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HOW DOES *PHYTOSEIULUS PERSIMILIS* FIND ITS PREY WHEN FORAGING WITHIN A BEAN PLANT?

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Abstract

The role of herbivore-induced volatile substances in prey-finding by phytoseiid mites has been repeatedly documented using an olfactometer. The objective of the present paper is to test the hypothesis that movement by *Phytoseiulus persimilis* is affected by these volatiles even on plants. Two series of laboratory experiments were carried out. In the first series we studied searching behavior of *P. persimilis* females on young bean plants in which a single leaf was infested with spider mites. The effect of spider mite colony location on the walking pattern of predatory mites while on a leaf was studied in the second series of experiments. We found that *P. persimilis* individuals were unable to discriminate between infested and uninfested leaves when they walked up the stem of a bean plant. On the other hand, results of the second series of experiments indicate that walk was not random once a predator was on the leaf surface since it was attracted to the spider mite patch, at least over a distance of 1 cm. These results thus demonstrate that herbivore-induced volatiles can be utilized by *P. persimilis* during search for prey also under conditions that mimic natural situations better than an olfactometer does.

Keywords

Acari, Phytoseiidae, Tetranychidae, herbivore-induced volatiles, prey location

Introduction

The role of volatile chemicals emitted from infested leaves and classified as herbivore-induced synomones in finding prey by phytoseiid mites was repeatedly documented using an olfactometer (Sabelis & van de Baan 1983, Dong & Chant 1986, Dicke *et al.* 1993). Under such test conditions predators are provided with a relatively constant and highly directional flow of kairomones and thus locating the volatiles source seems to be easier compared to real situations in the field. Results of our earlier experiments (Zemek & Nachman 1999) which better mimicked natural conditions revealed that *P. persimilis* does not disperse randomly to

the surrounding plants in a greenhouse but predominantly moves in the direction of nearby plants infested with two-spotted spider mites, *Tetranychus urticae* Koch. Janssen (1999) also tested whether *P. persimilis* locates spider mite-infested cucumber plants in greenhouse release experiments. His results confirmed that predatory mites released in the center of a hexagon of cucumber plants were indeed guided to the prey infested plants by herbivore-induced plant odors.

The objective of the present paper is to extend the previous studies by testing the hypothesis that predatory mites can use volatile chemicals emitted from infested leaves to localize spider mite

colonies within a plant. The results should contribute to our knowledge about the actual role of volatiles in finding prey by *P. persimilis*.

Materials and Methods

1 Plants and Mites

Lima beans, *P. vulgaris*, var. Katka were used for experiments as well as for rearing of spider mites. The plants were grown in a greenhouse and provided with artificial light when necessary to ensure long day photoperiod (at least 16L:8D). The plants were grown in plastic pots with 10.5 cm top diameter, 8.5 cm bottom diameter and 8 cm height filled with a sphagnum based growth medium. A single seed was sown in each pot to avoid competition for nutrients, space and light. No fertilizers and no pesticides were applied. The plants used in experiments were approximately 2-3 weeks old. Leaves applied for experiments were taken from the second node of the plants.

The culture of two-spotted spider mites originated from a local population collected in České Budějovice, Czech Republic, and have been reared at the Institute of Entomology for several years using beans as host plants. The culture of predatory mite *P. persimilis* was established from mites provided by Biocontrol Vodany, Czech Republic. Predatory mites were reared in a laboratory at temperature 22-24°C with long day photoperiod (18L:6D). They were kept in plastic trays (50x40x6 cm) within which a plastic platform was placed in the center surrounded by water. The water prevented the predatory mites inhabiting the platform from escaping and served to maintain a high humidity necessary for their successful development. The predators were supplied with spider mites via heavily infested detached bean leaves.

2 Design of experiments

Two series of laboratory experiments were carried out. In the first series we studied the searching behavior of *P. persimilis* females on a young bean plant in which one leaf was infested with spider mites.

The plants used were at the stage of the first two leaf triplets. One leaf triplet was infested by at least 40 adult *T. urticae* females and both petioles were treated with petroleum jelly to prevent migration of spider mites from the infested to the uninfested leaf. The plants were used for an experiment when the leaf damage index of the infested leaf reached approximately 3 according to the scale by Hussey & Parr (1963).

The observation of *P. persimilis* behavior was conducted in a metal box (90x60x60 cm) painted black inside and equipped with two fluorescent tubes fixed to the ceiling. A round mirror was placed behind the plant to allow for observations of predatory mites when they walked on back side of the plant. An experiment started by introduction of a single, one-day starved *P. persimilis* female on the plant about 10 cm below the bifurcation point of the petioles. Its behavior was then observed until the mite entered one of the petioles. Each plant and predatory mite were used only once. The position of infested and uninfested leaves in the box alternated to eliminate any potential factors affecting the direction of the predator's walk. The effect of the presence of a spider mite colony on the decision of *P. persimilis* to enter particular petiole was analyzed by a sign test (Siegel & Castellan 1988).

In the second series of experiments we studied the effect of spider mite colony location on the walking pattern of predatory mites while on a leaf. The observation arena was made up by a single bean leaf placed upside down on filter paper which in turn was placed on a water-saturated sponge in a Petri dish. Prey colony was established by introducing 40 adult females of *T. urticae* at one half of the leaf and isolated by covering them with a glass vial with an inner diameter of 2 cm. The leaf was kept for three days at 25°C and a photoperiod of 18L:6D prior to an experiment. After that period, the vial was removed and a thin black entomological pin was inserted at distance of 1 cm from the colony border so that it was perpendicular to the leaf surface. The dish with the leaf was then placed into a climate cabinet (25°C) equipped with a circular fluorescent tube placed horizontally in the center of the cabinet to ensure an even illumination of the observation arena. After 15 minutes which was expected to allow diffusion of kairomones into the surroundings, a single, one-day starved *P. persimilis* female was released on top of the pin. Each leaf and predatory mite were used only once.

The walking path of the mite was recorded by means of the computerized video tracking system EthoVision (Noldus Information Technology 1997). Data acquisition was started manually when the mite moved down, left the pin and started to walk on the leaf surface and finished automatically when the mite left a circular zone with a diameter of 2 cm. A mean heading direction was used as a parameter determining if predator movement was random or directed towards the prey colony. Although light was supposed to have a uniform intensity in all directions thanks to the circular

tube, the position of the prey colony was switched so that it in half of the experiments was oriented towards the back of the cabinet and in the other half towards the cabinet's door.

The obtained data were analyzed by means of circular statistics (Batschelet 1981). First, the mean vector was calculated and then Rayleigh test was applied to test if direction of walk of *P. persimilis* was random or not. Bi-modality of data was tested using the method described by Fisher (1993). A sign test (Siegel & Castellan 1988) was used to reveal whether significantly more mites walked in direction of the prey patch.

Results and Discussion

1 Searching for prey-infested leaves on a plant

The predators either immediately climbed up the plant stem after their release or they walked down first and then soon turned back. In a few cases predatory mites left the plant and entered the soil substrate. Such experiments were omitted from the analysis. In 16 cases out of 30 individual experiments *P. persimilis* walked to the petiole of the infested leaf. The preference was not statistically significant ($\chi_1^2=0.133$, $P=0.715$).

Thus, the results indicate that *P. persimilis* walking on the stem of a bean plant was unable to discriminate between infested and uninfested leaves. The reason might be that the concentration gradient of volatiles was not high enough as uninfested leaves of the spider mite-infested plant also produce predator-attracting infochemicals (Dicke *et al.* 1993). Moreover, the shape of the odor plum does not need to reflect plant morphology and/or could also be affected by the manipulation close to the plant when releasing a predatory mite. Another reason could be that a predator, when moving up the stem had relatively short time to perceive differences in odor concentration at the point of stem bifurcation. The conclusion is that we did not find any evidence of *P. persimilis* being capable of utilizing plant volatiles to improve its likelihood of discovering spider mite infested leaves within a plant.

2 Searching for a prey colony while on a leaf

The average speed of predatory mites was 0.22 cm/s ($n=58$). In most experiments predators usually left the observation zone within a short time as they moved more or less straight. In experiments in which a spider mite colony was turned to the back of the cabinet, the walking pattern of the predatory mites was significantly non-random and oriented towards areas infested

with spider mites (Tab. 1, 2). Contrary to this, some predators walked to, and some of them away from spider mite colony in experiments where the spider mite colony was oriented closer to cabinet door resulting in a statistically non-significant mean vector (Tab. 1). Since the latter data indicate that the predators have a symmetric bimodal distribution, we tested the bi-modality by subtracting 180° from data points lying in the arc (180°, 360°), then doubling all the angles and finally calculating the mean vector. The mean vector of the derived data turned out to be statistically significant (Tab. 1), giving an evidence of bi-modality and suggesting that the mean direction of predatory mites is along a 0°-180° axis, i.e. the mites moved both towards and away from the colony.

Table 1. Results of circular analysis of directions in which females of *P. persimilis* moved on the leaf arena.

Position	Φ^a	r^b	Φ^c	v^d	n^e	P^f
door	180	0.158	16.365	-0.151	29	0.468
back	0	0.396	28.776	0.347	29	0.007
door ^b	0	0.324	-6.51	0.322	29	0.045

^aThe angle in which the prey colony was located; ^bThe length of the mean vector; ^cMean angle; ^dThe component of the mean vector with respect to the position of a prey colony; ^eNumber of experiments; ^fSignificance - Rayleigh test; ^bData derived by reversing the angles between 180° and 360°, and doubling all measurements to convert them to vectors.

The sign test revealed that significantly more mites walked in the direction of the prey patch in experiments where the patch turned to the back of the cabinet but not in the experiments where it turned to the door (Tab. 2).

Table 2. Comparison of numbers of *P. persimilis* females walked in direction either towards or away from prey patch.

Position	Towards	Away from	P^a
door	10	19	0.136
back	22	7	0.008
door ^b	22	7	0.008

^aSign test; ^bData derived by reversing the angles between 180° and 360°, and doubling all measurements to convert them to vectors.

The bioassay used in this series of experiments was much less sensitive than an olfactometer for detecting predator responses to volatile kairomones, but it better mimicked natural conditions. According to Zhang & Sanderson (1992), the odor from a single spider mite colony

may not be strong enough to stimulate a significant response as a large number of spider mite-infested leaves is required for a significant response, even in an olfactometer (Sabelis & van de Baan 1983). Despite of that, our results indicate that the search path of *P. persimilis* once on a leaf was influenced by the location of spider mite colony. There might be two mechanisms underlying predator behavior: (1) predators either utilize volatile kairomones which are produced only at prey patch and then diffused into the air or (2) kairomones are produced by the whole leaf (Dicke *et al.* 1993) but their production decreases with the distance from the patch allowing the predators to follow the concentration gradient. Nevertheless, any air turbulences or wind might strongly affect such a concentration gradient. The results further show that some other factors like e.g. the reflection of the copper wall in the climate cabinet or an uneven temperature distribution may interfere with the effects of volatiles.

We can conclude that the spider mite-induced volatiles affect the searching pattern of predatory mites once they are on the leaf, at least over a distance of 1 cm. This paper thus demonstrates that *P. persimilis* at least to some extent is able to utilize prey-associated volatiles when searching for prey within a bean plant under relatively realistic conditions and not only in the rather artificial situation when studied in an olfactometer.

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