chemical cues with the presence of their prey and demonstrated the ability of *P. persimilis* to learn the association of *T. urticae* with a chemical cue (Herbivore Induced Plant Volatile: HIPV). Takabayashi & Dicke (1996) and Krips *et al.* (1999) also reported that rearing the predatory mite, *P. persimilis* on spider mite infested host plants induced a preference for these plant odours. Prolonged feeding of predators in the presence of HIPV blends also lead to olfactory preference for these HIPVs and help dispersing predatory mites to detect infested plants that they are already familiar with (Krips *et al.* 1999; van Wijk *et al.* 2008). Our interpretation for the attraction of naïve predators towards infested rose leaves is that the same constituents are certainly released by several plant species irrespective to their taxonomic characteristics (McCormick *et al.* 2012). Van Wijk *et al.* (2008) demonstrated that predatory mites could generalize their attraction behavior from previously experienced odours to similar odours that they have not experienced. De Boer & Dicke (2004 a, b) observed that *P. persimilis* (either satiated or starved) was attracted towards methyl-salicylate if the mites had previously experienced a methyl-salicylate containing odour blend, thereby showing that olfaction in *P. persimilis* could be elemental.

When *P. persimilis* females received clean air from one arm and odours related to thrips/*P. persimilis*-occupied rose leaves from the other arm, a significant number of predators moved towards the latter arm regardless of being experienced. The starvation time also did not affect the predator's olfactory response. *Frankliniella occidentalis* is considered as a heterospecific competitor for *P. persimilis* (Maleknia *et al.* 2012). Studies have also demonstrated that when a phytoseiid predator and its heterospecific competitor are present together, the former should adopt strategies in order to decrease

the predation risk from its competitor (Janssen *et al.* 2003; Magalhaes *et al.* 2005). Here, our explanation is that there was no spider mite present on the patches so that neither thrips nor the heterospecific predators could elicit production of alarm pheromones to aware the searching *P. persimilis* about the present risk (Janssen *et al.* 1999). On the other hand rose leaves' odours (discussed above) received by the searching *P. persimilis* were more attractive than clean air (alternative arm), therefore the female predators mostly moved towards the thrips/*P. persimilis* occupied patches.

Treatment group 2

In the olfactory experiments where *P. persimilis* females received odours related to clean rose leaves from one arm (fixed odour source) and odours related to *T. urticae*-infested leaves from another arm, the unexperienced predators with 5 hour-starvation showed no preference towards the arms while the ones with 10 hour-starvation moved significantly towards the *T. urticae*-infested leaves. It seems that for predators that had not experienced rose leaves previously, starvation time played an important role in the predator foraging behavior. Shimoda *et al.* (2000), previously observed that starvation time could prompt *P. persimilis* to prefer lima bean leaves infested with the non-prey caterpillars rather than their preferred prey (*T. urticae*), thereby contrasting with the results of the present research, although the mean time they kept *P. persimilis* starved for was ≈ 5 fold longer than that in our research. De Boer *et al.* (2004 a, b) argued that starvation level did not affect the responses of *P. persimilis* to MeSa. Also the experienced *P. persimilis* females in this study, preferred *T. urticae*-infested leaves regardless of the starvation time length. It seems that when the predators are examined on the same host plant on which they were previously reared, starvation time did not significantly affect their foraging behavior. Sznajder *et al.* (2010) compared the olfactory responses of 18 lines of *P. persimilis* to MeSa and reported that there was more variation, ranging from avoidance to preference, among the lines when the predators were starved although variation was also present in satiated mites.

In the olfactory experiments where *P. persimilis* females received odours related to clean rose leaves from one arm (fixed odour source) and odours related to rose leaves occupied by thrips from the other arm, experienced and naïve *P. persimilis* females which were kept starved for 5 hours did not make a significant choice between the arms, while when the starvation time increased to 10 hours, they significantly moved towards the clean leaves regardless of being naïve or experienced. Our interpretation is that in the latter case, *P. persimilis* should have recognized *F. occidentalis* as a competitor feeding on *T. urticae*, but one would wonder how this recognition happened as the predatory mites had not experienced odours related to thrips before. Magalhaes *et al.* (2005) had previously documented that the diet of intraguild predator could affect the behavior of the intraguild prey. However, investigations on the cues that may alert the intraguild predator about presence of its intraguild prey (here, competitor) is still lacking. Here, it is possible that *P. persimilis* received some information about the thrips' previous diet (*T. urticae*) so that it avoided the patch in which they (*F. occidentalis* and *P. persimilis*) would compete on a shared prey (*T. urticae*) and moved towards the clean leaves. It seems that keeping predators starved for 10 hours was triggering enough for both naïve and experienced *P. persimilis* to make them avoid the leaves occupied by thrips.

When *P. persimilis* received odours related to clean rose leaves from one arm and the odours related to leaves occupied by conspecifics from another arm, the five hour-

starved naïve predators made no choice between the arms (not starved enough to make them prefer one of the arms), while the 10 hour-starved ones and also the experienced predators both with 5 and 10 hour-starvation times moved significantly towards the clean leaves. This result is contrary with Janssen *et al.* (1997) who reported that odours produced by predatory mites (present upwind from a prey patch) were attractive for conspecifics. The difference between our experiment and that by Janssen *et al.* (1997) is that in the present research, there was no prey (*T. urticae*) present in the patches (odour sources), so that the olfactory response of searching *P. persimilis* was due to the odours related to conspecifics themselves. Several studies have demonstrated different responses of predatory mites to their conspecifics. According to Janssen *et al.* (1997) the odours related to conspecifics themselves should attract the searching *P. persimilis* females and would not make them move towards the clean leaves; which was opposite with our observations. The difference between Janssen *et al.* (1997) and our results might be due to the length of the starvation period prior to olfactory experiments (1 hour in Janssen *et al.* (1997) and 5 and 10 hours in our experiments, and the host plant species (Lima bean in Janssen *et al.* (1997) and rose in ours).

Treatment group 3

In the olfactory experiments in which *P. persimilis* females received odours related to *T. urticae*-infested rose leaves from one arm and odours related to leaves occupied by *F. occidentalis* from the other arm, the naïve predators which were kept starved for 5 hours did not make a significant preference between the arms (Our previous experiments in treatment group 1 also showed that when the searching *P. persimilis* were naïve and were kept starved for only 5 hours, they could not recognize *T. urticae* induced rose leaf volatiles), while the ones with 10 hour-starvation and the experienced predators with both 5 and 10 hour-starvation, preferred the odours related to *T. urticae*-infested leaves. Our interpretation for the latter observation is that either the odours related to *T. urticae*-infested leaves were attractive (Sabelis & van de Baan 1983; Vet & Dicke 1992; Janssen 1999), or odours related to thrips previous diet, *T. urticae*, (as discussed in treatment group 2) were alarming, leading to avoidance by *P. persimilis* implying that they were attracted towards *T. urticae* infested leaves.

When *P. persimilis* females received odours related to *T. urticae*-infested rose leaves from one arm and odours related to leaves occupied by conspecifics from the other arm, a significant number of predators moved towards *T. urticae*-infested leaves regardless of experience and starvation time. This is comparable with results from treatment group 2 when the predators received odours related to clean leaves vs leaves occupied by conspecifics. By replacing clean leaves by *T. urticae*-infested ones (a more attractive odour source) in the latter experiment, such an olfactory response from the searching *P. persimilis* was expectable. The *T. urticae*-infested rose leaves were so attractive that neither previous experience on rose leaves nor the length of starvation time affected the predator's olfactory responses.

Naïve *P. persimilis* starved for 5 hours did not show preference between odours from leaves with thrips or with conspecifics, while those with 10 hour-starvation and the experienced *P. persimilis* with both 5 and 10 hour-starvation significantly moved towards the leaves occupied by thrips. Again the odours related to conspecifics were so repelling that the searching *P. persimilis* preferred to move towards thrips although our previous experiments in treatment group 2 showed that when the starvation time increased to 10 hours, the predators avoided the leaves occupied by thrips. As discussed

before, this is in contrast with Janssen *et al.* (1997) and the difference might be due to either the host plant species or the length of starvation time. Our results are in support to Zahedi-Golpayegani *et al.* (2007) who reported similar avoidance behavior in *Zetzellia mali* (Ewing) when it received conspecific's odours alone.

The average speed of predatory mites in our study was 0.13 cm/s for the ones without previous experience and 0.16 cm/s for the experienced ones which were both lower than the 0.22 cm/s (n= 58) reported by Zemek *et al.* (2008). As these authors did not use a wind flow to allow the predators to follow the concentration gradient, it remained unclear why the predator speed recorded in our study was lower. In their study, Zemek *et al.* (2008) argued that other factors such as uneven temperature distribution could interfere with the predator's movements. (Zemek *et al.* 2008)

In conclusion, our study demonstrated that experience and starvation could both affect the predator's foraging behavior. Zhang & Sanderson (1992), studied the influence of predator's experience on foraging responses of *P. persimilis* on rose and bean leaves and observed that female predators born and reared on a given plant species (bean or rose) expressed no preference for each of the plants when exposed to odour of the other plant. They concluded that experience on different host plant could affect the foraging behavior of *P. persimilis* on a given plant species. Our results contrast, however, with the findings by (Xiao & Fadamiro 2010) who reported that starvation period did not affect the predation rate of three phytoseiid species, i.e. *Phytoseiulus persimilis*, *Neoseiulus californicus* and *Galendromus occidentalis* (Nesbitt) after a starvation periods of 24 and 48 hours. Our results are also in contrast with Blackwood *et al.* (2001) who demonstrated that starvation time had no effect on prey preference in 13 species of phytoseiid mite. However, field studies are needed to confirm our laboratory results and to evaluate the predation potential of *P. persimilis* in a broader range of food deprivation and with previous experience with more host plant species. It is also worthy to investigate the impact of starvation on the predator's fecundity to determine whether starvation could subsequently increase oviposition through an increase of the prey consumption rate.

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پاسخ بویایی کنه شکارگر (*Phytoseiulus persimilis* (Acari: Phytoseiidae) به

برگ‌های گیاه رز: تأثیر طول مدت گرسنگی و تجربه پیشین روی گیاه میزبان

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چکیده

در این پژوهش تأثیر تجربه پیشین بر گیاه میزبان و همین‌طور دوره‌های متفاوت گرسنگی بر واکنش بویایی کنه شکارگر *Phytoseiulus persimilis* روی برگ‌های گیاه رز در شرایط آزمایشگاه بررسی شد. هنگامی که هوای پاک به عنوان بازوی ثابت الفکتومتر در نظر گرفته شد، کنه *P. persimilis* صرف‌نظر از داشتن تجربه و طول مدت گرسنگی، گرایش معنی‌داری به سمت مواد فرار بویایی بازوهای متقابل از جمله برگ‌های سالم، برگ‌های آلوده به کنه تارتن دو لکه‌ای، برگ‌های آلوده به تریپس *Frankliniella occidentalis* و همین‌طور برگ‌های حاوی شکارگرهای هم‌گونه نشان داد. هنگامی که منبع هوای پاک با برگ‌های سالم گیاه رز جایگزین شد، شکارگرهای بی‌تجربه‌ای که ۱۰ ساعت گرسنه مانده بودند و همین‌طور شکارگرهای باتجربه با پنج و ۱۰ ساعت گرسنگی، گرایش معنی‌داری به سمت برگ‌های آلوده به کنه تارتن نشان دادند. هنگامی که کنه‌های شکارگر در برابر برگ‌های سالم از یک طرف و برگ‌های آلوده به تریپس از طرف دیگر قرار گرفتند، هیچ یک از کنه‌های باتجربه یا بی‌تجربه‌ای که پنج ساعت گرسنه مانده بودند، گرایش معنی‌داری به سمت بازوها نشان ندادند درحالی‌که کنه‌های شکارگری که ۱۰ ساعت گرسنه مانده بودند، صرف‌نظر از تجربه

پیشین، به‌طور معنی‌داری به سمت هوای پاک حرکت کردند. کنه‌های شکارگر بی‌تجربه *P. persimilis* با پنج ساعت گرسنگی و کنه‌های بی‌تجربه و باتجربه با ۱۰ ساعت گرسنگی، با دریافت مواد فرار بویایی مربوط به هم‌گونه‌ای‌های خود در مقابل مواد فرار بویایی مربوط به برگ‌های سالم، از هم‌گونه‌ای‌ها دوری کردند. شکارگرهای باتجربه‌ای که پنج ساعت گرسنگی دیده بودند و همین‌طور شکارگرهای بی‌تجربه و باتجربه‌ای که ۱۰ ساعت گرسنگی دیده بودند، هنگامی که از یک طرف مواد فرار بویایی مربوط به برگ‌های آلوده به کنه تارتن دو لکه‌ای و از طرف دیگر مواد فرار بویایی مربوط به برگ‌های آلوده به تریپس را دریافت کردند، به مقدار زیادی به سمت برگ‌های آلوده به کنه تارتن حرکت کردند. با دریافت مواد فرار متعلق به برگ‌های آلوده به کنه تارتن از یک سو و مواد فرار برگ‌های حاوی هم‌گونه‌ای‌ها از سوی دیگر، بیشتر شکارگرها صرف‌نظر از دوره گرسنگی و داشتن یا نداشتن تجربه، به سمت برگ‌های آلوده به کنه‌های تارتن حرکت کردند. شکارگرهای بی‌تجربه و باتجربه با ۱۰ ساعت گرسنگی و همین‌طور شکارگرهای باتجربه با پنج ساعت گرسنگی، با دریافت مواد فرار متعلق به برگ‌های آلوده به کنه‌های تارتن از یک سو و مواد فرار متعلق به برگ‌های آلوده به تریپس از سوی دیگر، به‌طور معنی‌داری به سمت بازوی اول حرکت کردند. صرف‌نظر از دوره گرسنگی کنه‌های شکارگر، تجربه پیشین آن‌ها تأثیر معنی‌داری بر زمان لازم برای عبورشان از بازوهای جانبی بوسنج نداشت. هم دوره گرسنگی و هم تجربه پیشین، بر میزان تخم‌گذاری کنه شکارگر مؤثر بود.

واژگان کلیدی: تجربه، منبع بو، رفتار کاوشگری، Phytoseiidae، مواد فرار.

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