Voluntary Falling in Spider Mites in response to Different Ecological Conditions at Landing Points

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Abstract We examined voluntary-falling behaviour by adult females of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) and one of its major predators *Neoseiulus californicus* McGregor (Acari: Phytoseiidae). Experiments were conducted using a setup in which mites could only move onto one of two landing points by falling. Significantly more *T. urticae* females fell onto available food leaves compared to non-food or heavily infested leaves, whereas significantly fewer females fell onto leaves with the predatory mite *N. californicus* compared to leaves without the predator. This suggests that spider mites can actively choose on which patch to land on the basis of food quality and predation risk on the patch. Using the same experimental setup, starved *N. californicus* females never fell, suggesting that falling *T. urticae* females gain the potential advantage of predator avoidance.

Keywords Decision-making ▪ downward vertical dispersal ▪ landing point ▪ *Tetranychus urticae* ▪ voluntary falling

Introduction

For small, terrestrial, wingless arthropods, common forms of dispersal are aerial dispersal using air currents as well as ambulatory dispersal (e.g., Washburn and Washburn 1984; Croft and Jung 2001; Duffner et al. 2001). Using ambulatory dispersal, animals can actively determine their destinations, often making detours to reach separate objects at a distance of more than one body length. In contrast, although aerial dispersal enables long-distance movement (e.g. Lawson et al. 1996; Uesugi et al. 2009), animals cannot actively determine the time or destination of wind-dependent dispersal. In cases of downward vertical dispersal, voluntary falling appears to offer both advantages of the aforementioned means of dispersal: directional movements and a beeline toward an object. However, voluntary falling as a means for dispersal in arthropods has been reported only in ants and aphids. Haemig (1997) argued that wood ants, *Formica aquilonia,* voluntarily fall from trees as a shortcut to the ground and/or to avoid predation by birds. Losey and Denno (1998) reported that pea aphids, *Acyrthosiphon pisum*, voluntarily fall from host plants in the presence of predators.

The body length of the spider mite, *Tetranychus urticae* Koch (Acari:Tetranychidae), is at most 0.5 mm. Therefore, observations of their behaviours are possible only under stereomicroscopes. This mite uses both ambulatory and aerial dispersal (Margolies and Kennedy 1985; Smitley and Kennedy 1985), and mated adult females are considered the dispersing stage (Margolies and Kennedy 1985). Aerial dispersal of *T. urticae* females is distinguished from accidental blow-offs by a specific posture they exhibit before taking off: lifting the first legs upright and raising the forebody (Smitley and Kennedy 1985; Li and Margolies 1993). When synthetic pesticides are applied to a host plant leaf, *T. urticae* females individually hang down by spinning threads, thereby avoiding the leaf (Penman and

Chapman 1983). Although this spindown behaviour likely represents a means of downward vertical dispersal, it has only been observed in response to pesticide applications (e.g., Penman and Chapman 1983; Suiter and Gould 1994).

Using video recordings, we conducted preliminary observations of *T. urticae* female behaviour. Mites did not spin down but would instead suddenly fall without exhibiting any specific postures as in cases of aerial dispersal (Ohzora, unpublished data), suggesting that distinguishing between voluntary and accidental falling of *T. urticae* females through behavioural observations may be impossible. An alternative approach to identifying voluntary falling may be to demonstrate adaptive decision-making of *T. urticae* females in response to different conditions. Therefore, we hypothesise that *T. urticae* females will fall more often on patches on which they are more likely to have a higher fitness.

Here, we examined the falling behaviour of *T. urticae* adult females depending on food availability and predators at landing points. Given the possible predator avoidance by *T. urticae*, we also examined the falling behaviour of the predatory mite, *Neoseiulus californicus*.

Materials and Methods

Test Animals

The study population of *T. urticae* was collected from a rose (*Rosa* cv. Rosaceae) garden in Kyoto, Japan, and maintained on expanded primary leaves of the kidney bean *Phaseolus vulgaris* L. (Leguminosae; hereafter referred to as “bean”) that were pressed onto water-saturated cotton in petri dishes (90 mm diameter, 14 mm deep). We also maintained a *T. urticae* population (stock culture) on potted bean plants. *Tetranychus kanzawai* Kishida

(Acari, Tetranychidae), a close relative of *T. urticae*, was collected from a strawberry (*Fragaria ananassa* Duch. Rosaceae) garden in Kyoto and maintained on bean leaves as described above. The study population of *N. californicus* was collected in Nagano, Japan, and reared on bean-leaf discs heavily infested with the stock culture of *T. urticae* (30–50 adult females and immature mites per leaf). All petri dishes were placed in a transparent plastic container and kept at 25ºC and 65% relative humidity, with a light:darkness photoperiod of 16:8 hours (hereafter, "laboratory conditions"). All experiments were conducted under these same conditions.

Experimental setup

We studied the falling behaviour of spider mites using dual choice tests. Two squares (25 × 25 mm) of either Parafilm (Parafilm M, American National Can, Chicago, IL) or leaf were placed on water-soaked cotton in a Petri dish (Fig. 1). A 15-mm diameter Parafilm circle containing the mite to be tested (either a mated female *T. urticae* or *N. californicus* with 2-4 days since maturation) was placed 10mm apart from the squares. The base of a circuit composed of vinyl-coated wire (0.7 mm in diameter) was placed on the Parafilm circle. The other end of the circuit was arranged 5 mm above the two squares. Because the circle and squares were surrounded by a water barrier, the mites could move to the squares only by falling from the circuit above. Preliminary experiments had confirmed that after falling onto the squares, mites never returned to the circuit above. This further indicates the falling behaviour is different from spindown in which the mites climb back up onto a place from which they had descended. When reusing the circuits, the wire was carefully wiped with a piece of water-soaked absorbent cotton to prevent mites from following *T. urticae* trails from previous trials (Yano 2008).

Preference of *T. urticae* females falling onto food vs. non-food patches

To examine whether *T. urticae* females fall onto food and non-food landing points with different probabilities, one Parafilm and one bean-leaf square were placed 5 mm apart on the experimental apparatus (Fig. 1). To control for potential environmental biases, the positions of the two squares were randomised. Fifty *T. urticae* females were then individually introduced onto 50 Parafilm circles. The experimental setup was then placed in a transparent plastic container to eliminate wind as a mode of aerial dispersal. After 48 h, we recorded which square contained females.

Preference of *T. urticae* females falling onto slightly vs. heavily infested leaves

To examine whether *T. urticae* females fall with different probabilities in response to the feeding damage of landing points, two bean-leaf squares differing in the degree of infestation were placed in the experimental setup. Damaged leaves were selected from the potted bean plants on which the stock culture of *T. urticae* was maintained. Bean leaves were chosen that had either injury scars on >50% (hereafter, “heavily infested”) or on <10% (hereafter, “slightly infested”) of the leaf area. Since injury scars of the mite can be clearly identified by their white color, the degree of damage was determined with the naked eye. We removed all stages of *T. urticae* from the leaf squares. Heavily and slightly infested leaf squares were then placed on the setup, and the falling behaviour of *T. urticae* females was tested as described above, using 40 replicates.

Preference of *T. urticae* females falling onto leaves with or without a predator

To examine whether *T. urticae* females fall with different probabilities in response to the presence of a predator on the landing points, bean-leaf squares were prepared with or without a *N. californicus* female. To retain the predator on a bean square, we introduced three *T. kanzawai* females as prey onto each leaf square in the experimental setup. After 24 h, a *N. californicus* female was introduced onto a randomly selected square in each setup. One *T. urticae* female was then introduced onto each Parafilm circle, and her falling behaviour was tested as described above, using 30 replicates. Because *T. kanzawai* was used as prey, we were able to easily discriminate fallen *T. urticae* females from prey mites.

Does *N. californicus* fall onto *T. urticae*–infested leaves?

To examine whether *N. californicus* females fall onto leaves infested by its prey, *T. urticae*, bean-leaf squares were prepared with or without *T. urticae* females. Five *T. urticae* females were introduced onto a randomly selected square on each experimental apparatus. After 24 h, a *N. californicus* female was introduced onto each Parafilm circle after having been starved in a 1.5-ml microtube (Treff AG, Degersheim, Switzerland) containing a water droplet for 48 h. The falling behaviour of *N. californicus* was then tested as described above, using 22 replicates.

Statistical analysis

Unless otherwise noted, experimental outcomes were analysed using two-tailed binomial tests, with the common null hypothesis that a *T. urticae* female would fall onto either of the two squares with equal probability (i.e., 0.5).

Results

Preference of *T. urticae* females falling onto food vs. non-food patches

Significantly more *T. urticae* females were found on bean squares than on Parafilm squares (23:3, binomial test: $P < 0.001$), indicating that *T. urticae* females preferred to fall onto a food patch rather than onto a non-food patch (Fig. 2). Twenty-four of 50 tested females were not found on either square (48.0%). Most of these females were drowned within the water barrier or remaining on Parafilm circles, suggesting that they had failed to enter the wire circuits.

Preference of *T. urticae* females falling onto slightly vs. heavily infested leaves

Significantly more *T. urticae* females were found on slightly infested bean squares than on heavily infested squares (16:2, binomial test: $P < 0.001$), indicating that when falling, *T. urticae* females can assess the relative food quality of landing points (Fig. 2). Twenty-two of 40 tested females were not found on either bean square (55.0%).

Preference of *T. urticae* females falling onto leaves with or without a predator

Significantly fewer *T. urticae* females were found on bean squares harbouring a *N. californicus* female than on squares without the predator (2:12, binomial test: $P = 0.0065$), indicating that upon falling, *T. urticae* females can assess the presence or absence of the predatory mite on a landing point (Fig. 2). Sixteen of 30 tested females were not found on either bean square (53.3%). The proportion of females that were not found on either square did not differ among the three experiments (Chi-square test: $\chi^2 = 0.48$, $P=0.79$), suggesting that it was due to the design of the experimental setup.

Does *N. californicus* fall onto *T. urticae*–infested leaves?

This experiment was originally intended to examine whether *N. californicus* females prefer to fall onto bean leaves infested by *T. urticae*. Although starved *N. californicus* females were observed crawling on wire circuits above the bean-leaf squares, no females (of 22 tested) were found on either square after 48 h, indicating that *N. californicus* does not fall.

Discussion

If *T. urticae* females only fall by accident, they should fall onto the two landing points with equal probability. However, the proportion of *T. urticae* females falling onto each square diverged significantly from 50%, indicating that females can control onto which patch they fall. Moreover, the patches on which they fell more frequently were consistently those on which their fitness is expected to be the highest (food vs. non-food, slightly vs. heavily infested, predator-free vs. with predators). This suggests that spider mites use information from the patches on which they can land to actively decide on which patch to fall. Because local food shortages reduce the fitness indices of *T. urticae* (Mitchell 1973; Oku et al. 2002) and *N. californicus* is one of the most effective predators of spider mites (Castagnoli et al. 1995), *T. urticae* is likely to have evolved the ability to avoid landing points with adverse ecological conditions.

The next question that arises is: which proximate cues could potentially be used by spider mites? A preliminary experiment (Ohzora, unpublished results) suggested that visual cues are not involved in the falling behaviour. In contrast, there is ample evidence that spider mites can perceive the condition of a distant food plant by detecting its odour. Adult females of *T. urticae* avoid the odour of lima bean plants heavily infested by conspecifics (Dicke 1986), while they prefer the odour of cucumber plants infested by conspecifics (Pallini et al. 1997). Moreover, spider mites can avoid predators on a distant plant by detecting the odour of predatory mites (Magalhães et al. 2002), the odour of injured conspecifics (Oku et al. 2003), and the odour of a plant infested by omnivorous predators (Pallini et al. 1997). Therefore, when falling, *T. urticae* females may similarly assess the food quality and predation risk of landing points by perceiving informative odours.

Our results provide evidence that *T. urticae* females voluntarily fall as a means of downward vertical dispersal. In this way, *T. urticae* females could easily move between neighboring plants that have overlapping leaves but are not in direct contact, i.e. separated at a distance of more than one body length (0.5 mm). Moreover, the falling behaviour may confer another advantage of predator avoidance on spider mites. Mated adult females are considered to be the dispersal stage of predatory mites (Pels and Sabelis 1999; Jia et al. 2002). Shimoda et al. (2005) demonstrated that starved *N. californicus* females respond to the odour of *T. urticae*–infested lima bean plants in Y-tube wind tunnels; thus, *N. californicus* females crawling 5 mm above *T. urticae*–infested bean squares were expected to perceive the odour of their prey. However, *N. californicus* females failed to fall onto either infested or noninfested bean squares, indicating that these mites do not fall. It is quite striking that *T. urticae* females can voluntarily fall and also lay threads while crawling to prepare for accidental falls (Saito 1977), yet the predatory mites neither lay threads nor exhibit such flexible falling behaviour. Thus, these experiments further suggest that falling *T.*

urticae females can escape from pursuit by *N. californicus* predators. Moreover, Yano and Osakabe (2009) recently reported that a predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), follows the trails left by *T. urticae* females. Therefore, *T. urticae* females that fall even a short distance may further reduce predation risk by interrupting their trail. The predator avoidance advantage of falling behaviour in *T. urticae* is similar to that of flying fishes, which fly above water not for purposes of migration but for avoiding sub-surface predators (Davenport 1994).

In future studies, our experimental setup should be altered to answer several additional questions. First, a considerable number of *T. urticae* females failed to enter the wire circuit, which reflects an incomplete design of the present setup. Second, the current experimental setup did not allow the examination of *T. urticae* falling in response to predators: when *T. urticae* and *N. californicus* females were both introduced onto a Parafilm circuit, the former would run into the surrounding water barrier instead of falling from the wire circuit. Such effect of spider mite’s experiences before falling should be examined using improvements to the experimental apparatus. The relative importance of falling vs. another dispersal means and the ability to fall from higher distances should also be examined in future experiments.

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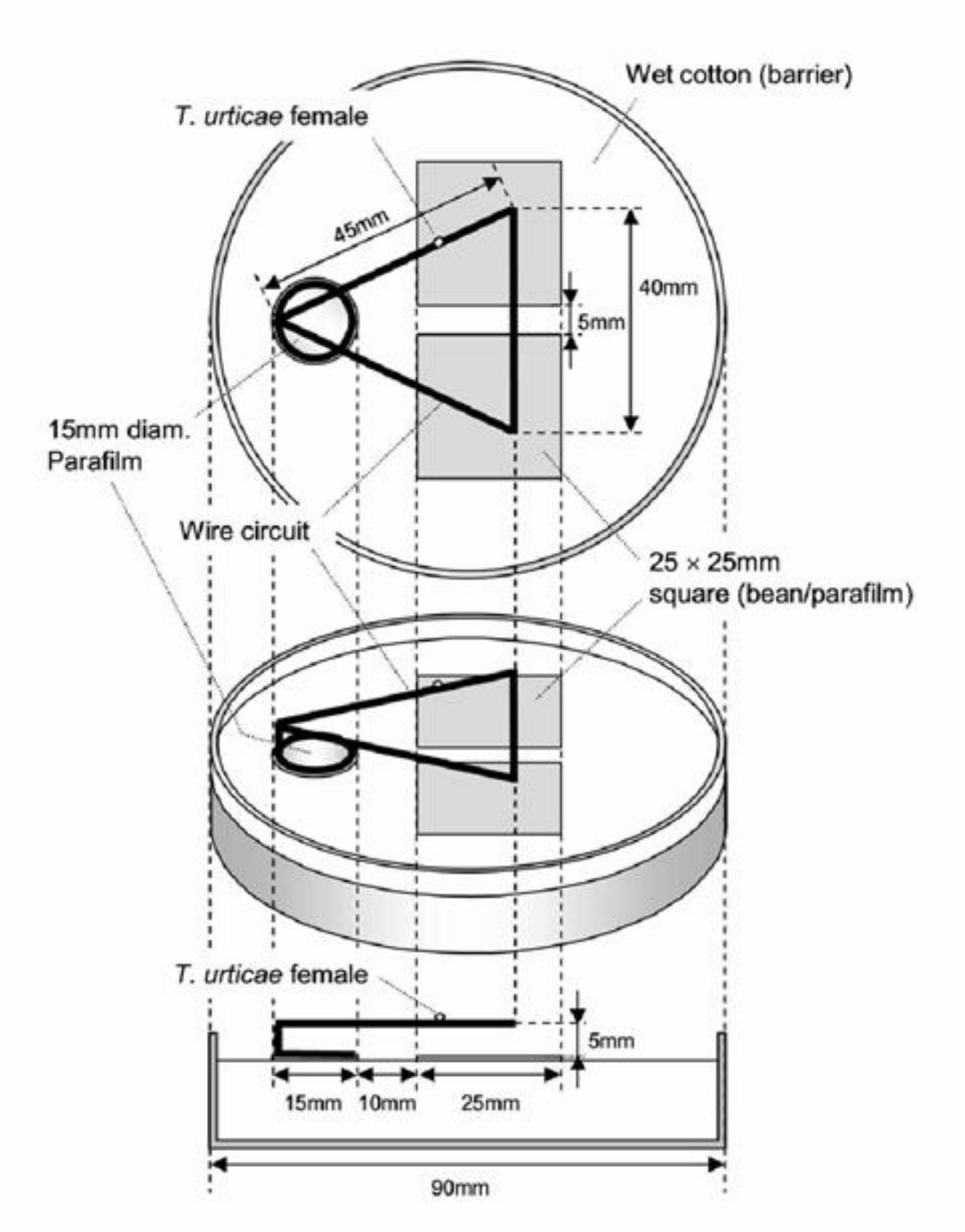
Figure captions

Fig. 1 Experimental setup used to examine the falling behaviour of *T. urticae* and *N. womersleyi* females. Females could move onto alternative landing squares only by falling from the wire circuit above.

Fig. 2 Fraction of *T. urticae* females falling onto a) food vs. non-food materials, b) slightly vs. heavily infested bean leaves, and c) leaves with vs. without a predatory mite, *N. womersleyi*.

Fig. 1


Wet cotton (barrier)
T. urticae female
45mm
40mm
5mm
15mm diam.
Parafilm
Wire circuit
25 × 25mm
square (bean/parafilm)
T. urticae female
5mm
15mm
10mm
25mm
90mm

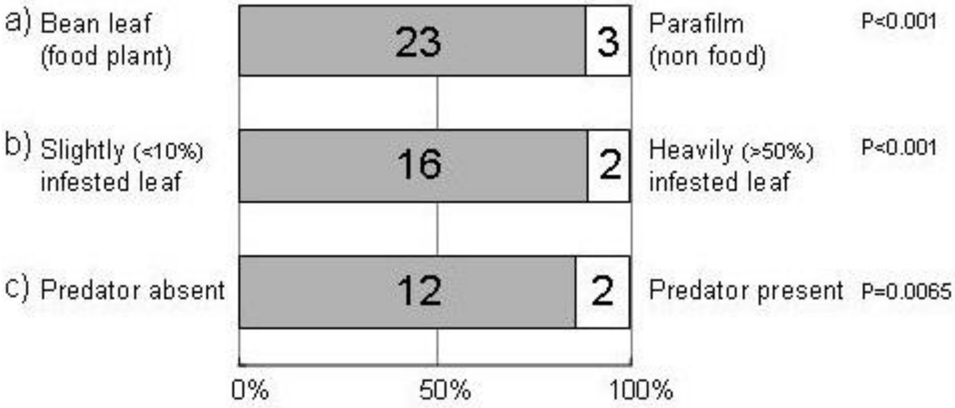

a) Bean leaf (food plant)
23
3
Parafilm (non food)
P<0.001
b) Slightly (<10%) infested leaf
16
2
Heavily (>50%) infested leaf
P<0.001
c) Predator absent
12
2
Predator present
P=0.0065
0%
50%
100%

Fig. 2