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Synomone-induced suppression of take-off in the phytoseiid mite *Phytoseiulus persimilis* Athias-Henriot

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**ABSTRACT**


**Key words:** Dispersal behaviour, Phytoseiidae, synonomes, semiochemicals.

Plant-inhabiting predatory mites in the family Phytoseiidae are known to disperse passively on air currents. In this article we analyse observations on the behaviour that initiates aerial dispersal, the so-called take-off behaviour. When starved for 24 hours at 25°C and 35% RH, about 80% of the females of *Phytoseiulus persimilis* Athias-Henriot became airborne during 10 minute exposure to wind velocities of 2 m s⁻¹ or higher. However, take-off was suppressed when females were exposed to volatile chemicals emanating from leaves that had been infested by two-spotted spider mites (*Tetranychus urticae* Koch) during one day preceding the experiments. This result is the first unambiguous proof that phytoseiid mites exert control over take-off. Interestingly, the females of the predator strain under study did not show the characteristic upright posture that was hypothesized to be important for take-off in two other species of phytoseiid mites (*Amblyseius fallacis* Garman and *Metaseiulus occidentalis* (Nesbitt)). These observations shed new light on the behaviour involved in controlling take-off. It is suggested that take-off control is exerted mainly via the grasp of the claws and the adhesive empodia in a way reminiscent of that described for aphids.

**INTRODUCTION**

Long-range dispersal in phytoseiid mites probably occurs to a large extent by passive dispersal on air currents (Sabelis and Dicke, 1985). Johnson and Croft (1979, 1981) recorded predatory mites in sticky traps positioned at various distances downwind from an apple orchard in Michigan. They found mostly adult females of *Amblyseius fallacis* (Garman) on traps up to ~70 m away from the orchard. Moreover, they observed a steep increase in predator densities in the groundcover vegetation exactly when prey became scarce in the trees, and inferred that the increase was due to ambulatory and aerial emigration of predatory mites as a consequence of starvation. Hoy (1982) suspected aerial dispersal of a carbaryl...
resistant strain of *Metaseiulus occidentalis* (Nesbitt), because the predators “had moved so far so quickly” (cit.) in a Californian almond orchard. She found large numbers of females on sticky panels located outside the almond orchard, and in a later study (Hoy et al., 1985) she reported carbaryl resistance in predator populations from almond orchards surrounding the sites where the resistant strain was originally released. Based on this type of evidence she inferred that the predators had dispersed over at least 800 meters in the period from 1981 to 1983. Dunley and Croft (1990) studied colonization rates on small groups of potted apple trees placed beneath the canopy of an apple orchard in Oregon and on distant groups at 10 m and 100 m away from an apple orchard in the open field. They found that the distant groups of trees were colonized faster by *M. occidentalis* than by *Typhlodromus pyri* Scheuten, whereas the ones placed beneath the orchard canopy were colonized at more or less equal rates by both phytoseiid species. The underlying causes for differential dispersal rates remain to be elucidated; this requires a combination of detailed laboratory studies on dispersal behaviour and carefully designed field studies to test the predictions from behavioural studies.

Aerial dispersal in the field has also been shown to occur in *Phytoseiulus persimilis* Athias-Henriot (Charles and White, 1988). However, preliminary observations on the dispersal behaviour of females of various strains of *P. persimilis* (Sabelis and Afman, 1984) suggested the absence of the dispersal posture described for *A. fallacis* and *M. occidentalis* (Johnson and Croft, 1975, 1976; Field and Hoy, 1985). This posture consists of an upright stance and is considered to promote successful take-off in that it helps crossing the low-wind-speed barrier in the laminar boundary layer above the leaf surface (Washburn and Washburn, 1984). Hence, it is of interest to study take-off behaviour in more detail and to make a comparison with the behaviour of *A. fallacis* under the same experimental conditions. In particular, we ask the question whether phytoseiid mites can exert control over the take-off event in response to cues related to feeding by their prey and whether the dispersal posture described by Johnson and Croft (1976) is essential to achieve such control.

**MATERIALS AND METHODS**

**Origin and state of the predator**
The strain of *P. persimilis* was obtained from the rearing unit of Koppert Ltd (Berkel en Rodenrijs The Netherlands) and was subsequently maintained in the laboratory on Lima bean leaves infested by *Tetranychus urticae* Koch. The experiments were carried out with females of *P. persimilis* that were in the oviposition phase for 2 to 6 days. They were in a well fed state when collected, and then starved individually in Munger cages for one day at 25°C and 35% RH, unless explicitly stated otherwise for experiments designed to investigate the effect of these factors.

The Munger cages comprised of a rectangular piece of Perspex (10 × 5 × 1 cm) with a hole in the middle (2.5 cm diameter) that was closed at one side by a glass
plate and at the other side by gauze with a mesh width small enough to prevent the escape of predators from the cage and to allow free exchange of air. The Munger cages were not provided with a leaf.

**Wind tunnel**

The wind tunnel (Fig. 1) consisted of a Perspex tube (1 m length; 5 cm diameter) with a free air-outlet at one end and with the other end connected via plastic tablets (2 m length; 1 cm diameter) to an air compressor and an air cooling system. Humidity was controlled by shunting part of the air stream through a water filled Erlenmeyer. In all experiments temperature and relative humidity in the air stream were maintained at 25 ± 1°C and 70 ± 5 % RH respectively. Wind speed in the tube was measured by a hot-wire anemometer; control of wind speed was achieved by tuning an extra air outlet in the connecting tubing. The wind tunnel was placed in a climatic chamber at 25°C and 70% RH and with fluorescent light tubes behind a screen of cheese-cloth to create a diffuse light environment.

**Experimental procedure**

The predators were taken from the Munger cages and placed on the upperside of a small rose leaflet isolated by a ring of tanglefoot around the petiole. To prevent wilting the leaf was left connected via the petiole to a 5 cm part of the stem which was placed in a small water-filled vial closed with Parafilm. After at least 30 minutes the leaf with the predator was inserted into the wind tube via a slit.
Leaf insertion was performed under conditions of still air, and wind speed was gradually increased from 0 to 8 m s⁻¹ during the first 5 minutes of the experiment and then back again during the next 5 minutes. Thus, the predators were exposed to a variable wind speed during 10 minutes (unless explicitly stated otherwise).

Initially, two types of experiments were carried out to assess the optimal conditions for take-off. In the first series the conditions during pretreatment of the predators were varied; the take-off response was measured for phytoseiid mites starved for different periods (0, 8, 24, 48 hours) under a range of humidity conditions (90, 75, 55, 35 %RH). In the second series the conditions inside the wind tunnel were manipulated. Wind speed was varied by setting different upper limits and creating a faster approach to the maximum wind speed (within 1 minute).

After assessing the optimal conditions for take-off it was investigated whether the take-off frequency can be reduced in response to cues directly from two-spotted spider mites or indirectly via feeding activities on the leaves. In one series the rose leaflets were previously infested for 24 hours by 25 females of the two-spotted spider mite, *T. urticae*. Just prior to use in the wind tunnel experiment the females, the eggs, the web and most of the faeces were brushed from the leaf so that it would provide similar grip as a clean leaf. Each replicate experiment was carried out using a new predator and a new leaf system (thus avoiding the presence of predator-products deposited during earlier visits). By comparing the take-off responses on uninfested and cleaned, previously infested leaves it was possible to test whether take-off is a matter of being passively blown off, or whether take-off is controlled by the predator in response to damage-related cues (i.e. products of damaged leaf, spider mites or both).

The effect of volatiles emanating from an infested leaf was studied by putting the infested leaf in an envelope made of fine-mesh gauze. By using two such envelopes per leaf it is virtually impossible for the predators to contact the leaves, but they may well perceive the odours emanating from the substrate. Each replicate experiment was done with a freshly prepared leaf system, but unfortunately the gauze envelopes had to be reused for 5 (sometimes 10) consecutive replicate experiments. By wrapping uninfested and infested leaves it was investigated whether take-off can be controlled in response to leaf-damage caused by two-spotted spider mites.

**Classification of behaviour**

A subsample (~10%) of the experiments was subject to more detailed behavioural analysis. To facilitate observation of behaviour the Perspex wind tube was provided with a glass window positioned exactly opposite the slit and the platform for take-off. Behavioural observations were made by using a binocular microscope connected to a video-system (camera, recorder, monitor). Behavioural features were scrutinized by (repeated) frame-by-frame inspection of the relevant records on video-tape. Five types of behaviour were distinguished: (1) walking, (2) crouch-
ing, (3) standing with first pair of legs raised and waving in the air, (4) standing on hindlegs with the soma raised and the two pairs of frontlegs in the air (Johnson and Croft, 1975, 1976), and (5) resting with the soma in normal position and first legs withdrawn. In addition the side of the predator facing the wind was recorded.

RESULTS

Take-off response
The take-off response was calculated as the percentage of predators leaving the platform during the 10 min. exposure to an air stream. The results in Table 1 show that the take-off response increased with increasing starvation and decreasing humidity during starvation. Take-off was observed most frequently when predators were starved for one day at 25°C and 35% RH. Hence, this pretreatment seemed to offer a good starting point for all further experiments to identify factors suppressing take-off.

To specify the optimal conditions for take-off within the wind tunnel additional experiments were carried out in which wind speed was varied (Table 2); take-off was very low at 0.1 and 0.5 m s⁻¹, whereas it was intermediate at 1 m s⁻¹ and maximal at 2 m s⁻¹ or higher. It is concluded that to create optimal conditions for take-off wind speed should exceed 1 m s⁻¹. It is interesting to note that these results indicate a predator's decision to stay when wind speed is too low. However, they leave open the alternative explanation that the wind speed is not strong enough to dislodge the mites and lift them into the air. Indeed, take-off may be a passive event, determined by the balance between predator vigor and the force exerted upon the predator by the wind.

TABLE 1

Assessment of the optimal conditions for take-off of young females of *Phytoseiulus persimilis* in terms of the period of starvation and humidity during starvation (at 25°C); N = 50 for each experiment.

A test of independence was carried out in a 4 × 4 table; the G-statistic adjusted for Williams-correction (Sokal and Rohlf, 1987) was significant at P << 0.001. Hence, we reject the null hypothesis that the take-off response is independent of starvation period and ambient humidity during starvation.

<table>
<thead>
<tr>
<th>RH(%)</th>
<th>0 hr</th>
<th>8 hrs</th>
<th>1 day</th>
<th>2 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>10</td>
<td>12</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>75</td>
<td>4</td>
<td>18</td>
<td>38</td>
<td>64</td>
</tr>
<tr>
<td>55</td>
<td>18</td>
<td>19</td>
<td>44</td>
<td>72</td>
</tr>
<tr>
<td>35</td>
<td>8</td>
<td>54</td>
<td>82</td>
<td>78</td>
</tr>
</tbody>
</table>
The null hypothesis on passive dislodgement by the wind was tested in an experiment in which the response to spider-mite cues was studied under conditions that are otherwise optimal for take-off (i.e. starvation for one day under low humidity; exposure in tunnel to wind speeds exceeding 1 m s⁻¹; Table 1 and 2). This null hypothesis can be rejected however, as the presence of cues related to the recent feeding activities of the spider mites led to a strong reduction in the take-off frequency, whereas it increased again when leaves were used with the remnants of feeding activities of more than 6 days earlier (Table 3). Take-off frequency was

**TABLE 2**

The effect of wind speed on the take-off response of young adult females of *Phytoseiulus persimilis*, starved for one day at 25°C and 35% RH.

A test of independence was carried out in a 2 × 6 table; the G-statistic adjusted for Williams-correction (Sokal and Rohlf, 1987) was significant at $P << 0.001$. Hence, we reject the null hypothesis that take-off response is independent of wind speed.

<table>
<thead>
<tr>
<th>Wind speed (m s⁻¹)</th>
<th>Take-off response¹ (%)</th>
<th>N²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>1.0</td>
<td>65</td>
<td>100</td>
</tr>
<tr>
<td>2.0</td>
<td>86</td>
<td>50</td>
</tr>
<tr>
<td>4.0</td>
<td>79</td>
<td>100</td>
</tr>
<tr>
<td>8.0</td>
<td>88</td>
<td>50</td>
</tr>
</tbody>
</table>

¹ Percentage of females dispersed from the leaf during 10 minute exposure to air currents.
² N = Number of individual females observed.

**TABLE 3**

The effect of previous leaf infestation by spider mites and volatile chemicals emanating from spider-mite-infested leaves on the take-off response of young adult females of *Phytoseiulus persimilis*, starved for one day at 25°C and 35% RH.

Tests of independence were carried out in two-way tables; the G-statistic adjusted for Williams-correction (Sokal and Rohlf, 1987) was significant at $P << 0.001$. Hence, we reject the null hypothesis that take-off response is independent of previous leaf infestation.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Take-off response (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfested leaf</td>
<td>78</td>
<td>80</td>
</tr>
<tr>
<td>Previously infested¹ leaf</td>
<td>7</td>
<td>80</td>
</tr>
<tr>
<td>same leaves, 6 days after cleaning</td>
<td>62</td>
<td>80</td>
</tr>
<tr>
<td>Gauze-wrapped uninfested leaf</td>
<td>82</td>
<td>50</td>
</tr>
<tr>
<td>Gauze-wrapped infested leaf</td>
<td>8</td>
<td>50</td>
</tr>
</tbody>
</table>

¹ Mites and their web were brushed off prior to the experiment, thereby also removing most (but not all) of the faeces.
only 7% on rose leaflets that were fed upon by 25 females of the two-spotted spider mite during the preceding day and that had the spider mites, the web and most of their faeces removed just before the wind tunnel experiment. These previously infested, but recently cleaned leaves are not likely to provide a better grip than the clean leaves, but certainly not better than the previously infested leaves that were cleaned 6 days before the experiment. Thus, the sheer presence of prey-related cues suppresses the predator’s tendency to take off, even though the predator is starved to such an extent that it would become airborne otherwise. Essentially the same result was obtained when the infested leaves were wrapped in an envelope of fine mesh gauze. Here, the presence of odours emanating from the spider-mite infested leaves were the only possible cues that led to suppression of take-off (Table 3). Hence, it can be concluded that take-off is largely under the control of the predator.

Take-off behaviour

Video-aided observations on behaviour preceding take-off events showed that females of *P. persimilis* did not exhibit a raised body stance preceding take-off, as observed by Johnson and Croft (1981) for *Amblyseius fallacis* (Garman). In fact, such behaviour was observed only once at low wind speed (0.5 m s⁻¹) and then it lasted for only 2 seconds. To check whether experimental conditions induced the absence of characteristic behaviour preceding take-off, additional observations were done on starved females of *A. fallacis*, obtained from a culture kindly provided by Dr. B.A. Croft in 1984. However, as these predator females showed the raised body stance frequently under our experimental conditions, we have no reason to suspect inappropriate experimental conditions. Raising of the the first and/or second pair of legs of *A. fallacis* was observed quite frequently, but especially at low wind speeds when take-off rarely occurred. At high wind speeds, these movements with first legs were also observed but not specifically preceding the take-off event. In a number of cases the females of *A. fallacis* maintained the raised stance at high wind speed for several minutes or even up to an hour without subsequent take-off (M.W. Sabelis, personal observation).

A behavioural response observed very frequently in response to increasing wind speed is a reduction in walking speed up to a complete standstill and bending of the legs such that the soma comes more close to the substrate. This behaviour is termed ‘crouching’. It probably helps the predator to decrease exposure to the wind as it brings the predator’s body within the laminar boundary layer where wind speed decreases nearly linearly to zero at the level of the substrate (Schlichting, 1968). Indeed, when observing the predators on excised small parts of pubescent apple leaves (var. Schone van Boskoop), which have a much more thick boundary layer, crouching behaviour was observed much less frequent and became manifest at much higher wind speeds than on glabrous rose leaves.

Predators were very occasionally observed dangling while maintaining holdfast to the leaf by a single leg. Apparently, they were unable or unwilling to release
the tarsal claws. Also it was observed three times that the predators had anchored themselves to the substrate by their mouth appendages (probably the fixed and/or movable digits of the chelicerae). In the latter case it was not easy to dislodge the predators, even by use of a brush.

DISCUSSION

The experiments are the first demonstration of an ability of phytoseiid mites to control the initiation of aerial dispersal. The sheer presence of odours emanating from a leaf previously infested by two-spotted spider mites led to a significant reduction in take-off frequency. It is now well established that these odours are produced by the host plant in response to feeding activity of two-spotted spider mites (Sabelis and van de Baan, 1983; Sabelis et al., 1984a; Sabelis and Dicke, 1985; Dicke et al., 1990ab; Dicke et al., 1993; Dicke, 1994); following the terminology of Dicke and Sabelis (1988) these volatile chemicals are called synomones. Synomone-induced suppression of take-off may be one of the mechanisms contributing to arrestment of phytoseiid mites in a cluster of leaves infested by spider mites. The cloud of odour surrounding this cluster of spider-mite infested leaves may provide the stimulus not to disperse aerially when predators have left a particular spider-mite infested leaf in the cluster and pass plant area without any prey, inbetween the infested leaves. Presence of the odour is a signal that will lead to the continuation of ambulatory dispersal and an increased probability of finding another spider-mite infested leaf nearby. Together with other arrestment mechanisms, such as klinotactic and klinokinetic responses to odour gradients (Sabelis et al., 1984b) and state-dependent and odour-conditioned anemotaxis (Sabelis and van der Weel, 1993), synomone-induced suppression of take-off may help explain how phytoseiid mites manage to stay in or near clusters of spider-mite infested leaves (Sabelis and van der Meer, 1986; Charles and White, 1988; Sabelis, 1992).

How phytoseiid mites resist dislodgement by wind and gain control over take-off, is a question of great importance in understanding the influence of the plant on the performance of predators (relative to herbivores) (Juniper and Southwood, 1986). Spider mites may resist dislodging by way of producing a silken thread to anchor themselves to the leaves, but phytoseiid mites do not have the ability to produce silk. Probably, the crouching posture serves to reduce the drag because of the lower fluid momentum close to the substrate. However, it is very unlikely to be a major feature of take-off control, because the experimental conditions (small leaf size, gradual increase in wind speed up to a maximum 8 m s\(^{-1}\)) cause the laminar boundary layer to become very small; thus, at times the predator may extend well above the laminar boundary layer. We hypothesize that take-off control is achieved via the claws and possibly the empodia. By analogy with claws and pulvillus in aphids (Lees and Hardie, 1988; Kennedy, 1986) the claws may be more effective on rough surfaces whereas the empodia may be effective on smooth surfaces. Whether oily secretions assist the empodia in surface tension adhesion, is not clear
yet, because there is no evidence for tarsal oil glands nor for empodial oils (Evans, 1992).

It is noteworthy that females of *P. persimilis* did not show the dispersal stance observed in *A. fallacis* (Johnson and Croft, 1975, 1976) and in *M. occidentalis* (Field and Hoy, 1985). As we made comparative observations between *A. fallacis* and *P. persimilis* in the same experimental set-up and compared various strains of *P. persimilis*, including a 'wild' strain shortly after collection in Morocco (Sabelis, personal observation), we conclude that there are interspecific and/or intraspecific differences. Evidently, the upright body stance is not a prerequisite for take-off, albeit certainly helpful as it increases the drag because of greater fluid momentum higher in the boundary layer (Washburn and Washburn, 1984). Actually it is not clear why the upright posture is required because the predators could move to the upwind edge of the leaf where the boundary layer is vanishingly small. It is also not clear why phytoseiid mites (and also crawlers of coccids; Washburn and Washburn, 1984) adopt a standing posture with the dorsal side upwind (Johnson and Croft, 1976). Actually, a predator could gain more drag by heading the wind. Indeed, one should be cautious in referring to the upright body stance as “the dispersal posture”, because (1) there may be other behaviour that can initiate aerial dispersal, and because (2) the posture may serve other functions. In this respect one may think of inspection of the wind velocity profile, the perception of odour or both. Also, one may speculate on a function in phoretic transport, because the upright posture is reminiscent of the so called questing behaviour described for phoretic stages of some astigmatic mites (Schulze, 1924; Hall, 1959; Houck and O'Connor, 1991) and for nymphs of ixodoid ticks (Lees, 1948). Phoretic transport has been reported for some phytoseiids (Krantz, 1973; Sabelis and Dicke, 1985; Fain and Krantz 1990) and may well be more widespread than currently thought. For all these reasons we advocate a more critical assessment of the possible functions of the upright posture.

ACKNOWLEDGEMENTS

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