



The effect of predation risk on an acarine system

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Current theory on trait-mediated interactions in tri-trophic food chains shows that antipredator behaviour of the middle species can cause similar indirect effects giving rise to trophic cascades as mediated by density changes. In this article, the effect of predation risk in a tri-trophic food chain (bean plant—two-spotted spider mite prey—predatory mite, *Phytoseiulus persimilis*) is measured, both on plants (changes in leaf damage) and spider mites (changes in egg numbers, mortality rate and dispersal behaviour), under two risk scenarios. In the predator risk treatment a predatory mite was caged in an experimental cell that was placed above a leaf disc with spider mites. This prevented the predator from subduing prey while the predator was perceived by spider mites as a potential threat. In the predator cues treatment a predatory mite was introduced to the leaf disc for 24 h before placing the spider mites on the disc. Compared to control without predators, after four days we observed in both risk treatments a nonsignificant increase in plant damage per spider mite and a significant decline in spider mite fecundity. No significant effect of predation risk on spider mite mortality was observed. We also showed that the above effects are not uniformly distributed in time. For example, the spider mite fecundity and dispersal rate in the predator cues treatment were significantly different from the control only the first day. We also found that under predator cues treatment, walking activity of the spider mites increased significantly.

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The ability to detect and avoid predators is a crucial trait that influences survival, a key component of animal fitness. Behavioural traits related to predation risk are: habitat or diet shift, increase use of refugia, changes in time and/or activity budget (reviewed in Lima & Dill 1990; Bolker et al. 2003), delayed oviposition (Hoffmeister & Roitberg 1997) and diapause induction (Kroon et al. 2004, 2005). Abrams (1996) introduced the idea that trait responses of consumers to predators can lead to a wider

range of indirect interactions (i.e. interactions between resources and predators that are mediated by consumers) in food webs than those that are mediated via direct predation effects. A classical example of indirect density-mediated interactions in food chains are trophic cascades where an increase in top predators leads to an increase in resources due to decrease in consumer densities (Paine 1980; Carpenter et al. 1985; Polis 1999; Polis et al. 2000). However, recent studies (reviewed in Schmitz et al. 2004) suggest that behavioural changes in the intervening species can also give rise to the cascading effect. In fact, whereas density effects are limited by physiological constraints of predators (the gut capacity, the rate of metabolism, etc.), trait-mediated effects caused by mere presence of predators (that need not feed on herbivores) are not limited by these constraints, and influence the entire prey population. Thus, potentially, they can lead to a strong cascading effect (Peacor & Werner 2001).

In this study we consider a tri-trophic food chain consisting of bean plants, *Phaseolus vulgaris* L., var. Katka

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(resource), the two-spotted spider mite, *Tetranychus urticae* Koch. (herbivorous consumers), and the predatory mite, *Phytoseiulus persimilis* A.-H. (predators). To study the effects of predation risk we created two types of risk treatments. In the first treatment ('predator risk'), a predatory mite was caged in a cell above the leaf disc preventing it from feeding on spider mites, while in the second treatment ('predator cues'), leaf discs were exposed to predators before placing the herbivores on them. Since direct contact of herbivores with predators was not possible in either type of risk treatment, spider mites could be warned about the potential risk by olfactory stimuli and/or by perceiving predators moving by sight (Helle & Sabelis 1985). The effects of chemical substances (called info chemicals) on the behaviour of prey mites were reviewed by others (Dicke 1986; Janssen et al. 1997; Kriesch & Dicke 1997; Pallini et al. 1997, 1999; Grostal & Dicke 1999; Oku et al. 2003a, b). The chemical compounds are either volatile and/or contact. While the former are perceived without contacting the source, the latter evoke a behavioural response only after a direct physical contact with the solid or liquid form. Our two types of risk treatments were designed to study the effect of these chemical compounds on spider mite behaviour separately. In all treatments we observed the damage caused by herbivores to the bean leaf, the total number of spider mite eggs per leaf disc, the number of dead spider mites on the disc, and the number of spider mites that escaped from the disc. In separate experiments we observed the walking pattern of spider mites on a predator-exposed leaf disc and time dynamics of *T. urticae* fecundity, mortality and dispersal rate on a leaf disc pre-exposed to predator.

Based on some recent studies, we expect that predation risk in our acarine system can cause: (1) a trophic cascading effect (Schmitz et al. 1997), (2) a decrease in fecundity (e.g. due to changes in energy budget caused by predation risk, Grostal & Dicke 1999; Oku et al. 2004, 2006), (3) increased tendency of spider mites to escape from leaf discs (Werner & Peacor 2003; Oku et al. 2003a, b, 2004), (4) an increase in herbivore mortality (Schmitz et al. 1997), (5) changes in the walking pattern of spider mites due to changes in activity level (Werner & Peacor 2003), and (6) increase in fecundity of spider mites after exposition to predation risk (postponed oviposition, Hoffmeister & Roitberg 1997).

METHODS

There were three series of laboratory experiments. The first series studied the effects of predation risk on plant damage, *T. urticae* fecundity, mortality and dispersal. The second series of experiments focused on the effect of predator cues on walking pattern of *T. urticae*. The third series of experiments evaluated the temporal effect of predation risk on *T. urticae* fecundity, mortality and dispersal.

All series of experiments used a specially designed experimental unit. The unit consisted of a disc (diameter 13 mm) cut from a bean leaf and put individually on a piece of plastic foam (35 × 35 mm and 10 mm high approximately) in a petri dish filled with water to supply the leaf disc with moisture. A predatory mite cell, assembled for the predator risk treatment, was placed on top of the leaf disc. The cell was made of two rings cut from a white plastic sheet 1-mm thick (upper one with 35 and 16 mm outer and inner diameter, respectively; bottom one with 35 and 22 mm outer and inner diameter, respectively) and glued together (Fig. 1). Fine mesh (0.2-mm opening) was glued between the rings and on top of the upper ring to form a small cell. When the cell was put above the leaf disc, there was approximately 1 mm distance between the mesh and the leaf surface allowing unrestricted movement of the herbivorous mites on the leaf disc. The predatory mite inside the cell was not able to catch the herbivorous mites, but the distance between the predator and its prey was small enough to ensure that the odour of predatory mite, i.e. volatile info chemicals released by *P. persimilis*, was perceived by spider mites as a potential threat. The cell did not physically prevent spider mites to leave the leaf disc (Fig. 1) in which case the spider mites drowned on the water saturated plastic foam.

All experiments were carried out in climate controlled cabinets at a constant temperature of $25 \pm 1^\circ\text{C}$ and a photoperiod of 18:6 h light:dark cycle. We used one cabinet per treatment to avoid possible interactions via volatile substances between treatments.

Predation Risk

Design of experiments

The experiment was arranged in a randomized block design with four experimental treatments per block. Leaf

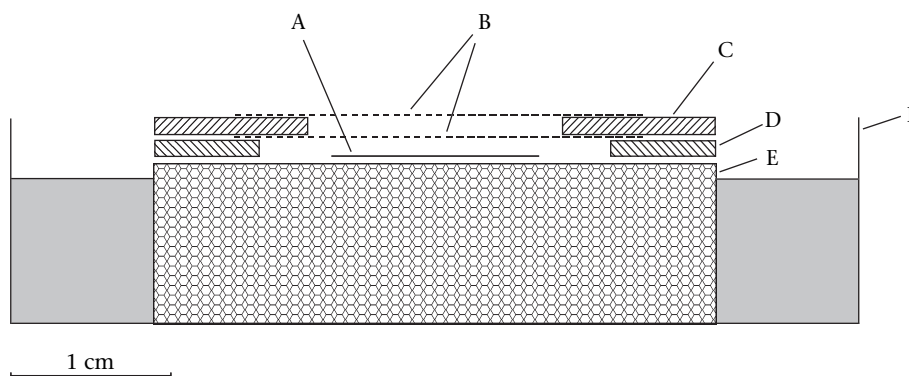


Figure 1. Side view of the experimental unit with a predatory mite cell. A = leaf disc; B = fine mesh; C = upper plastic ring; D = bottom plastic ring; E = water saturated plastic foam; F = petri dish filled with water.

discs (taken from the second node of 2- or 3-week-old plant) used in treatments within one block were taken from the same bean leaf to account for between-leaf variability. Each treatment was replicated 30 times. Leaf discs within a block were randomly assigned to the following treatments: (1) no mites (empty disc); (2) spider mites only (P-); (3) spider mites and a predatory risk mite (Rp+), and (4) spider mites and predator cues (Cp+). The last two treatments represented experimental set-up for studying trait-mediated indirect interactions. Treatment P- consisted of 10 adult *T. urticae* females only. In the predator risk treatment (Rp+) one well-fed *P. persimilis* female was introduced into the cell above the leaf disc. For the predator cues treatment (Cp+), the leaf disc was first exposed to one adult *P. persimilis* female for 24 h to ensure that the expected contact info chemicals remain on the leaf surface. Then the predatory mite and all its eggs (if any) were gently removed from the disc using a hair loop and 10 adult *T. urticae* females were added. A new predatory female was used for each leaf disc. To ensure the same experimental conditions, the leaf discs for all treatments were cut one day before spider mites were added.

One disc was left free of mites and was used only to normalize damage inflicted by spider mites to other discs (see below). Empty predatory mite cells were placed above all leaf discs to achieve the same experimental conditions for all treatments. Experimental units were then placed individually in four climatized cabinets (one cabinet per treatment) and were inspected every 3–6 h to add water to petri dishes as necessary. The experiments lasted for 4 days (96 h exactly). The four cabinets were allocated at random to the treatments at each run.

Observed variables and statistical analysis

The total number of eggs per leaf disc was counted using a dissection microscope and a mechanical counter. The total number of dead spider mites (excluding drowned spider mites) per leaf disc was also counted and mortality (i.e. the proportion of dead spider mites out of 10 initially introduced mites) was calculated. To assess the effect of predation risk treatments on spider mite fitness we corrected data on fecundity for observed mortality of *T. urticae*. We calculated fecundity per average surviving female as $F = E / [(S_0 + S_T) / 2]$ where E is the total number of eggs per disc, S_0 (=10) and S_T are numbers of live spider mites at the start and end of the experimental period, respectively. Leaf discs were then gently cleaned of organisms using a fine artists' brush, scanned, and the normalized proportion of leaf area damaged (NPLAD) was calculated for every experimental treatment according to the method described by Škaloudová et al. (2006). NPLAD was defined as the ratio of the number of leaf image pixels in the damaged area (A) to the total number of pixels of the entire leaf image (B) corrected for false positively marked pixels using leaf discs without mites ($NPLAD = (A_T/B_T - A_C/B_C) / (1 - A_C/B_C)$, where treatment is denoted by T and the empty leaf disc by C . We remark that NPLAD of the empty leaf disc is zero). Then we calculated NPLAD per an average surviving female ($NPLAD / ((S_0 + S_T) / 2)$).

As there were a few missing data, we used unbalanced multivariate analysis of variance (MANOVA) using the Generalized Linear Models procedure (PROC GLM) in SAS (SAS Institute 2000). Statistical differences between control (P-) and predation risk (Rp+ and Cp+) and between Rp+ and Cp+ group means were obtained with CONTRAST statement in GLM procedure. When the MANOVA analysis was significant ($P < 0.05$), univariate ANOVAs for the individual effects and Tukey–Kramer post hoc tests to detect significant differences between particular treatments were conducted. Since NPLAD per surviving female, mortality and proportion of drowned mites data are not normally distributed, the arcsine square-root transformation (Sokal & Rohlf 1969) was applied to normalize the data before statistical analysis. All statistical tests use two-tailed probabilities.

The Effect of Predator Cues on Herbivore Behaviour

Design of experiments

We studied the effect of predator cues (i.e. contact info chemicals) on walking behaviour of spider mites. Two discs were cut from a single bean leaf and each formed one experimental unit as described in the previous series. One leaf disc was exposed to *P. persimilis* female for 24 h while the other one was left clean. The experimental set-up was similar as in the previous series except that no predatory mite cells were placed above the leaf disc, which allowed us to monitor the behaviour of spider mites. Locomotory activity of spider mites was observed in an open arena made of a small petri dish (6 cm diameter) filled with water preventing spider mites from escaping. Both the discs, i.e. the clean disc and the disc with predator cues, were transferred to these experimental units in which a piece of black cloth was placed on the plastic foam to ensure a dark background necessary for automatic measurement of spider mite walking activity. A single adult female of *T. urticae* was introduced on the first disc and the unit was placed in a climate controlled cabinet where activity was observed. The cabinet was equipped with a circular fluorescent tube providing even illumination of the experimental arena.

Walking patterns of spider mites were observed by means of an analogue B&W camera Panasonic, Model WV 1350AE/C equipped with a 50-mm lens and a macro ring. The camera was fixed vertically 20 cm above the centre of the experimental arena. A composite video signal was fed into a computerized video tracking system consisting of a video monitor, a personal computer with a frame grabber (Targa Plus, True Vision) and EthoVision software (Noldus Information Technology 1997). To increase contrast between the background and the spider mite, a small amount of magenta fluorescent dust (Radiant color NV, type JST 18) was applied on the dorsal part of the spider mite's body with a fine brush and a red photographic filter was placed in front of the camera lens. Using this set-up the mite looked like a white spot on the dark background. The location of the spider mite was determined automatically. The co-ordinates of the centre of each animal's body ('centre of gravity') were

calculated using a spatial resolution of 254×238 pixels, corresponding to 0.465 and 0.366 mm in the x - and y -direction, respectively. The tracking was done at a frequency of 10 samples/s, which gave a sufficiently accurate representation of the track.

Two recordings of activity were obtained for each spider mite: one on the clean disc and the other one on the disc with predator cues. Half of the experiments started with the clean disc and half with the predator cues disc to rule out possible 'learning effect'. Measurements began after a 10-min habituation period and they lasted for another 10 min. Then the spider mite was transferred to the second disc and the measurement was repeated. The experiment was repeated 20 times at 25°C; new *P. persimilis* and *T. urticae* were used for each repetition.

Observed variables and statistical analysis

Five behavioural parameters were calculated from the digitized paths of the spider mites: (1) the total path length (TPL); (2) the mean velocity over the entire observation time (MV); (3) the absolute angular velocity (AAV); (4) the absolute meander (AM); and (5) percentage of time spent moving (PTSM). The TPL is defined as the sum of distances, measured in a straight line, moved by a mite between two consecutive samples. The MV denotes a distance moved by a mite per unit time (s). The AAV is defined as the absolute change in direction of movement of a mite per time unit and ranges between 0 and 180°/s. The AM is the absolute change in direction of movement of a mite relative to the distance moved. It indicates tortuosity of walking path and ranges from 0 to 180°/cm. The PTSM is defined as a percentage of time in which a mite moves. For detailed algorithms of parameter calculation see Noldus Information Technology (1997). The differences in these characteristics for the two different treatments (i.e. with and without predator cues) were tested using Wilcoxon signed-ranks test (Siegel & Castellan 1988). In addition, we tested if the order of leaf discs (clean first versus cues first) had any effect on the behaviour of spider mites. For this purpose the ratio between PTSM on the cues disc and PTSM on the clean disc was calculated and the two series were compared using Mann–Whitney test (Siegel & Castellan 1988).

Temporal Effects of Predator Cues

Additional experiments were conducted to reveal how and if response of spider mites to predator cues attenuates over time. The experimental set-up was similar to the predation risk experiments described above, except that no predatory mite cells were put above the leaf discs to enable daily inspection of discs. There were two treatments: control leaf discs not exposed to a predatory mite (P–) and the risk treatment with predator cues-treated leaf discs (Cp+). Data on spider mite fecundity, mortality and proportion of drowned mites were collected for 4 consecutive days.

The obtained time series data were analysed using a doubly multivariate repeated measures design (repeated measures MANOVA). When the analysis was significant

($P < 0.05$), univariate repeated measures ANOVAs for the individual effects and Tukey tests were conducted. Data on mortality and proportion of drowned spider mites were normalized using the arcsine square-root transformation before the statistical analysis. Computations were done by means of PROC GLM in SAS/STAT package (SAS Institute 2000) with option statement REPEATED.

RESULTS

Predation Risk

Fecundity and NPLAD per an average surviving *T. urticae* female, proportion of drowned spider mites and their mortality rate are shown in Fig. 2. Results of the MANOVA indicate that there was a highly significant overall treatment effect (Wilk's $\lambda = 0.554$, $F_{8,90} = 3.87$, $P = 0.0006$) and a highly significant overall block (leaf) effect (Wilk's $\lambda = 0.050$, $F_{116,181} = 1.75$, $P = 0.0003$). Overall contrast between control treatment (P–) and predation risk (Rp+ plus Cp+) treatments was also highly significant (Wilk's $\lambda = 0.585$, $F_{4,45} = 8.00$, $P < 0.0001$). No significant differences were found between Rp+ and Cp+ treatments (Wilk's $\lambda = 0.941$, $F_{4,45} = 0.70$, $P = 0.594$).

The effect on NPLAD

An increase of NPLAD per a live female was observed in both Rp+ and Cp+ treatments compared to the control (Fig. 2a). The univariate ANOVA model for NPLAD was highly significant ($F_{31,48} = 4.60$, $P < 0.0001$) and explained 75% of the variability. The ANOVA revealed a highly significant leaf effect ($F_{29,48} = 4.38$, $P < 0.0001$) on NPLAD while the treatment effect was marginally nonsignificant ($F_{2,48} = 2.54$, $P = 0.089$). Contrasting predation risk treatments (i.e. when data for Rp+ and Cp+ treatments were grouped together) with the herbivore-only treatment (P–) gave significant differences ($F_{1,48} = 4.88$, $P = 0.032$).

The effect on *T. urticae* fecundity

The univariate ANOVA model for fecundity was also highly significant ($F_{31,48} = 3.09$, $P = 0.0002$) and explained 67% of the variability. The ANOVA revealed a highly significant leaf and treatment effect ($F_{29,48} = 2.83$, $P = 0.0007$ and $F_{2,48} = 8.67$, $P = 0.0006$, respectively). The Tukey–Kramer test indicated significantly ($P = 0.0015$ and $P = 0.0053$, respectively) lower spider mite fecundity in Rp+ and Cp+ treatments, respectively (Fig. 2b). There was no significant ($P = 0.992$) difference between Rp+ and Cp+ treatments on *T. urticae* fecundity.

The effect on *T. urticae* mortality

The univariate ANOVA model for mortality was not significant ($F_{31,48} = 1.24$, $P = 0.245$) and explained 45% of the variability. Although a slight increase in mortality was observed in Cp+ treatment (Fig. 2c), the ANOVA revealed no significant leaf and treatment effect ($F_{29,48} = 1.23$, $P = 0.261$ and $F_{2,48} = 1.70$, $P = 0.193$, respectively). Contrast between predation risk treatments (Rp+ and Cp+) and the control (P–) was also not significant ($F_{1,48} = 1.97$, $P = 0.167$).

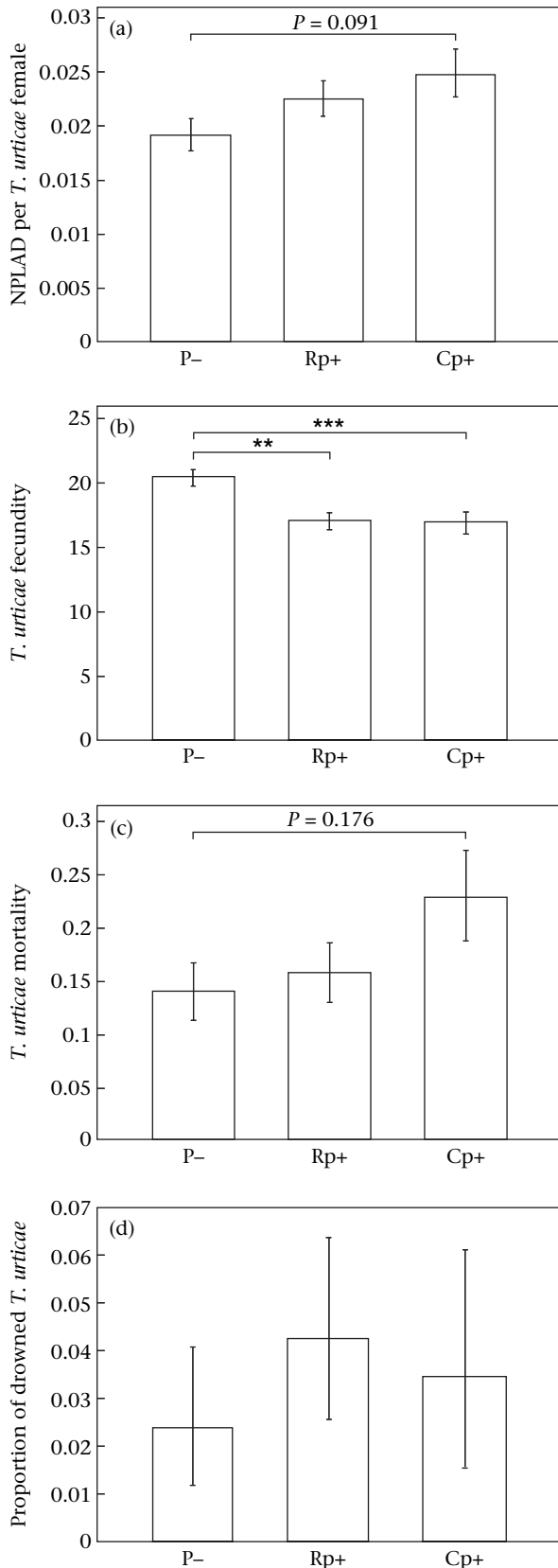


Figure 2. The normalized proportion of leaf area damaged (NPLAD) per an average surviving *T. urticae* female (a), *T. urticae* fecundity per an average surviving female (b), mortality of spider mites (c) and proportion of drowned spider mites (d) under different treatments.

The effect on *T. urticae* dispersal

The univariate ANOVA model for proportion of drowned spider mites was not significant ($F_{31,48} = 0.90$, $P = 0.620$) and explained 37% of the variability. Neither the treatment effect ($F_{2,48} = 0.30$, $P = 0.743$) nor the leaf effect ($F_{29,48} = 0.90$, $P = 0.609$) was significant. Contrast between predation risk treatments (Rp+ and Cp+) and the control (P-) was also not significant ($F_{1,48} = 0.45$, $P = 0.506$).

The Effect of Predator Cues on Herbivore Behaviour

The effects of predator cues on various walking parameters are summarized in Table 1. Out of five observed characteristics, only the percentage of time spent moving (PTSM) significantly increased with predator cues.

No significant differences were found in PTSM between experiments in which spider mites were first exposed to clean discs and experiments in which spider mites were first exposed to discs with predator cues (Mann–Whitney test: $U = 31$, $P_{\text{two-tailed}} = 0.1897$).

Temporal Effects of Predator Cues

Fig. 3a, c, e shows changes in *T. urticae* fecundity, mortality and proportion of drowned spider mites in the experiment. Repeated measures MANOVA revealed a highly significant main effect of time (Wilk's $\lambda = 0.204$, $F_{9,21} = 9.08$, $P < 0.0001$) and a significant main effect for treatment (Wilk's $\lambda = 0.699$, $F_{3,27} = 3.87$, $P = 0.020$). The analysis revealed also a highly significant interaction effect of treatment*time (Wilk's $\lambda = 0.285$, $F_{9,21} = 5.86$, $P < 0.0004$) and leaf*time (Wilk's $\lambda = 0.552$, $F_{9,49} = 4.41$, $P < 0.0003$).

Univariate ANOVA for *T. urticae* fecundity revealed a highly significant effect of time and treatment*time interaction ($F_{3,87} = 21.45$, $P < 0.0001$ and $F_{3,87} = 9.20$, $P < 0.0001$, respectively). Significant decrease in fecundity was found on the first day (Fig. 3a). For mortality, the ANOVA revealed significant effect of time ($F_{3,87} = 4.11$, $P = 0.0089$) but no significant differences between P- and Cp+ (Fig. 3c). Univariate ANOVA for the proportion of drowned spider mites revealed significant effects of treatment and time ($F_{1,29} = 5.06$, $P = 0.032$ and $F_{3,87} = 7.37$, $P = 0.0002$, respectively) and nonsignificant treatment*time interaction ($F_{3,87} = 1.73$, $P = 0.167$). A significant increase in proportion of drowned mites was found on the first day only (Fig. 3e).

To facilitate comparison with the result of the first experiment we also calculated the cumulative effect of Cp+ and P- treatments, respectively (Fig. 3b, d, f). Results

Data points and vertical lines indicate least squares means \pm SE (b) and backtransformed least squares means \pm SE obtained from arcsine square-root transformations of original data (a, c, d). P- = no predator, Rp+ = predator risk (a predator caged in the cell above the leaf disc), Cp+ = predator cues (predator cues present on the leaf disc). Horizontal lines and asterisks indicate differences based on Tukey–Kramer adjustment for multiple comparisons (** $P < 0.01$; *** $P < 0.005$, otherwise P is given explicitly).

Table 1. Effects of predator cues (Cp+) on moving pattern of spider mites

Observed parameter (units)	Control	Predator cues	Wilcoxon test	
	Mean±SE	Mean±SE	Z-statistics	P
PTSM (%)	37.37±4.65	54.54±4.55	-2.613	0.045*
TPL (cm)	7.06±1.35	8.12±1.48	-0.672	1.000
MV (cm/s)	0.03±0.004	0.03±0.003	-0.635	0.526
AAV (°/s)	24.07±0.85	22.12±0.78	-1.704	0.352
AM (°/cm)	846.88±76.16	865.47±68.19	-1.136	0.768

PTSM = percentage of time spent moving; TPL = total path length; MV = mean velocity; AAV = absolute angular velocity; AM = absolute meander. *P* values (two-tailed) were adjusted according to the modified Bonferroni method for multiple comparisons (Jaccard & Wan 1996). Significant differences ($\alpha = 0.05$) are shown in bold.

of the MANOVA revealed that there was a significant overall treatment effect (Wilk's $\lambda = 0.684$, $F_{3,27} = 4.16$, $P = 0.015$) and nonsignificant overall block (leaf) effect (Wilk's $\lambda = 0.095$, $F_{87,82} = 1.12$, $P = 0.296$). Interestingly, the effect of predator cues on *T. urticae* fecundity was marginally nonsignificant (univariate ANOVA: $F_{1,29} = 2.56$, $P = 0.120$, Fig. 3b versus Fig. 2b). Moreover, there was a significant effect of the treatment on spider mite dispersal (univariate ANOVA: $F_{1,29} = 4.81$, $P = 0.036$, Fig. 3f versus Fig. 2d) but nonsignificant effect on *T. urticae* mortality (univariate ANOVA: $F_{1,29} = 0.40$, $P = 0.534$, Fig. 3d).

DISCUSSION

We showed that predation risk had a significant effect in our acarine tri-trophic food chain consisting of plants, spider mites *T. urticae* and top risk predator mites *P. persimilis*. Below we discuss the six questions we asked in the Introduction.

Does Predation Risk Cause Trophical Cascading Effect?

Increasing predation risk either by introduction of a caged predator mite or by predator cues on the leaf disc led to a marginally significant increase in plant damage (NPLAD) per *T. urticae* female (Fig. 2a). When data from both two treatments were pulled together, the increase in plant damage was significant. It is interesting that under predation risk spider mites increased their feeding. This differs from other studies (reviewed in Bolker et al. 2003; Werner & Peacor 2003; Schmitz et al. 2004) where antipredator behaviour caused reduced feeding. In the context of trait-mediated cascading effect in food chains, our results suggest that predation risk has the opposite effect on plants when compared with direct predation. The classical density-mediated cascading effect where top predators feed on the middle species in tri-trophic food chains predicts positive correlation between plant biomass and top predator numbers. If the middle species decreases its feeding due to antipredator behaviour, the effect is the same. For example, Schmitz et al. (1997) observed that introduction of risk predatory spiders caused a marginally significant increase in plant biomass in a plant–grasshopper system. However, in our experiment

predation risk led to a decrease in plant biomass, which is just the opposite of the trophical cascading effect. The increase in spider mite feeding can be caused by increased activity of spider mites (see section below), which leads to a higher energetic demand, and thus to increased food consumption.

Does Predation Risk Decrease Herbivore Fecundity?

Increasing predation risk either by introduction of a caged predator mite or by predator cues on the leaf disc decreased *T. urticae* fecundity (Figs 2b and 3b). The decrease was significant in the case of the caged predator. In the case of predator cues the decrease was significant in the first experiment (Fig. 2b) but marginally nonsignificant when the experiment was repeated (Fig. 3b). This discrepancy can be caused by the fact that spider mites were in different physiological states because in the first case *T. urticae* fecundity was much lower than in the latter case. The temporal dynamics of spider mite fecundity in cues treatment (Cp+, Fig. 3a) shows that there was a significant difference on the first day only. In the next 3 consecutive days, the fecundity did not differ significantly from the control (leave disc without predator cues). This suggests that predator cues evaporate quickly which causes the attenuation of the effect after the first day. This can also explain differences between results shown in Figs 2b and 3b. The predatory mite cell used in the first experiment can restrict air circulation above the leaf disc, which can prolong the effect of predator cues on spider mites and lead to a significant decrease in fecundity in Cp+ treatment.

Grostal & Dicke (1999) studied the effect of predator cues on prey oviposition. They introduced six adult *P. persimilis* females on a leaf disc (1 cm diameter) for 24 h, and a paired leaf disc was left without predator cues and served as a control disc. One randomly selected *T. urticae* female was placed on either a predator-exposed or an unexposed leaf disc and eggs laid by the spider mite were counted after 24 h. They found no significant difference between the number of eggs on predator-exposed discs and on unexposed discs. Discrepancies between their results and our results show that whether or not predator cues influence oviposition behaviour of *T. urticae* depends on details of experimental set-up. For example, Grostal & Dicke (1999)

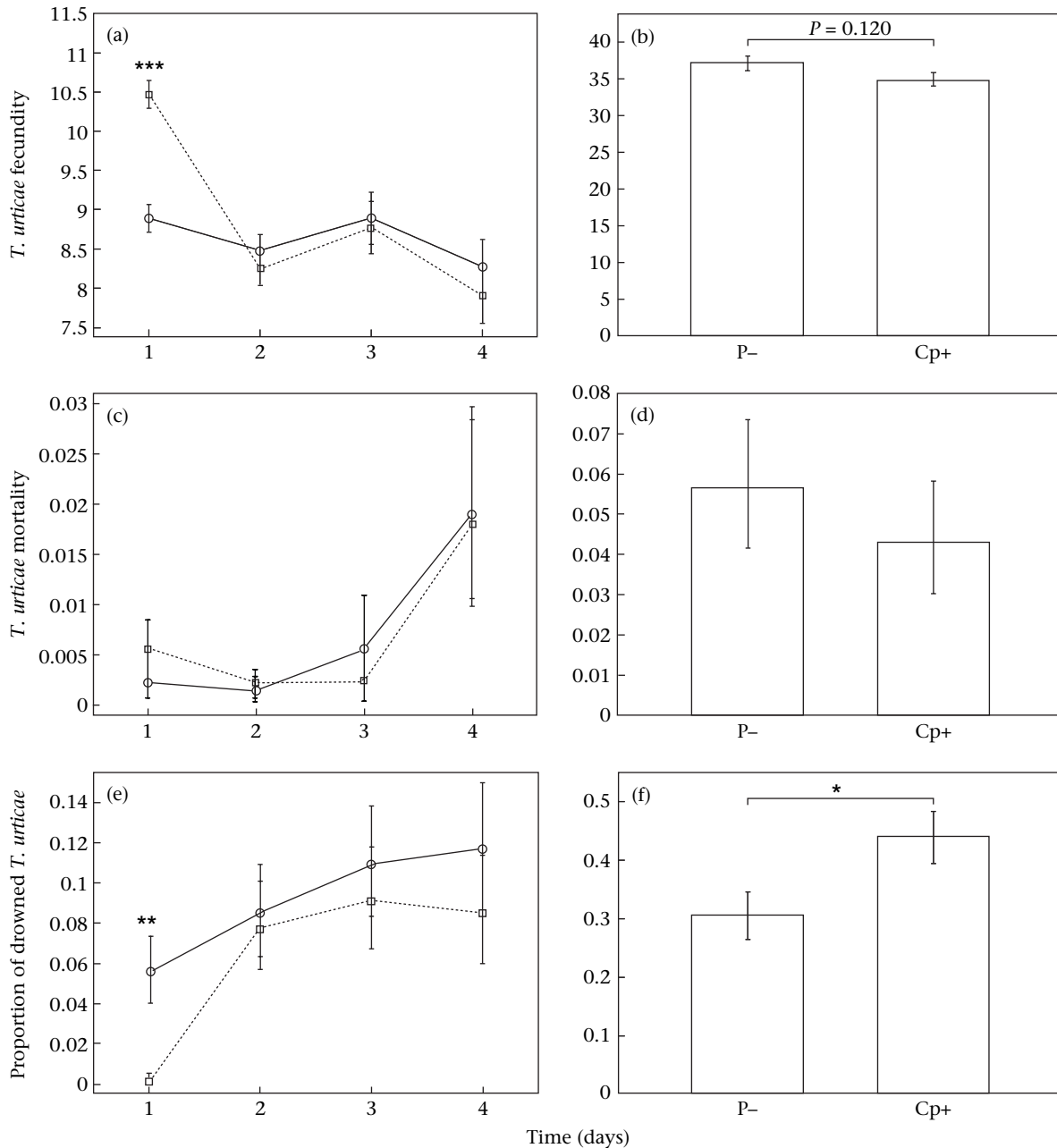


Figure 3. Temporal changes in fecundity (a, b), mortality (c, d) and proportion of drowned spider mites (e, f) on leaf discs with (Cp+, solid line, dots) and without (P–, dashed line, squares) predator cues. The cumulative results for the two treatments are shown in b, d, and f. Data points and vertical lines indicate least squares means \pm SE (a, b) and backtransformed means \pm SE obtained from arcsine square-root transformations of original data (c–f). Horizontal lines and asterisks indicate significant differences ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$) based on Tukey tests.

tested the response of only a single spider mite when compared to 10 spider mites used in our study. Thus, spider mites could receive information about predation risk from conspecifics that are under predation stress and the effect can amplify (Kunert et al. 2007).

Does Predation Risk Increase Herbivore Dispersal?

It was observed by many authors (e.g. Oku et al. 2003a, b, 2004; Werner & Peacor 2003) that organisms avoid

predation risk by moving to safer areas. Despite that leaving the disc in our experimental setting necessarily led to spider mite drowning, we observed a slight nonsignificant increase in the proportion of drowned spider mites under both predation risk treatments (Fig. 2d). In the experiment where we studied the temporal effects of predator cues, the proportion of drowned mites was significantly different from the control. This discrepancy can be caused by the fact that in the latter experiment there was no predatory mite cell attached to the leaf disc. The predatory mite cell in the first experiment can influence spider mite movement out of the disc (although it does not prevent

them to leave the disc). In fact, the mortality due to drowning was more than 10 times higher in the experiment without the cage (cf. Figs 3e, f and 2d).

Does Predation Risk Increase Herbivore Mortality?

There were no significant changes in spider mite mortality under predation risk (Figs 2c and 3c, d). In the first experiment the spider mite mortality rate slightly increased in both risk treatments while in the experiment that evaluated the temporal effect of predator cues on spider mite mortality, there was a slight decrease in mortality. We remark that in Fig. 3c, d spider mite mortality rate was much lower when compared with Fig. 2c. However, this lower mortality rate was compensated by a higher emigration rate. Once again, these effects can be caused by the fact that in the experiment shown in Fig. 3 there was no predatory mite cell on the leaf disc.

Does Predation Risk Cause Changes in Spider Mite Activity Level?

We found a statistically significant increase in spider mite walking activity (percentage of time spent moving) when compared with the control (unexposed leaf disc) in the predator cues treatment. The increase in locomotion activity decreases the time an individual can use for feeding and resting (Oku et al. 2004). Then, how is it possible that under predation risk our spider mites increased leaf damage? This discrepancy can be due to the short observation time of spider mite activity (10 min only) while other experiments lasted for 4 days. It is likely that after a longer time the effect of predatory cues attenuates exactly as in the experiment that evaluated temporal effects of predator cues.

Janssen et al. (1997) measured the time spider mites spent by feeding when predators were present on the leaf disc. They observed a significant decline in the percentage of time spider mites fed (15% reduction). Similar results were obtained by Oku et al. (2004) with another spider mite, *T. kanzawai* Kishida, under predation risk (*Neoseiulus womersleyi* Schicha). In this case predatory mites were present but they did not feed on spider mite adults (they fed on their eggs only). Janssen et al. (1997) also studied the effect of predatory volatiles on feeding activity of *T. urticae*. In contrast to the previous experiments they did not find any significant effect.

Does Fecundity of Spider Mites Increase After Predation Risk Ceases?

Some insects delay oviposition when they perceive predation risk (Hoffmeister & Roitberg 1997). Our additional experiments (Fig. 3a) did not corroborate the assumption that the reduced fecundity under predation risk is because of the adaptive behaviour of spider mites and that they are 'saving' their eggs for habitats or times with no predation risk.

Conclusions

The acarine system shows some of the complexities associated with evaluating the effect of predation risks on prey and on direct and indirect interactions in food webs. On the contrary to the generally accepted view in which herbivores decrease their feeding activity under predation risk, which leads to a trait-mediated cascading effect, we observed that predation risk increased spider mite feeding on plants (i.e. negative cascading effect). Moreover, in systems where predation risk is perceived by odours, details of experimental set-up can lead to conflicting results. First, we observed that the effect of predatory cues on spider mite fecundity attenuated very quickly (after 1 day) in time. This shows that the length of experiment can influence the results. Second, we observed that empty predatory mite cell over the leaf disc most probably influenced spider mite dispersal rate. As it is quite common that in risk treatments various manipulations are necessary (e.g. various cages, glued mouthparts), these can possibly influence the experimental results. The experimental manipulations can be especially important in those systems where predation risk is perceived by odours. Our results also show that the strength and direction of trophical cascading effects caused by trait-mediated interactions can depend on life histories of the intervening species. If so, this would make it difficult to predict the combined effects of trait- and density-mediated interactions in systems where separately these two have opposite effects. Our article calls for more studies to better understand ecological consequences of predation risk.

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References

- Abrams, P. A. 1996. Dynamics and interactions in food webs with adaptive foragers. In: *Food Webs: Integration of Patterns and Dynamics* (Ed. by G. A. Polis & K. O. Winemiller), pp. 149–159. New York: Chapman & Hall.
- Bolker, B., Holyoak, M., Krivan, V., Rowe, L. & Schmitz, O. J. 2003. Connecting theoretical and empirical studies of trait-mediated interactions. *Ecology*, **84**, 1101–1114.
- Carpenter, S. K., Kitchell, J. F. & Hodgson, J. R. 1985. Cascading trophic interactions and lake productivity. *BioScience*, **35**, 634–639.
- Dicke, M. 1986. Volatile spider mite pheromone and host-plant kairomone, involved in spaced-out gregariousness in the spider mite *Tetranychus urticae*. *Physiological Entomology*, **11**, 251–262.

- Grostal, P. & Dicke, M. 1999. Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. *Behavioral Ecology*, **10**, 422–427.
- Helle, W. & Sabelis, M. W. 1985. *Spider Mites: Their Biology, Natural Enemies and Control* Vol. 1. Amsterdam, The Netherlands: Elsevier.
- Hoffmeister, T. S. & Roitberg, B. D. 1997. Counterespionage in an insect herbivore–parasitoid system. *Naturwissenschaften*, **84**, 1–3.
- Jaccard, J. & Wan, C. K. 1996. *LISREL Approaches to Interaction Effects in Multiple Regression*. Thousand Oaks, California: Sage Publications.
- Janssen, A., Bruin, J., Jacobs, G., Schraag, R. & Sabelis, M. W. 1997. Predators use volatiles to avoid prey patches with conspecifics. *Journal of Animal Ecology*, **66**, 223–232.
- Kriesch, S. & Dicke, M. 1997. Avoidance of predatory mites by two-spotted spider mite *Tetranychus urticae*: the role of infochemicals. *Proceedings of the Section Experimental and Applied Entomology, N.E.V. Amsterdam*, **8**, 121–126.
- Kroon, A., Veenendaal, R. L., Bruin, J., Egas, M. & Sabelis, M. W. 2004. Predation risk affects diapause induction in the spider mite *Tetranychus urticae*. *Experimental and Applied Acarology*, **34**, 307–314.
- Kroon, A., Veenendaal, R. L., Egas, M., Bruin, J. & Sabelis, M. W. 2005. Diapause incidence in the two-spotted spider mite increases due to predator presence, not due to selective predation. *Experimental and Applied Acarology*, **35**, 73–81.
- Kunert, G., Trautsch, J. & Weisser, W. W. 2007. Density dependence of the alarm pheromone effect in pea aphids, *Acyrtosiphon pisum* (Stenorrhyncha: Aphididae). *European Journal of Entomology*, **104**, 47–50.
- Lima, S. & Dill, L. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**, 619–640.
- Noldus Information Technology. 1997. *Ethovision: Video Tracking, Motion Analysis and Behavior Recognition System. Reference Manual. Version 1.90*. Wageningen, The Netherlands: Noldus Information Technology.
- Oku, K., Yano, S. & Takafuji, A. 2003a. Spider mite's use of a refuge during the quiescent stage in the presence of a predator. *Entomologia Experimentalis et Applicata*, **108**, 71–74.
- Oku, K., Yano, S., Osakabe, M. & Takafuji, A. 2003b. Spider mites assess predation risk by using the odor of injured conspecifics. *Journal of Chemical Ecology*, **29**, 2609–2613.
- Oku, K., Yano, S. & Takafuji, A. 2004. Nonlethal indirect effects of a native predatory mite, *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae), on the phytophagous mite *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). *Journal of Ethology*, **22**, 1090–1112.
- Oku, K., Yano, S. & Takafuji, A. 2006. Host plant acceptance by the phytophagous mite *Tetranychus kanzawai* Kishida is affected by the availability of a refuge on the leaf surface. *Ecological Research*, **21**, 446–452.
- Paine, R. T. 1980. Food webs: linkage, interaction strength and community infrastructure. *Journal of Animal Ecology*, **49**, 667–685.
- Pallini, A., Janssen, A. & Sabelis, M. W. 1997. Odour-mediated responses of phytophagous mites to conspecific and heterospecific competitors. *Oecologia*, **110**, 179–185.
- Pallini, A., Janssen, A. & Sabelis, M. W. 1999. Spider mites avoid plants with predators. *Experimental and Applied Acarology*, **23**, 803–815.
- Peacor, S. D. & Werner, E. E. 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. *Proceedings of the National Academy of Sciences, U.S.A.*, **98**, 3904–3908.
- Polis, G. A. 1999. Why are parts of the world green? Multiple factors control productivity and the distribution of biomass. *Oikos*, **86**, 3–15.
- Polis, G. A., Sears, A. L. W., Huxel, G. R., Strong, D. R. & Maron, J. 2000. When is a trophic cascade a trophic cascade? *Trends in Ecology & Evolution*, **15**, 473–475.
- SAS Institute. 2000. *The SAS System for Linux, Release 8.2. SAS Online doc. Version 8*. Cary, North Carolina: SAS Institute.
- Schmitz, O. J., Beckerman, A. P. & O'Brien, K. M. 1997. Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology*, **78**, 1388–1399.
- Schmitz, O. J., Krivan, V. & Ovadia, O. 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecology Letters*, **7**, 153–163.
- Siegel, S. & Castellan, N. J., Jr 1988. *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill.
- Škaloudová, B., Krivan, V. & Zemek, R. 2006. Computer-assisted estimation of leaf damage caused by spider mites. *Computers and Electronics in Agriculture*, **53**, 81–91.
- Sokal, R. R. & Rohlf, F. J. 1969. *Biometry*. San Francisco, California: Freeman.
- Werner, E. E. & Peacor, S. D. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology*, **84**, 1083–1100.