How counter-attacking prey influence foraging and oviposition decisions of a predatory mite
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Attacking prey is not without risk; predators may endure counterattack by the prey. Here, we study the oviposition behaviour of a predatory mite (Iphiseius degenerans) in relation to its prey, the western flower thrips (Frankliniella occidentalis). This thrips is capable of killing the eggs of the predator. Thrips and predatory mites—apart from feeding on each other—can also feed and reproduce on a diet of pollen. Because thrips may aggregate at pollen patches, such patches may be risky for oviposition by the predatory mites. We found that, in absence of thrips, predatory mites lay their eggs close to pollen, but further away when thrips are present. Predatory mite eggs near pollen were killed more frequently by thrips than when they were deposited further away.

The oviposition behaviour of the predatory mite was also studied in absence of thrips, but in presence of the alarm pheromone of thrips. This pheromone is normally secreted upon contact with predators or competitors. When applied close to the pollen, predatory mites oviposited significantly further away from it. When the alarm pheromone was applied away from the food source, most eggs were found near the pollen. These results indicate that female predatory mites show flexible oviposition behaviour in response to the presence of their counter-attacking prey.

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Predation is one of the main factors influencing the distribution and dynamics of populations. Most studies on predator-prey interactions concern the direct removal of prey by predators from the ecological system. However, predators can also have indirect, non-lethal effects on prey (Sih et al., 1985; Sih, 1987; Kats & Dill, 1998; Lima, 1998). Avoidance of predation is considered to be such an indirect effect (Kats & Dill 1998; Janssen et al., 1998). This behaviour entails benefits and costs. Whereas the benefits result from decreased predation risk, costs arise from time and energy spent in avoiding predation at the expense of other vital tasks (Lima & Dill, 1990; Dugatkin & Godin, 1992; Lima, 1998). Many of the anti-predator defences are induced by chemical cues from potential predators. These cues help prey to assess predation risk (Havel, 1987; Kats & Dill, 1998; Venzon et al., 2000).

In many species, eggs are vulnerable to predation. Females that avoid oviposition at sites that are accessible to predators would reduce the risk of egg predation, thereby gaining a selective benefit (Kats & Sih, 1992). Demonstration of such avoidance mainly comes from studies on aquatic animals (Chesson, 1984; Resetarits & Wilbur, 1989; Petranka & Fakhoury, 1991; Kats & Sih, 1992; Knapp, 1993). For example, adult mosquitoes and
&midges avoided ovipositing in pools that contained chemical cues of predatory bluegill sunfish (Petranka & Fakhoury, 1991), and bicolour damselfish avoid spawning at sites that contain the odours of an oophagous predator (brittlestar) (Knapp, 1993).

Not only prey, but also predators have to take predation risk into account. Some prey counter-attack and may even kill predators. Saito (1986), for example, found that male spider mites attack and kill the larvae of the predator that feeds on spider mites. In this article, we consider a similar predator-prey system, consisting of a predatory mite, *Iphiseius degenerans* (Berl.) (Acari: Phytoseiidae), western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), and pollen. The predator eggs are vulnerable to counter-attack by thrips (Faraji *et al.*, in prep.). We studied the effect of counter-attacking thrips on the decision of the predatory mites where to lay eggs.

**THE 'PREDATOR-PREY' SYSTEM**

The predatory mite is used to control western flower thrips in greenhouses in the Netherlands. The interaction between the 'predator' and the 'prey' is of a special nature: all active stages of the thrips are capable of killing eggs of their predator, whereas the predatory mites only kill young thrips larvae. On plants, the predatory mites frequently visit flowers to feed on thrips larvae and pollen (van Houten & van Stratum, 1995). Despite frequenting flowers, the females prefer to lay eggs in leaf domatia (Faraji *et al.*, in prep.). Since the female predatory mites are able to produce a few eggs per day and feed in flowers in-between ovipositions on leaves, they have to commute between flowers and leaves repeatedly. We have shown that predator eggs in flowers run a higher risk of predation by thrips than eggs on leaves (Faraji *et al.*, in prep.). This is because western flower thrips, and many other predators, concentrate in flowers due to the presence of pollen and prey. Pollen, however, also rains down on the leaves directly below the flowers. In case thrips and other predators are absent from such pollen patches, they would offer safer oviposition sites than flowers, but in their presence, these patches may again be risky sites for eggs. Hence, it is expected that females save time and energy, both for themselves and for their offspring, by ovipositing close to pollen patches without thrips. However, when thrips are present at the patch with pollen, it would pay the female to avoid ovipositing there. To test this, we studied the oviposition behaviour of predatory mites on patches of pollen in absence and presence of thrips. Moreover, we investigated whether predatory mites use chemical cues from thrips to assess predation risk for their offspring. Western flower thrips produce an alarm pheromone that is released in droplets of anal fluid in response to disturbance by predators and competitors (Teerling *et al.*, 1993a,b). This anal fluid also acts as a defensive allomone that, upon contact, causes the predators of thrips to withdraw and groom their head and first pair of legs (Bakker & Sabelis, 1986, 1989). Moreover, the pheromone can act as a kairomone because it betrays the presence of thrips to predators (Teerling *et al.*, 1993b). In the following, we will consistently use the term "pheromone", which refers to chemical communication within a species (in this case a prey), even when addressing communication between species (in this case predator and prey) (*i.e.* a kairomone sensu Dicke & Sabelis, 1988).
MATERIALS AND METHODS

REARING

The strain of *I. degenerans* used in this study originates from Rabat, Morocco (van Houten et al., 1995) and was reared on rectangular arenas (van Rijn & Tanigoshi, 1999) on a diet of birch pollen. Arenas consisted of a grey PVC sheet (30 x 21 cm) on a wet sponge in a water-containing tray. Paper tissue was wrapped over the edges of the arena, serving as a water source as well as a barrier. To provide more water for the animals, each arena was divided into four sections by wet filter paper strips (1 x 30 cm). A thread of sewing cotton (0.5 mm thick, 2 cm long, black) was provided as oviposition substrate on each section. *Iphiseius degenerans* oviposited the vast majority of eggs on these threads. Mated female predators (10-14 days old) were used for the experiments.

The laboratory population of thrips was established on chrysanthemum in 1993 at the Section Population Biology with individuals collected from chrysanthemum at the Centre for Plant Breeding and Reproduction Research in Wageningen, the Netherlands.

EFFECT OF PRESENCE OF THRIPS ON EGG DISTRIBUTION OF *I. DEGENERANS*

Due to the small size of predator eggs, it is difficult to estimate egg mortality and oviposition on plants simultaneously. We therefore carried out experiments in the laboratory on an artificial substrate that allows easy discrimination between intact and killed eggs at the end of the interaction period. We used artificial substrate rather than leaf tissue, because it is impossible to construct the kind of arena's we used (see below) from leaf material without including water barriers that make it difficult for the predators to move from one part to the other. Killed eggs can be recognised because they become whitish and shrivelled and because the egg chorion is left behind. Since experiments lasted only 2 days, eggs could not hatch within the experimental period.

To obtain indications for the effects of volatile pheromones of thrips (as opposed to effects of physical encounters between thrips and predators), a U-shaped chain consisting of 20 connected plastic discs (26 mm diameter) with 10-mm wide interconnections was used as experimental arena (Fig. 1). The arenas were cut from PVC sheets (green, Kangaro Co., Zutphen, The Netherlands) and were placed on wet cotton wool in plastic trays. Ample birch pollen was supplied on the first disc and a piece of cotton thread (0.5 mm thick, 1.5 cm long) on each disc to serve as oviposition substrate. One female predatory mite as well as five adult thrips and five second-instar thrips larvae were released on the second disc of each chain. Thrips of these stages cannot be attacked by the predatory mites, which simplified interpretation of the experiments. Thus, thrips can feed on pollen as well as on predator eggs, whereas predators can only feed on pollen. Most thrips stayed on the disc with pollen; only a few of the adults were found on the other discs. Air could move freely over the experimental arenas, and we therefore assumed that the concentration of volatiles decreases with distance from the odour source. Therefore, use of the U-shaped arena allowed us to discriminate between the effects of physical encounters between adult mites and thrips, which would result in oviposition further away, up to the end of the chain, and the effect of volatiles produced by thrips, of which concentrations would be lowest around disc 10, further away from the first disc with thrips than disc 20 (Fig. 1). The same set-up was used for control experiments without thrips. Ten replicates were done for treatment and control.
After two days, the numbers of eggs laid by the predatory mites and eggs killed by thrips were recorded per disc.

To measure the distance of predator eggs to the food source (i.e. the 1st disc), we calculated the oviposition distance \( D \), expressed in disc rank number, for each female with the following formula:

\[
D = \frac{\sum_{d=1}^{20} dn_d}{N},
\]

where \( d \) is the rank number of a disc (1 to 20, with disc 1 containing the pollen, Fig. 1), \( n_d \) is the number of eggs on disc \( d \), and \( N \) is the total number of eggs. The oviposition distances of females in the presence of thrips were compared to those from the control using the two-tailed Mann-Whitney U-test.

**EFFECT OF THRIPS ALARM PHEROMONE ON EGG DISTRIBUTION OF *I. DEGENERANS***

We followed the methods described by Teerling et al. (1993a,b) for preparation of the synthetic alarm pheromone of western flower thrips. An amount of 0.1 µg of synthetic western flower thrips alarm pheromone (decel acetate and dodecyl acetate in a molar ratio of 1.5:1 in 2 µL of pentane) was applied to a triangular filter paper wick (base=2 mm, sides=7 mm, see Teerling et al. (1993a,b) for details). The wicks were placed on the arenas after the solvent had evaporated. Besides serving as an alarm pheromone for thrips, this amount of volatile is known to be attractive to two predators of the thrips, the predatory bug *Orius tristicolor* and the phytoseiid *Neoseiulus cucumeris* (Teerling et al. 1993b). We were not only interested in the effect of applying alarm pheromone close by the food source, but also away from it, to detect whether the presence of alarm pheromone results in oviposition away from the food source or in oviposition away from the odour source. Because the concentration of alarm pheromone is expected to decrease with linear distance to the paper wick, and not with walking distance, it was more appropriate to use a linear chain consisting of 9 discs, rather than a U-shaped disc (see Fig. 3). In one experiment, we placed the wick with the alarm pheromone close to the food source to mimic the presence of thrips on the disc with pollen. As a control we put a similar wick, but only with pentane on the first disc. Ten replicates were done of both control and treatment. Subsequently, we tested the effect of the position of the alarm pheromone relative to the food source by supplying either the 1st or the 9th disc with a wick with alarm pheromone. Twelve replicates were done for both control and treatment. To ensure continuous presence of the pheromone, wicks were replaced with freshly prepared wicks every two hours, taking care not to disturb the predators. At 6.30 PM, the numbers of eggs were counted and their position was scored. Two females in the control, six females with alarm pheromone close to the pollen and three females with pheromone at the other end did not produce any eggs and had to be eliminated from the results. The same formula as mentioned above was used to calculate the oviposition distance relative to the pollen and the Mann-Whitney U test was used to test the null hypothesis.
PREDATORY MITES AVOID OVIPOSITING NEAR COUNTER-ATTACKING PREY

RESULTS

EFFECT OF PRESENCE OF THRIPS ON EGG DISTRIBUTION OF *I. degenerans*

Predatory mites oviposited similar numbers of eggs in presence and absence of thrips (a total of 43 eggs in presence of thrips and 45 in absence). Figure 1 shows the mean percentage of eggs deposited by *I. degenerans* females on arenas consisting of 20 interconnected discs in the presence and absence of thrips. This includes eggs that were killed by thrips during the experiment. Without thrips, female predatory mites tended to lay eggs close to the food source whereas in presence of thrips they oviposited further away. The oviposition distance relative to the pollen in the absence of thrips is significantly lower ($Dc=2.35$) than that in presence of thrips ($Dt=6.38$, two-sided Mann-Whitney U Test, $P=0.00004$). An aggregation of oviposition around disc 10 can be observed (Fig. 1). This cannot be explained as an effect of the physical interaction between thrips and predators, which would lead to a gradual increase or decrease of oviposition with walking distance along the U-shaped arena. The aggregation may be caused, however, by a response of the predators to thrips volatiles, such as alarm pheromone, since the linear distance of disc 10-12 to the disc with pollen and thrips (disc 1) is longest, and the concentration of thrips volatiles would be lowest on these discs.
Figure 2. Mean percentage (+ s.e.) of predator eggs (*I. degenerans*) killed by thrips (*F. occidentalis*) as a function of distance from the food source. Data of 4 adjacent discs are taken together. Numbers above the bars refer to the total number of eggs.

Because some discs received no eggs, neighbouring discs were combined in groups of 4 to calculate predation risk (Fig. 2). Predation on predator eggs by thrips occurred more frequently on the discs near the food source (Fig. 2, Kruskal-Wallis one-way ANOVA by ranks on first three groups of discs: KW=7.51, df=2, P<0.05; because very few eggs were laid on the fourth and fifth group of discs these were excluded from the analysis). It can be concluded that the presence of thrips changes the oviposition behaviour of *I. degenerans*, which leads to decrease of predation risk with distance from the food source.

**EFFECT OF THRIPS ALARM PHEROMONE ON EGG DISTRIBUTION OF I. DEGENERANS**

The previous experiment suggests a role of volatile cues in the avoidance of oviposition near thrips by predators. We therefore carried out experiments with the alarm pheromone of thrips, using a linear chain of discs rather than a U-shaped chain. We found no difference in the oviposition pattern in the two experiments when the alarm pheromone was applied close to the food, and therefore pooled these results for ease of comparison (Fig. 3A). With alarm pheromone present close to the food, females oviposited further away from the food source (Fig. 3A). The oviposition distance is significantly higher in the presence of thrips alarm pheromone (*D*=6.7) than the control (*D*=3.12, Fig. 3B) (two-sided Mann-Whitney U Test, *P*=0.008). When the thrips alarm pheromone was placed away from the food on disc 9, the females avoided ovipositing near the alarm pheromone and they laid eggs on the first four discs (Fig. 3C). The oviposition distance is significantly lower when the alarm pheromone was placed on disc number 9 (*D*=2.4), than when it was placed close to the food (two-sided Mann-Whitney U Test, *P*=0.00003). These data suggest that females can acquire information concerning the presence of their egg predators on a patch by perceiving chemical cues associated with them.
PREDATORY MITES AVOID OVIPOSITING NEAR COUNTER-ATTACKING PREY

FIGURE 3. Distribution of predator (*I. degenerans*) eggs on each of 9 interconnected discs (see inset for experimental set-up) in presence of thrips (*F. occidentalis*) alarm pheromone on disc 1 (A), in absence of alarm pheromone (B), and with alarm pheromone on disc 9 (C). Food was invariably provided on disc 1. Pieces of cotton thread, serving as oviposition site, were placed on every disc. The number of replicates was 8 for the control, 16 for 'pheromone on disc 1', and 9 for 'pheromone on disc 9'. The mean oviposition distance *D* is indicated by an arrow below the X-axis (see text for further details).

DISCUSSION

In another paper, we show that eggs of *I. degenerans* that were put in flowers run a higher risk of being killed by thrips than eggs on leaves (Faraji *et al.*, in prep.), and predators avoid ovipositing in flowers even in absence of thrips. This explains the frequent commuting of adult females from flowers, where they feed, to leaves to oviposit. In this paper, we investigated a scenario where pollen is present outside flowers, which happens frequently
because pollen rains down from flowers on leaves. In case thrips and other predators are absent, these pollen patches would offer safer oviposition sites than flowers, but in their presence, these patches may again be risky sites for eggs. Using the chain of discs, we simulated such a situation where pollen is present locally (disc 1) and oviposition sites everywhere (all discs had cotton threads). Thus, predators could oviposit close to the food source, thereby saving time and energy that would otherwise be spent in commuting between a food source and oviposition sites. Moreover, their offspring would have food nearby. However, thrips may also visit the patch with pollen. The predatory mites therefore have to balance the benefits of nearby pollen against the costs of egg predation and travel energy.

Our experiments showed that, in absence of thrips, female predatory mites indeed lay eggs close to the food source whereas females avoided oviposition near the disc with pollen when thrips was around. The percentage of eggs killed decreased with increasing distance to the disc with pollen, but the risk only decreased gradually with disc number and did not approach zero, since there were always some eggs killed. Hence, females reduce the risk of predation on their eggs by laying them further away from the disc with pollen. For the predator larvae emerging from eggs that were oviposited away from the food source, this obviously entails costs of finding food. As in many other phytophagous mites, larvae of *I. degenerans* do not need food (van Rijn & Tanigoshi, 1999). Moreover, the larvae and nymphs of this species are particularly mobile, and were found to move quickly within plants (Faraji et al., in prep.). We therefore expect the juveniles not to have many difficulties in finding food for further development.

To further study the underlying mechanisms, we used the alarm pheromone of thrips as a chemical cue that is produced by thrips when disturbed by other thrips or attacked by predators. Synthetic alarm pheromone was applied either on the disc with pollen or at the opposite end of the chain of discs. In absence of the pheromone, females oviposited close to the pollen, whereas they avoided ovipositing near the pollen when the pheromone was present. When the pheromone was applied at the opposite end of the chain of discs, predatory mites again oviposited close to the pollen. It can be concluded that the females of this predatory mite use the alarm pheromone of thrips to avoid ovipositing at places where thrips form a threat for the eggs of the predatory mite. Strikingly, the thrips alarm pheromone led to oviposition further away than when thrips themselves were present close to pollen, even though the alarm pheromone was provided in concentrations that are supposed to be realistic according to Teerling et al. (1993a,b). This may be due to the mode of application of the alarm pheromone: while thrips may produce alarm pheromone intermittently, it evaporates continuously when applied manually.

Interestingly, another predatory mite (*N. cucumeris*) uses the alarm pheromone of western flower thrips as a prey-finding kairomone (Teerling et al., 1993). These authors showed that the predatory mite spent a larger proportion of time on leaf discs with pheromone, but they did not assess whether these predators also oviposited on these discs. We hypothesise that the alarm pheromone is used for prey location when predators have to feed and for prey avoidance when predators search for oviposition sites. The fact that the response to the alarm pheromone could be dependent on the state of the predators, is reminiscent of the state-dependent response of the same predator to flower and leaf odours: starved females prefer flower odours, while females with an oviposition drive prefer leaf odours (Faraji et al., in prep.).

Our results show that the oviposition behaviour of the predators is flexible, and can be adjusted to predation risk. It is an open question why the predatory mites do not show the same flexible behaviour with respect to oviposition in flowers; flowers are never used for
oviposition, irrespective of whether thrips were present or not (Faraji et al., in prep.). One explanation for such fixed avoidance is that flowers are risky places to oviposit even when no egg predators are present yet, since these predators will inevitably visit flowers in the future. Another possible explanation is that many flowers, including those of sweet pepper, are short-lived and, after shedding, the predatory mite larvae emerging from the eggs will have to migrate back onto the host plant (van den Meiracker & Sabelis, 1993).

Since defence tactics entail costs, one might expect the organism to assess the risk of predation risk as accurate as possible (Kats & Dill, 1998). Chemical cues of predators are important information sources for such an assessment. This is shown especially for aquatic systems (Kats & Dill 1998), but evidence is increasing that volatile and non-volatile chemical cues are also important for predator avoidance in terrestrial arthropods (Grostal & Dicke, 1999; Pallini et al., 1998, 1999; Venzon et al., 2000, Persons et al., 2001). Our work on I. degenerans suggests that these mites use the chemical cues of their prey, which is a potential egg predator, to assess the predation risk and respond accordingly by oviposition away from risky places.

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