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State-dependent and odour-mediated anemotactic responses of the predatory mite *Phytoseiulus persimilis* in a wind tunnel

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Abstract. Anemotaxis in the predatory mite *Phytoseiulus persimilis* (both well-fed and starved), has previously been studied on a wire grid under slight turbulent airflow conditions yielding weak, yet distinct, gradients in wind speed and odour concentration (Sabelis and Van der Weel 1993). Such conditions might have critically influenced the outcome of the study. We repeated these experiments, under laminar airflow conditions on a flat surface in a wind tunnel, thereby avoiding variation in wind speed and odour concentration. Treatments for starved and well-fed mites were (1) still-air without herbivore-induced plant volatiles (HIPV) (well-fed mites only), (2) an HIPV-free air stream, and (3) an air stream with HIPV (originating from Lima bean plants infested by two-spotted spider mites, *Tetranychus urticae*). Well-fed mites oriented in random directions in still-air without HIPV. In an air stream, starved mites always oriented upwind, whether plant odours were present or not. Well-fed mites oriented downwind in an HIPV-free air stream, but in random directions in an air stream with HIPV. Only under the last treatment our results differed from those of Sabelis and Van der Weel (1993).

Introduction

Predatory mites, such as *Phytoseiulus persimilis* Athias-Henriot, disperse passively with the wind, to displace themselves over long distances. In this phase they have no opportunity for orientation towards prey, but after landing their search for prey starts (Sabelis and Dicke 1985; Charles and White 1988; Sabelis and Afman 1994). This ambulatory search phase can be divided into three sub-phases: (1) search for spider mite patches near the landing site, (2) search for spider mite colonies in the patch, and (3) search for spider mites within a colony (Sabelis and Dicke 1985). In each of these three phases, *P. persimilis* may exploit herbivore-induced plant volatiles (HIPV; Dicke et al. 1990a, b) to increase the likelihood of finding spider mites, but the orientation mechanisms used after perception of HIPV are not yet fully understood.

One orientation mechanism is the ability to orient upon gradients in the concentration of HIPV. Both well-fed and starved predatory mites showed a chemotactic response to steep gradients of HIPV and this may help them to stay in the odour plume (Sabelis et al. 1984). However, this is only effective close to the odour

source where odour gradients are relatively static. The lack of ability to track moving odour gradients was demonstrated in a vertical airflow olfactometer. Here, the airflow was perpendicular to a fine mesh gauze serving as a substrate for mite movement and the mite's response was assessed towards the odour coming from an HIPV-emitting cylinder that was moved underneath the gauze in the same or in the opposite direction of mite displacement. In this way, the mite's response was studied towards a moving odour gradient in absence of an airflow yielding directional cues. The predatory mite showed right-about turns in all experiments, that is, irrespective of the direction of gradient displacement (Sabelis et al. 1984). Hence, orientation to odour gradients alone is only effective close to the odour source and there must be other cues that help the mite to orient towards the odour source from longer distances.

This has led to experiments aiming to assess the role of wind as a directional cue (Sabelis and Van der Weel 1993). On a wire grid positioned downwind of a row of wind fans, well-fed female predators moved upwind in the presence of HIPV, but downwind in absence of these odours. Starved predators moved upwind whether HIPV was present or not (Sabelis and Van der Weel 1993). The method used in these experiments needs scrutiny. First, the airflow showed a weak unidirectional gradient of HIPV concentrations increasing towards the source. Thus, either upwind or downwind movement may have been influenced by differences in wind speed and odour concentration. Second, the wire grid that served as a substrate, had cross-junctions constructed in such a way that orientation to wire-edges would cause the predators to move up- or downwind with higher probability, than to move crosswind (Sabelis and Van der Weel 1993). On a plant, *P. persimilis* is guided by stems, veins and leaf rims (Sabelis 1981; Sabelis and Dicke 1985) and it is possible that such edge structures were essential for the anemotactic responses observed by Sabelis and Van der Weel (1993).

In this article, we present the results of experiments, similar to those of Sabelis and Van der Weel (1993), after modifying their set-up: (1) by using a flat substrate, instead of a wire grid, and (2) by using a constant laminar airflow in a wind tunnel, thereby avoiding (especially longitudinal) gradients in wind speed and odour concentration altogether. Using this set-up, we test whether *P. persimilis* shows odour-mediated and feeding state-dependent anemotactic responses in the absence of edged structures and under constant laminar airflow conditions. We test well-fed and starved adult *P. persimilis* females for its orientation behaviour in (1) still-air without HIPV (well-fed mites only), (2) an HIPV-free air stream, and (3) an air stream with HIPV (originating from Lima bean plants infested with two-spotted spider mites, *Tetranychus urticae* Koch).

Material and methods

Predator and prey

The predatory mites, *P. persimilis*, were reared on detached Lima bean (*Phaseolus lunatus*) leaves infested with two-spotted spider mite (*T. urticae*) colonies on water-

soaked clay pots in a climate room at 25 °C and 60% RH. As soon as the predatory mites reached the stage of first egg deposition, they were transferred to petri-dishes with detached Lima bean leaves infested with *T. urticae* colonies on moisturized cotton wool. They were stored in a climate room at 5 °C for 7–14 days. We only used adult female *P. persimilis* for our experiments, all in the oviposition phase and 4–8 days old since their final moult.

Either starved mites or well-fed mites were used. Starved mites were obtained by transferring female mites from the rearing unit individually into a gel capsule (6 mm × 10 mm), where they were kept without food for 18–22 h at 23 °C and 60% RH. Well-fed mites were obtained by transferring females from the rearing unit into a petri-dish, where they were kept for 18–22 h on infested Lima bean leaves with ample supply of prey at 23 °C and 60% RH.

Wind tunnel

The wind tunnel consisted of two equal-sized segments (1 m × 0.75 m × 0.51 m) connected to each other, the first of which had six wind fans (three rows of two fans; 20 W ITHO desk model) covering the open side of the tunnel, and a table for plants positioned directly downwind, whereas the second segment contained the substrate for the mites. The first segment was separated from the second by nylon netting (mesh width 0.7 mm, thread width 0.2 mm) resulting in a slight over-pressure in this segment of the wind tunnel. Fluctuations in wind speed are now spread out by the nylon netting, reducing variation in wind speed. The second segment of the wind tunnel contained a glass plate floor positioned so as to receive the odour emanating from the plants (i.e., somewhat higher than the plant table in the first segment). A constant light intensity, temperature (23 °C) and relative air humidity (80%) was applied. The wind speed monitored at 5 mm above the substrate, using a hot wire anemometer, was 0.25 ± 0.01 m/s. The glass plate was provided with a reference grid drawn with a marker pen on the underside. This reference grid facilitated drawing of the walking paths on graph paper.

The use of a glass plate (0.95 m × 0.51 m × 0.4 m) as a substrate, instead of a wire grid is another modification to the set-up used by Sabelis and Van der Weel (1993). This was done to exclude the possibility of thigmotactic responses of *P. persimilis* along the wires of the grid, as explained in the Introduction section.

HIPV blend

As an odour source we used Lima bean plants in the 2–6 leaf stage, infested with *T. urticae*. We placed 2–3 bean plants per pot, with a total leaf area of 250–300 cm². Each plant was infested with 80–125 adult *T. urticae* females plus eggs and other developmental stages. For treatments with HIPV, a total of 18 pots

were placed on the table in front of the fans in the first segment of the wind tunnel.

Test series

Five series of experiments, each consisting of 20 replicates, were conducted according to the following scheme: (1) well-fed mites in an HIPV-free and still-air environment, (2) well-fed mites in an HIPV-free air stream, (3) well-fed mites in an air stream with HIPV, (4) starved mites in an HIPV-free air stream, and (5) starved mites in an air stream with HIPV. After releasing a mite from the gel capsule (starved mites) or a fine brush (well-fed mites) at the centre of the glass plate (0,0), the walking path was recorded for 20 min or until the mite reached any of the borders of the arena in the wind tunnel. Path records were obtained by drawing observed paths on a copy of the reference grid on paper (paper : substrate size = 1 : 5).

Parameters and statistical analysis

On each path record we projected a unit circle with a diameter of 45 cm and its centre (0,0) set equal to the mite's release point. For each mite we determined a path vector (R_i) from the start coordinates (0,0) to the final coordinates (x_i, y_i) on the circle (the first cross-section of the walk path with the unit circle). The resultant vector (R_n) from n replicates can be calculated as: $R_n = \sqrt{X^2 + Y^2}$ with $X = \sum_{i=1}^n x_i$ and $Y = \sum_{i=1}^n y_i$. The mean vector length ($r = R_n / n$) is an indicator for directedness within a treatment. The circular mean direction (Φ) is defined by the direction of the resultant vector and is calculated as:

$$\Phi = \begin{cases} \arctan(\frac{Y}{X}) & \text{if } X > 0, Y \geq 0, \\ 90^\circ & \text{if } X = 0, Y > 0, \\ \arctan(\frac{Y}{X}) + 180^\circ & \text{if } X < 0, \\ \arctan(\frac{Y}{X}) + 360^\circ & \text{if } X \geq 0, Y < 0. \end{cases}$$

For more detailed information on circular statistics, see Batschelet (1981), Fisher (1993), and Rao and SenGupta (2001).

For treatment 1 we use a Raleigh's circular uniformity test under the alternative of non-uniformity, that is, no specified mean direction. For those treatments where we hypothesize a specified direction (Φ_A) as the alternative to uniformity (downwind, i.e., $\Phi_A = 180^\circ$, for treatment 2; and upwind, i.e., $\Phi_A = 0^\circ$, for treatments 3–5) we used the V -test. The Watson's F -test is used to determine if the mean angles between the series differ significantly from each other in a pair-wise fashion. This test was only performed between those treatments that showed a significant mean direction. Our datasets were analysed using the CircStats package for circular analysis (Rao and SenGupta 2001) using R statistical software (Ihaka and Gentleman 1996) and Oriana[©] software for circular analysis (Oriana 1999). Levels of significance in all statistical tests were set at $\alpha = 0.05$.

Figure 1. Circular representation of the distribution of the mean directions of walks of *well-fed* (B and C) and *starved* (D and E) *P. persimilis* females ($n = 20$) in a wind tunnel on a glass plate under laminar airflow conditions. Stimulus conditions for A: HIPV-free in still-air, B and D: an HIPV-free air stream, and C and E: an air stream with HIPV.

Results

In a still-air environment without HIPV, well-fed mites did not show a significant deviation from a uniform distribution of directions (Figure 1A, Raleigh's uniformity test: $p = 0.46$, $r = 0.20$). Well-fed mites oriented downwind when an HIPV-free air stream was offered (Figure 1B, V-test for uniformity with $\Phi_A = 180^\circ$: $p < 10^{-2}$, $r = 0.47$, $\Phi = 156 \pm 35^\circ$). Well-fed mites did not show a significant deviation from a uniform distribution of directions in an air stream with HIPV (Figure 1C, V-test for uniformity with $\Phi_A = 0^\circ$: $p = 0.97$, $r = 0.29$). Starved mites showed a positive anemotactic response in an HIPV-free air stream (Figure 1D, V-test for uniformity with $\Phi_A = 0^\circ$: $p < 10^{-5}$, $r = 0.60$, $\Phi = 12 \pm 26^\circ$) or in an air stream with HIPV (Figure 1E, V-test for uniformity with $\Phi_A = 0^\circ$: $p < 10^{-7}$, $r = 0.75$, $\Phi = 354 \pm 19^\circ$).

The mean direction of well-fed mites in an HIPV-free air stream differed significantly from the mean direction of starved mites towards an air stream with HIPV (Watson's F -test, d.f. = 38, $F = 52.51$, $p < 10^{-7}$) and from the mean direction of starved mites in an HIPV-free air stream (Watson's F -test, d.f. = 38, $F = 38.15$, $p < 10^{-6}$). No significant differences between the mean angles were found between starved mites in an HIPV-free air stream and starved mites in an air stream with HIPV (Watson's F -test, d.f. = 38, $F = 1.13$, $p = 0.29$). We conclude that starved predatory mites on a flat substrate responded to a constant laminar air stream by positive anemotaxis. This response was not modified when the air stream was

permeated with HIPV. Well-fed mites responded by a negative anemotaxis to an air stream without HIPV, but with HIPV no anemotactic response was observed. Thus, the anemotactic responses of *P. persimilis* were feeding state-dependent and odour-mediated, but only odour-mediated in the sense that the anemotactic response was lost in well-fed mites in the presence of HIPV.

Discussion

Starved predatory mites showed a positive anemotactic response towards both an HIPV-free air stream and an air stream with HIPV, using a glass substrate under constant laminar airflow conditions in a wind tunnel. These results are in agreement with earlier experiments where either a wire grid served as a substrate under slight turbulent air conditions (Sabelis and Van der Weel 1993) or a smooth sphere in a locomotion compensator was used under laminar airflow conditions (Van Tilborg et al. 2003). Thus, the structure of the substrate and the turbulence of the air stream are not decisive factors for starved predatory mites to walk in upwind direction.

It is easy to understand why starved mites, when perceiving HIPV, move in upwind direction, because it will bring them closer to the source of the odour plume. Why starved mites also walk upwind in the absence of HIPV, instead of downwind or crosswind, is less obvious. Sabelis and Schippers (1984) hypothesized that under variable wind directions, over a range larger than 30°, either upwind or downwind searching is advantageous over crosswind searching, in terms of the area scanned for prey odour. Clearly in our experiments we had no variation in wind direction, thus making crosswind search more favourable, but – in the field – wind directions vary and their range may easily surpass the critical threshold of 30°, making up- or downwind search more effective. Thus, the observation that starved mites do not search crosswind can be explained as an innate response, selected for under field conditions. As far as the starved mites show upwind instead of downwind search, we hypothesize that it is just simpler to search upwind because no anemotactic switch is required when entering a plume of HIPV, a condition under which upwind searching behaviour is performed. This would therefore lead to selection for a generalized, innate upwind response of starved predatory mites.

Well-fed predatory mites show negative anemotactic responses in a laminar air stream without HIPV on a glass substrate. This result is in agreement with earlier experiments where either a wire grid served as a substrate in a slightly turbulent air stream (Sabelis and Van der Weel 1993) or a smooth sphere in a locomotion compensator in a laminar air stream (Van Tilborg et al. 2003). Thus, the structure of the substrate and the turbulence of the air stream are not decisive factors for well-fed predatory mites to walk in downwind direction in HIPV-free air.

Why downwind search is profitable in absence of prey odour, is discussed by Sabelis and Van der Weel (1993). They state that a well-fed predator must be close to its prey. Under those conditions a negative anemotactic response in HIPV-free air is profitable, because a predator that happened to move out of a spider mite patch at the windward side of the patch may increase the likelihood of returning to the

patch. In case the predator would not succeed to return, it would still scan the area for prey odour in the most efficient way (i.e., more efficient than crosswind and equally efficient as upwind search).

Whereas all previous treatments did not show differences with those obtained by Sabelis and Van der Weel (1993), there is one treatment that differs: well-fed adult *P. persimilis* females in an air stream with HIPV showed random orientation in our experiments, but – depending on HIPV concentration – an upwind response in those of Sabelis and Van der Weel (1993). Our result is in agreement with Van Tilborg et al. (2003), who also studied the mites under conditions of a laminar airflow and a smooth substrate. To explain the deviating response observed by Sabelis and Van der Weel (1993), we hypothesize that turbulent air matters to the anemotactic response. This is because turbulence creates a fine scale structure in odour filaments (Murlis and Jones 1981). The test mite may therefore receive odour as a series of odour puffs, which in turn may trigger another response than in a laminar airflow, as has been shown in flying moths (Mafra-Neto and Cardé 1994; Vickers and Baker 1994), walking lobsters (Moore et al. 1991) and walking silkmoth males (Kramer 1992). Future experiments that need to be done include studies on the effect of a variable wind flow on the orientation behaviour of *P. persimilis*. Variation in wind flow can be mimicked in a controlled manner by offering a repetitive series of air pulses, either with or without HIPV, in for example still-air. Such experiments might contribute to the understanding of variation in wind as a directional cue and in the requirements for upwind anemotaxis to HIPV for the predatory mite, *P. persimilis*.

The absence of a positive anemotactic response of well-fed mites in a laminar air stream with HIPV may represent a good search strategy for a predatory mite if one realizes that a laminar airflow is perceived by the mite as a homogeneous odour field. Such conditions, with a continuous high concentration of HIPV, will under the more turbulent field conditions only be encountered very close to the odour source. As long as the mite experiences HIPV everywhere and at the same concentration, it may well be best to search at random. However, when the distance to the prey patch is larger, concentrations of HIPV will due to turbulence undergo short term, abrupt changes. Discontinuous perception of HIPV might therefore be a cue for a larger distance from the source (i.e., prey) and under these conditions upwind orientation is the expected response.

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