Combatting whiteflies: predatory mites as a novel weapon

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How do predatory mites find plants with whitefly prey?

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We investigated the searching behaviour of two species of predatory mites, *Typhlodromips swirskii* and *Euseius scutalis*, both predators of immature stages of the whitefly *Bemisia tabaci*. When released in a greenhouse in a circle of cucumber plants that were either infested with immature whiteflies or clean, the mites took several days to find plants, and *T. swirskii* were recaptured significantly more frequently on plants with whiteflies. This suggests that the mites are able to discriminate between plants with and without whiteflies from a distance. Since the mites have no eyes, they possibly use volatile cues. *Euseius scutalis*, also took several days to find plants in the greenhouse, and numbers recaptured on clean plants and infested plants did not differ much. This suggests that this species does not discriminate between clean and infested plants. In a Y-tube olfactometer, *T. swirskii* was not attracted to the odours of cucumber or cotton plants with whiteflies, suggesting that these mites do not use volatiles to discriminate between infested and clean plants, or that the olfactometer set-up as used here is not suitable to detect the response of this species to odours. The efficiency with which predatory mites find plants with whiteflies is much lower than the efficiency of whiteflies searching for new plants. We expect this to give rise to spatial pattern formation in the dynamics of predator and prey. We discuss the consequences of this for biological control of whiteflies with predatory mites.

Many arthropod predators and parasitoids use volatiles to find plants with their herbivorous arthropod prey (Sabelis and van de Baan 1983; Dicke and Sabelis 1988; Dicke 1994; Turlings *et al.* 1995; Sabelis *et al.* 1999a,b; Janssen 1999; Keßler and Baldwin 2001). Predatory mites are no exception to this; there are several examples of phytoseiids that respond to herbivore-
induced plant volatiles (HIPV's) (Sabelis and van de Baan 1983; Dicke et al. 1990; Janssen 1999) and this response is either innate or can be learned (Takabayashi et al. 1994; Krips et al. 1999; Dicke et al. 2000; Drukker et al. 2001). The volatiles are often produced by plants in response to damage by herbivores (Dicke and Sabelis 1988; Dicke 1994; Turlings et al. 1995; Sabelis et al. 1999a,b; Janssen et al. 1999; Keßler and Baldwin 2001; Janssen et al. 2002).

In this publication, we investigate the response of predatory mites to odours emanating from plants that are attacked by the whitefly *Bemisia tabaci*. It is unclear whether plants are induced to produce volatiles through attacks by whiteflies (van Lenteren et al. 1996; Walling 2000). Heinz and Parrella (1998) found that 4 out of 9 strains of whitefly parasitoids (2 out of 5 species) spent significantly more time in odour fields of poinsettia and cotton leaves that were infested with *Bemisia argentifolii*-infested than in odour fields of clean leaves. This response could be due to HIPV or to volatiles of whitefly by-products such as honeydew.

Several species of predatory mites are associated with whiteflies on various crops (Nomikou et al. 2001) and 2 of these species are considered suitable candidates for biological control of *B. tabaci*: they can control local populations of whiteflies on isolated cucumber plants (Nomikou et al. 2002). The predatory mites can only disperse by walking or through air currents while whiteflies can disperse by flying. It is expected that this results in differences in dispersal capacity of prey and predators with the prey being the better disperser. Such differences are likely to lead to spatial pattern formation (Maron and Harrison 1997; Brodmann et al. 1997; Maron et al. 2001) and to hide-and seek dynamics of predator and prey, with the better-dispersing prey being able to escape temporarily from predators by migrating to predator-free plants (Nomikou et al., submitted). The long-range searching behaviour (i.e. the searching for plants with prey) of the predators is therefore of interest both from a theoretical point of view and for development of strategies for biological crop protection.

We studied the ability of two species of predatory mite, *Typhlodromips swirskii* and *Euseius scutalis*, both predators of whiteflies, to find plants with whiteflies in a greenhouse. Moreover, we tested the response of *T. swirskii* to odours emanating from cucumber and cotton plants infested with the whitefly *B. tabaci*, and how this response affected was by experience of predators with plants infested with whiteflies.

**MATERIALS AND METHODS**

**Cultures**

Cucumber plants (var. Ventura RZ®, RijkZwaan, De Lier, The Netherlands) were grown from seeds in pots (2 l) with soil in a climate room (25°C;
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Bemisia tabaci strain B (J. Fransen, pers. comm.) were obtained from a culture on poinsettia from the Research Station for Floriculture in Aalsmeer, The Netherlands, in March 1995, and they were reared in climate boxes (27°C; l:d = 16:8) on cucumber plants. A culture of whiteflies on cotton was started with adult females from the same strain.

The predatory mite Typhlodromips swirskii (Athias-Henriot) was collected in Israel (location Revodim) in 1997 from cotton plants that were infested with B. tabaci (Nomikou et al. 2001). It was reared on plastic arenas (8 x 15 cm) placed on a wet sponge in a plastic tray containing water (Overmeer 1985). Strips of wet tissue were placed on the plastic arena along its periphery so that the predators could obtain water, and glue barriers were applied on the wet tissue to prevent escapes and contamination. A piece of transparent plastic sheet (1-2 cm²) bent in the shape of a tent was placed on each arena, to provide shelter for the mites. A few cotton threads were put underneath the shelter to serve as a substrate for oviposition. Mites were fed by brushing broad bean (Vicia faba L.) and cattail (Typha latifolia L.) pollen on the arenas twice per week. The predatory mite cultures were maintained in a climate room (25°C, 60% RH).

The other predatory mite, Euseius scutalis (Athias-Henriot), was collected in Jordan from Ricinus communis L. infested with whiteflies in 1998 (Nomikou et al. 2001). It was cultured on lima bean (Phaseolus lunatus L.) leaves, placed upside-down on wet cotton wool in a plastic tray. Glue barriers were applied along the inner side of the tray and on the wet cotton wool around the leaf to prevent escapes and contamination. A shelter and pollen were supplied as described above.

Release-recapture

Six potted cucumber plants (4-5 weeks old) were placed in a greenhouse (conditions set at 25°C, 60% RH, 16-8h photoperiod) inside a plastic tray (l x w x h: 174 x 100 x 19) filled with soil, so that they occupied the six corners of a hexagon with a diameter of 80 cm (Janssen 1999). The rim of the pots was just below the soil surface and an additional layer of soil was added so that the pots were buried and the soil surfaces inside and outside the pots were equal. Prior to the experiment, half of the plants had been incubated in a whitefly culture on cucumber plants for 7-10 days (starting when they were 3-3.5 weeks old). Adult whiteflies were removed from these plants before the experiment to prevent contamination of the clean plants. Hence, infested plants had only whitefly eggs and crawlers. Because uninfested plants grow faster than plants in the whitefly cultures, we used slightly younger (3-7 days) clean plants to correct for size differences. Plants with
different treatments had alternate positions in the hexagon resulting in two different configurations of the set-up, one with plants with predators in positions 1, 3 and 5 and one with these plants in positions 2, 4 and 6. In each series of experiments, both configurations of the set-up were used an equal number of times.

One day after introducing the plants into the greenhouse, a petri dish (diameter 8 cm) with 131-188 adult female predatory mites was put in the centre of the hexagon from where they could walk to the plants. Plants were checked for predators 1-6 times per day during the subsequent 4 days (see results for exact frequencies). Four replicates, each with a new set of plants, whiteflies and mites, were done for each predator species. Differences in numbers of mites found back on either of the two types of plants were tested using the two-sided binomial test.

**Olfactometer**

A Y-tube olfactometer was used to study the response of adult female predators (Sabelis and van de Baan 1983). It consists of a glass tube in the form of a Y, with a black Y-shaped metal wire in the middle to railroad the mites. The base of the tube was connected to a pump that produced airflow from the arms of the tube to the base. Potted plants, either cucumber or cotton, were placed in a tray (l x w x h = 46 x 30 x 8 cm) that was put inside a second tray containing water (60 x 40 x 4.5 cm). A Plexiglas container (50 x 36 x 43 cm) was put over the plants so that it rested in the outer water-containing tray. In this way a water barrier was created to prevent escape of whiteflies, with the water serving as an airtight seal (see Janssen 1999). Three plants, infested with whiteflies for 7-10 days, were put in one container, while the other container received 3 uninfested plants of the same age. The containers had an air inlet and outlet (diameter 4 cm) in opposing walls, covered with fine gauze. The air outlet was connected with a plastic hose to either arm of the olfactometer. A hot-wire anemometer was used to measure wind speeds in both arms of the olfactometer. The wind speed was calibrated using valves that were inserted into the hoses connecting the containers to the olfactometer. When wind speeds in both arms are equal (0.5 m / s), the odours form two neatly separated fields in the base of the Y-tube with the interface coinciding with the metal wire (Sabelis and van de Baan 1983).

Adult female predatory mites were starved for 3.5 to 24 h prior to the experiments. In some experiments, mites were given an experience with plants with whiteflies prior to the olfactometer experiment, by putting them for 3 days to one generation (10 days) on leaves (either cotton or cucumber) infested with whitefly immatures. These leaves contained sufficient prey (whitefly eggs and crawlers) for the predators and were replaced with fresh leaves whenever the condition of the leaf deteriorated or the amount of prey became too low. Mites were introduced one at a time, by disconnecting
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the pump and putting one female on the metal wire at the base of the Y-tube. After reconnecting the pump, the female started moving upwind to the junction of the wire, where she had to choose one of the two arms. Each individual was observed until she had reached the end of the arm or for a maximum of 5 min and was subsequently removed. Each replicate lasted until 20 predators had made a choice for one of the two odour sources. The odour sources were switched to the opposite arm of the olfactometer after each five mites that made a choice to correct for any unforeseen asymmetry in the experimental set-up. Differences in numbers of mites choosing for any of the odour sources were tested using the two-sided binomial test.

RESULTS

Release-recapture

During the first day, only few predators of both species were recaptured on the plants (Figure 1). We therefore refrained from hourly sampling of the plants and from removing predators that were found on the plants. A 26.5 ± 2.6% (mean ± s.e.) of all released *T. swirskii* and 23.3 ± 4.1% of all *E. scutalis* were found on the plants at the end of the experiments.

Overall, the numbers of *T. swirskii* on plants with whiteflies increased with time, but sometimes decreased over subsequent counts (Figure 1A), suggesting that some mites moved off the plants. Lower numbers of predators were recaptured on plants without whiteflies, with a gradual decline towards the end of the observation period (Figure 1A). Except for one replicate, the fraction of *T. swirskii* on infested plants was mostly higher than that on clean plants (Figure 2A), suggesting that the mites showed a preference for infested plants within the first day since release. However, this difference was not significant during the first few hours of observation due to the low numbers of mites on the plants. Therefore, we excluded all samples with a lower total number than 8 mites from further analysis (7 out of 8 mites need to be recaptured on the same type of plant to detect a significant difference in the binomial test). Of all remaining data points, only a single one was not significant (Figure 2A).

The number of *E. scutalis* on plants with whiteflies generally increased with time, but sometimes decreased over subsequent counts (Figure 1B), suggesting that some mites of this species also moved off the plants. Although lower numbers of mites were recaptured on clean plants than on infested plants, the difference was not as large as for *T. swirskii*. This is reflected in the fraction of mites found on infested plants (Figure 2B), which was mostly not significantly higher than that on clean plants. Moreover, the fraction of *E. scutalis* on clean plants did not decrease towards the end of the experiments (Figure 1B).
Figure 1 The number of predatory mites recaptured through time on clean cucumber plants (open symbols and hatched lines) and on cucumber plants infested with immatures of the whitefly B. tabaci (closed symbols and drawn lines). At time zero, 131-188 mites were released in the centre of a hexagon of plants, with each plant neighbouring two plants of the other type. Subsequently, the mites on the plants were counted repeatedly. A. T. swirskii; B. E. scutalis. Within a panel, series with the same symbol (but open and closed) refer to the same replicate.
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Figure 2 The fraction of predatory mites that was found on plants with whiteflies through time. A. T. swirskii; B. E. scutalis. The dotted line corresponds to the expected fraction under the hypothesis that the mites have no preference for clean or infested plants. Black symbols concern fractions that differ significantly from this expected fraction (binomial test). Open symbols refer to data points with fewer than eight mites recaptured in total, hence a significant difference is unlikely to be found. Gray symbols refer to data points with > 8 mites, hence a significant difference could be expected given that the mites would have a preference. The symbols correspond to those used in Figure 1.
Part 3 - Between-plant mobility of predator and prey

![Diagram showing mobility of predator and prey](image)

Figure 3 The fraction of predatory mites (*T. swirskii*) that chose for either clean plants or plants infested with the whitefly *B. tabaci* in an olfactometer. Mites were offered a choice between odours emanating from 3 plants (either cucumber or cotton) infested with *B. tabaci* or clean plants of the same species. Each bar represents an experiment with 20 predatory mites that made a choice using a separate set of plants. Predatory mites were either naïve with respect to plants and whiteflies, or experienced. *: significant preference for plants infested with whiteflies (Binomial test, \( P < 0.05 \)).

More *T. swirskii* were recaptured on the plants that were positioned east-north-east relative to the release point, while more *E. scutalis* were recaptured east-north-east and west-south-west (Chi-square test, \( P < 0.05 \)). The reason for directionality is unknown, but is not uncommon in phytoseiids (Janssen 1999; Zemek and Nachman 1999). Since we took care to use both configurations of the set-up an equal number of times, we compensated for the effects of this directionality.

**Olfactometer**

Most of the *T. swirskii* predators walked upwind in the olfactometer and only 6% of the mites did not reach the end of one of the arms in 5 minutes.
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The mites did not show a significant preference for odours of cucumber plants infested with whiteflies (Figure 3, top 2 bars). Experience on cucumber leaves infested with whiteflies did not lead to an increase in preference (Figure 3, 2nd group of bars). Overall, 48% of the predators chose for odours of infested cucumber plants. The period of starvation prior to the experiment did not affect this response.

Because the mites were collected from cotton fields, they may not recognise odours of infested cucumber. Therefore, we decided to test their response to odours of cotton with whiteflies as well. Without prior experience with infested cotton, mites again did not show a preference for such odours (Figure 3, 3rd group of bars). Overall, 42.2% of all mites tested chose for odours of cotton plants infested with whiteflies. Predators with prior experience with infested cotton showed a significant attraction to odours of infested cotton only in 2 out of 9 replicates (Figure 3, 4th group of bars), but the total response over all replicates was not significant (54.2% of the females chose for odours of infested cotton).

DISCUSSION

Most predatory mites of the species *T. swirskii* were recaptured on plants with whiteflies. It took them very long to reach plants with or without whiteflies, only a quarter of the predators found a plant and it took them several days. In a similar experiment, another phytoseiid predator, *P. persimilis*, was recaptured in higher percentages (30-60%) in one day (Janssen 1999). The fraction of *T. swirskii* recaptured on plants with whiteflies was significantly higher than that on clean plants (Figure 2A). This can be the result of differential attraction to infested and clean plants or of differential arrestment. Because the fraction of *T. swirskii* on plants with whiteflies was high from the beginning (Figure 2A), we suspect that the mites are attracted to plants with whiteflies and thus can discriminate between plants with and without prey from a distance. Because mites have no eyes, the most likely cue that can be used from a distance consists of volatiles, either produced by infested plants or by whiteflies (honeydew) or both.

The other predatory mite species released in the greenhouse, *E. scutalis*, was also recaptured more frequently on plants with whiteflies, but this was significant only towards the end of the experiments and only in 2 out of 4 replicates. This suggests that these predatory mites have a very low or even no ability to discriminate between plants with and without whiteflies compared to *T. swirskii*.

Because *T. swirskii* showed a clear and consistent preference for plants with whiteflies in the release-recapture experiments, we decided to study the response of this species to odours emanating from plants with whiteflies in an olfactometer. It was not attracted by volatiles emanating
from cucumber and cotton plants infested with *B. tabaci*, despite the fact that the strain used was collected from cotton plants with whiteflies (Nomikou et al. 2001). We also tested several other species of phytoseiids, including the whitefly predator *T. athiasae* and the spider mite predator *P. persimilis* but none of these species were attracted to odours of plants with whiteflies (Nomikou, unpublished results). Moreover, *T. swirskii* that had experience with leaves of either cotton or cucumber with whitefly prey also did not show any preference. Possibly, plants with whiteflies are not induced to produce volatiles. There is not much evidence that phloem-feeding herbivores, such as whiteflies, induce plants to produce volatiles (Walling 2000). Indeed, there are several other studies (reviewed in van Lenteren et al. 1996) that show a lack of response of the notorious parasitoid of whiteflies *Encarsia formosa* to plants with *Trialeurodes vaporariorum*, another species of whitefly. However, Heinz and Parrella (1998) found that 4 out of 9 strains of whitefly parasitoids (2 out of 5 species) spent significantly more time in odour fields of poinsettia and cotton leaves that were infested with *Bemisia argentifolii*-infested than in odour fields from clean leaves. Moreover, whiteflies produce honeydew, and predators could use the smell of honeydew to find their prey. This leads to the conclusion that either this species does not use volatiles to search for plants with prey, or that the olfactometer may not be a suitable set-up to detect olfactory responses of *T. swirskii*. Since the results of the release-recapture experiments suggest that *T. swirskii* is capable of discriminating between plants with and without whiteflies from a distance, they probably do use some cue that can be perceived some distance away from the plants, and odours are the most likely candidates. Possibly, the time span in which the predator has to choose in the Y-tube is too short to reveal attraction.

We found no evidence that *E. scutalis* is capable of discriminating between plants with and without prey from a distance. Moreover, a consistent large fraction of mites of this species was found on clean plants. It is possible that mites of this species do not disperse from plant to plant frequently. Predatory mites can often use other food types besides their prey, such as pollen and extrafloral nectar (van Rijn and Tanigoshi 1999a,b) and populations of predators can therefore persist on plants without prey. The two species released here are no exception to this; they both thrive on pollen. Moreover, *E. scutalis* cannot be reared without plant tissue (M. Nomikou, pers. obs.), suggesting that it may feed on plant tissue as well as on prey. This was further confirmed by the observation that this species has high mortality on plants that were treated with a systemic pesticide (Nomikou et al., in prep.). Hence, it is possible that *E. scutalis* has not been subject to strong selection to find plants with prey, but, rather, occupies all plants on which they can find pollen or other alternative food.

The efficiency with which the predatory mites find host plants with prey is low compared to that of adult whiteflies (86.7% of the released whiteflies was recaptured in one day; Nomikou et al., submitted). Adult
whiteflies learn to discriminate between plants with predators and whiteflies and plants with only whiteflies (Nomikou et al., submitted) and the first offspring that whitefly produce on plants without predators may reach an invulnerable stage before predators arrive. Combined with the slow dispersal of predatory mites, this is expected to result in spatial dynamics with prey moving away from plants with predators and predators slowly following prey, resulting in a front of prey-infested plants that moves outward from the focus of infestation. These spatial dynamics may be undesirable when attempting to control whiteflies in crops. Because the predators can also feed on pollen, predator populations can be established on plants without whiteflies but with pollen (Nomikou et al. 2002) and this strategy can be used to prevent the spatial game of hide and seek.

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REFERENCES


Part 3 - Between-plant mobility of predator and prey


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