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Geotaxis and leaf-surface preferences mitigate negative effects of a predatory mite on an herbivorous mite

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ABSTRACT

Reproductive success and population growth of an herbivorous mite are limited by activities of phytoseiid predators. However, occurrences on upper vs. lower leaf surfaces are sometimes mismatched between these prey and predators. The mismatch potentially mitigates predation risk for the prey species. We assessed factors that affect mite distributions on leaf surfaces, testing whether the presence of the phytoseiid mite *Phytoseius nipponicus* alters the leaf-surface distribution and reproductive success of the herbivorous false spider mite *Brevipalpus obovatus*. The host plant was *Viburnum erosum* var. *punctatum* (Adoxaceae). Leaves were set in natural (TRUE) and reversed (upside down; INVERTED) orientations using experimental devices. Both surfaces were accessible to mites. We detected lower and abaxial leaf-surface preferences in *P. nipponicus*. In contrast, upper and adaxial surfaces were preferred by *B. obovatus*. Thus, prey and predatory mites accumulated on different sides of leaves. Presence of the predator also indirectly decreased egg production in *B. obovatus*. *Brevipalpus obovatus* females actively avoided leaf surfaces with elevated predator numbers; these females shifted their distributions and changed oviposition sites to leaf surfaces with fewer predators. In consequence, *B. obovatus* eggs on the upper sides of leaves were less frequently preyed upon than were those on lower sides. We suggest that upper leaf-surface exploitation in this particular herbivorous mite species mitigates predation risk from phytoseiid mites, which prefer lower leaf surfaces.

INTRODUCTION

The reproductive success and population growth of herbivorous mites may be limited by the activities of phytoseiid predators. However, occurrences on upper and lower leaf surfaces are sometimes mismatched between prey and predators. Such a difference in spatial distribution is likely to mitigate predation risk on the prey species.

Many plant-dwelling mites occur on lower abaxial leaf surfaces (Sudo and Osakabe 2011). However, substantial numbers of *Brevipalpus obovatus* Donnadieu [a false spider mite (Tenuipalpidae)] individuals occur on upper adaxial leaf surfaces of *Viburnum erosum* Thunb. var. *punctatum* Franch. et Sav. (VEP; Adoxaceae), which is a major component of forest vegetation in western Japan (Sudo and Osakabe 2011). Phytoseiid and many other forms of mites occur preferentially on the lower sides of leaves. Field observations confirm that nearly 98% of motile phytoseiids

(mainly *Phytoseius* spp.) are distributed on lower leaf surfaces (Sudo and Osakabe 2011). Hence, exploitation of VEP upper leaf surfaces by *B. obovatus* may decrease predation pressure from phytoseiid mites.

Hairs and/or trichomes that obstruct prey searching and predation (Krips et al. 1999; Loughner et al. 2010) are generally denser and better developed on abaxial than on adaxial leaf surfaces (Chien and Sussex 1996). Accordingly, predation efficiency may be greater on the adaxial sides of leaves. Indeed, more *B. obovatus* eggs were consumed by *Phytoseius nipponicus* Ehara (Acari, Phytoseiidae) on adaxial than on abaxial surfaces in laboratory experiments using leaf disks as test substrata (Sudo and Osakabe unpublished data). Under these circumstances, preference for adaxial leaf surfaces would be detrimental for *B. obovatus* in the face of predation risk from *P. nipponicus*. However, predation is not the only factor affecting risk for prey mite species that can choose between leaf surfaces. Abiotic threats such as

rainfall, high temperature, desiccation, and solar ultraviolet radiation are more severe on upper leaf surfaces and may also limit distributions of many plant-dwelling mites to lower leaf surfaces. Among these factors, solar ultraviolet radiation has recently been demonstrated to have serious impacts on mite survival and spatial distribution (Weintraub et al. 2007; Ohtsuka and Osakabe 2009; Suzuki et al. 2009; Sakai and Osakabe 2010; Onzo et al. 2010).

Loughner et al. (2010) showed that the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) balances a behavioral preference for complex habitat, such as a plant surface with dense trichomes, against a positive geotaxis to increase egg survival. Phytoseiid mites in the genus *Phytoseius*, which occur abundantly on VEP leaves, are generalist predators that depend on a range of alternative food sources (McMurtry and Croft 1997). Because the prey–predator relationship between *B. obovatus* and *P. nipponicus* is not specific, it may not have a role in leaf-surface choices in prey populations. However, if upper leaf-surface exploitation mitigates predation risk, *B. obovatus* should respond behaviorally to the presence of *P. nipponicus*.

In this study, we determined factors that affect leaf-surface distribution and tested whether the presence of *P. nipponicus* alters the leaf-surface distribution and reproductive success of *B. obovatus*. We used VEP leaves as substrata and deployed devices that made both surfaces accessible to the mites. VEP leaves were set in natural and inverted (upside down) positions to explore the effects of gravity and heterogeneous microstructure (density of leaf hairs) of leaf surfaces on intra-leaf distributions of the prey–predator system.

MATERIALS AND METHODS

Mites

We collected *B. obovatus* occurring on VEP leaves in a secondary broadleaf forest site at Iwakura on the outskirts of Kyoto City (Japan, 35°5'28"N, 135°46'42"E, 150–160 m a.s.l.) in September 2009. The mites were reared on VEP leaf disks (abaxial surfaces) placed on water-soaked cotton in Petri dishes in a laboratory at 25°C and a 16-h light (L):8-h dark (D) cycle. We used *B. obovatus* females that were 40–50 days old (measured as the duration of time since the mothers had oviposited the eggs from which they

hatched).

Phytoseius nipponicus, a generalist phytoseiid species that is predominant in the foliar mite community on VEP in Kyoto (Sudo et al. 2010), was collected from VEP leaves in Iwakura and reared on VEP leaf disks for 2 days prior to experiments. *Phytoseius nipponicus* adults (both females and males) were identified by pairs of thick dorsal setae on the coarse plate (Ehara and Gotoh 2009).

Host-plant leaves

VEP leaves have stellate hairs on both adaxial and abaxial surfaces. Stellate hairs and thick straight hairs grow on veins on abaxial leaf surfaces. Hairs growing from closely neighboring veins hang over the vein axil and form so-called hair-tuft domatia (O'Dowd and Willson 1989).

We collected VEP leaves from four shrubs in Iwakura 2 days before each experiment. Simultaneously, we collected phytoseiid mites described above. Leaves were inserted in plastic bags, brought to the laboratory, and assembled into experimental configurations within 6 hours of collection. Predaceous mites (Phytoseiidae and Stigmaeidae), insects (aphids, gall midges, and thrips), and debris (>1 mm) were removed from the leaves with forceps prior to the experiments. Fungivorous mites, herbivorous mites (Eriophyoidea), pollen, and fungi were left on leaf surfaces.

Experimental setups

To explore the ways in which mites use upper/lower or adaxial/abaxial surfaces, VEP leaves were held in a plastic frame (leaf holder: LH; 29.4 × 8.4 × 3.0 cm; Fig. 1) that permitted changes in leaf orientation. Two VEP leaves were held in a LH, and the basal halves of both leaf blades were wrapped in cotton fabric (15 × 7 cm). The cotton fabric was fastened onto the frame with adhesive tape (Mask Light Tape #730, Sekisui Chemical, Osaka). Of the two leaves held on a single LH, one was set with the adaxial surface upward (TRUE gravity) and the other with the abaxial surface upward (INVERTED gravity). To prevent leaves from flooding with water, we painted acrylic emulsion adhesive (#10824, Konishi, Osaka) where the leaf blade met the cotton fabric (Fig. 1). To prevent mites escaping, we coated leaf surfaces 4 mm distant from the cotton fabric with sticky grease (Tangle B grease; Fuji Yakuhiin Kogyo,

Tokyo). Available leaf spaces (distal part of the leaf blade measured from the boundary of the sticky grease) were $10.52 \pm 6.12 \text{ cm}^2$ (mean \pm SD per leaf surface), $9.60 \pm 2.89 \text{ cm}^2$, $8.62 \pm 1.82 \text{ cm}^2$, and $9.34 \pm 2.32 \text{ cm}^2$ in the TRUE-gravity configuration without predators, TRUE-gravity configuration with predators, INVERTED-gravity configuration without predators, and INVERTED-gravity configuration with predator treatments, respectively. Areas were measured with the Histogram Function of Photoshop Elements ver. 2 software (Adobe Systems, San Jose, CA, USA).

Two LHs (bearing four leaves in total) were placed in rectangular plastic tray ($33.5 \times 25 \times 5 \text{ cm}$) containing distilled water 1 cm deep. Leaves held in the LHs were supplied with water via the cotton fabric. We maintained LHs on a laboratory shelf at 25°C under a L16:D8-h photoperiod. LHs were illuminated with 40 W fluorescent lamps fixed at 40-cm intervals on a side wall 30 cm distant from the shelf. We used a crossed design with predators (absent/present) and gravity (TRUE/INVERTED) as independent variables. Two LHs in a single vat were used as duplicates, and leaves collected from the same branch were used as replicates.

Experimental design

We used a two-way factorial design in August, October,

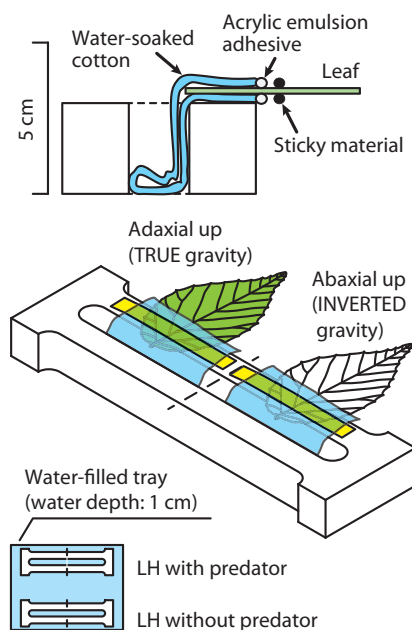


Fig. 1 Leaf holder (LH) used to test the intra-leaf distribution of mites.

and November 2010. In each experimental period, we prepared four trays containing two LHs (eight LHs in total). Each tray contained a series of VEP leaves assigned to (1) predator-absent TRUE-gravity, (2) predator-absent INVERTED-gravity, (3) predator-present TRUE-gravity, (4) predator-present INVERTED-gravity treatments.

The day before the beginning of experiment (day zero), we introduced three adult *P. nipponicus* onto two leaves held in one LH (predator-present TRUE/INVERTED-gravity) assembly but not onto the other LH (predator-absent TRUE/INVERTED-gravity) on the same tray. The following day (day one), three adult *B. obovatus* females were introduced onto all VEP leaves in the four trays. At daily intervals over the next 10 days (days 2–11), we recorded leaf-surface distributions of mite adults (*P. nipponicus* and *B. obovatus*) on each VEP leaf. We first counted the mite adults on the upper sides (whether adaxial or abaxial) of the leaves on each LH using a stereomicroscope with 13 \times magnification; we then turned over the LH and immediately counted mites on the lower sides of leaves. Introduced *B. obovatus* females were removed from the leaves on day 11. If *B. obovatus* females had escaped from the leaves before day 10, we compensated by adding fresh female conspecifics of the same age. If the number of *P. nipponicus* mites had decreased, we added fresh individuals every day until the egg-hatching rate of *B. obovatus* had been determined. Eggs of *P. nipponicus* were removed from the leaves immediately after we found them.

We recorded the locations of newly-laid *B. obovatus* eggs on the leaves every 24 hours. Subsequently, all eggs were inspected daily until the fate of each egg had been identified as (i) hatched, (ii) lost to predation, or (iii) lost to other causes. Egg vitality was assessed by checking whether each egg was empty and by determining eggshell status. We noted in preliminary observations that hatching larvae clipped their eggshells and left rounded breaks. In contrast, *P. nipponicus* did not leave conspicuous breaks on the eggshells of *B. obovatus* when sucking out egg contents. Hence, when there was a round break on the eggshell and contents had disappeared, we recognized the specimen as a hatched egg. When hatching of a larva was interrupted for more than 24 hours for any reason, the egg was categorized as dead. When all or some contents had disappeared without eggshell clipping, we classified the egg as preyed upon.

In the experiments set up in August 2010, we continued

observation of *B. obovatus* eggs until day 41, which is beyond the maximum incubation period of *B. obovatus* eggs at 25°C (22 days), which we had determined in our preliminary observations. In October and November, we discontinued observations if no eggs had hatched during the previous 7 days across all treatments; we made this decision because no eggs had hatched after a 7-day no-hatching interval during the August experiments. Eggs that had not hatched by the end of the observation were categorized as dead.

Six leaves withered before the experiments were completed. Thus, we used data collected from 10 leaves in the predator-absent TRUE-gravity and predator-present INVERTED-gravity treatments; data were collected from 11 leaves in the predator-present TRUE-gravity and predator-absent INVERTED-gravity treatments.

Data analyses

Brevipalpus obovatus eggs that were flooded before hatching or predation (48 of 944 eggs in total) were excluded from egg-fate categorization and included only in the analyses of fecundity. Eggs for which the cause of death was neither predation nor flooding were included in analyses of hatching rate but excluded from comparisons of predation rates between leaf surfaces.

We constructed generalized linear models (GLMs) and generalized linear mixed models (GLMMs) with modules “glm” and “glmmML” (in package glmmML by Broström and Holmberg 2011) of R software (version 2.10.1; R Development Core Team 2009), respectively. We calculated 95% confidence intervals (95% CI) for rates based on the binomial distribution.

Effects of gravity direction and leaf surface on intra-leaf distribution of the predator and *B. obovatus* adults

GLMMs assuming the binomial distribution (logit-link) were used to evaluate (i) the effects of gravity direction (TRUE/INVERTED) on intra-leaf distribution (lower/upper side of leaves) of *P. nipponicus* adults and (ii) the effects of gravity direction and predator (absence/presence of *P. nipponicus*) on the intra-leaf distribution of *B. obovatus* adults. Because the counting was repeated 10 times (separate days) on every leaf, leaves were assigned random-effect status in mixed modeling to prevent pseudo-replication. We formulated all combinations among explanatory variables and their two-way interactions into the models, and then selected the appropriate versions based on Akaike’s information criterion (AIC).

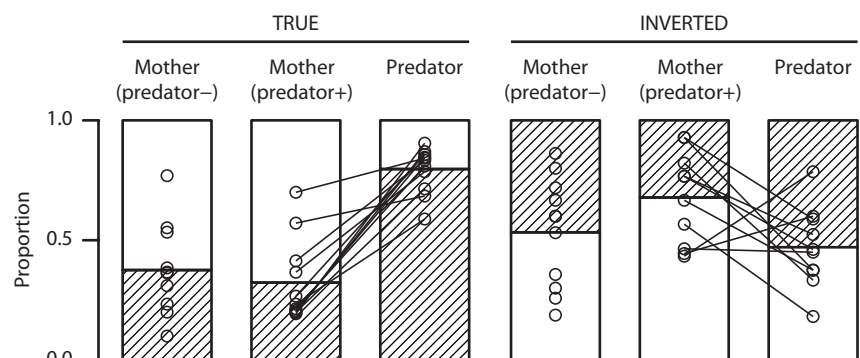
Avoidance of predator-abundant leaf surfaces by *B. obovatus* females

The predator-present INVERTED-gravity treatments explored the effects of predator frequency on upper/lower distribution of *B. obovatus* females; this test evaluated whether *B. obovatus* females avoided leaf surfaces with abundant predators. The cumulative number of predators (explanatory variable) and *B. obovatus* females (response variable) on upper and lower leaf sides were used in a GLM analysis assuming binomial distribution (logit-link) following a Wald test.

Indirect effects of predators on fecundity of *B. obovatus*

To explore the effects of predator and gravity direction on the fecundity of *B. obovatus*, we used the total number of eggs laid on a single leaf during the first 10 days of the experiment (days 2–11) as the subject variable for GLM

Fig. 2 Distribution of mite adults on VEP leaves, showing proportions of individuals on upper and lower sides of leaves (total frequency through the first 10 days of the experiment). Open and shaded bars indicate proportions of mites on adaxial and abaxial leaf surfaces, respectively. Each circle in the bars signifies the distribution of mites on each leaf, and the segments between circles in “Mother (predator+)” and “Predator” indicate that data came from same leaf.



analysis, assuming a Poisson distribution (log-link); we selected suitable models by their AICs.

Factors affecting oviposition-site choice by B. obovatus

To determine factors affecting oviposition-site (leaf surface) choice by *B. obovatus*, we formulated all combinations among surface (leaf surface: adaxial/abaxial), side (direction of leaf surface facing: lower/upper), predator, and their interactions into the GLMMs (log-link, Poisson error) and then selected those with the lowest AIC values. We used total fecundity over 10 days on each leaf surface as the subject variable and offset this by the cumulative observed frequencies of *B. obovatus* mothers on the surface over 10 days. The data obtained from the two surfaces of an identical leaf were clustered for mixed modeling.

Effects of leaf surfaces and direction of gravity on B. obovatus egg hatchability

We used GLMMs (logit-link, binomial error) and model selection by AIC value to explore effects of gravity direction and side on egg predation (preyed upon or not based on individual *B. obovatus* eggs) in the predator-present treatments. Eggs laid on the same leaf were clustered for

mixed modeling.

RESULTS

Effects of direction of gravity and leaf surface on intra-leaf distribution of *P. nipponicus* and *B. obovatus* adults

Median frequencies of *P. nipponicus* adults on upper sides (leaf surface facing upward) of VEP leaves were 16.7 and 54.4% in TRUE (adaxial leaf surfaces) and INVERTED (abaxial leaf surfaces) gravity treatments, respectively (Fig. 2). The model selection supported a version containing gravity direction as an explanatory variable for the intra-leaf distribution of *P. nipponicus* adults (Table 1). The coefficient of intercept was negative, whereas the coefficient of gravity direction was positive, but it did not override the effect of the intercept, suggesting that *P. nipponicus* adults prefer the lower sides to the upper sides of VEP leaves (i.e., these mites have positive geotaxis) and tend to favor abaxial over adaxial leaf surfaces.

In predator-absent treatments, medians of *B. obovatus* adult frequencies on the upper sides of VEP leaves were 63.5 and 40.0% in the TRUE- and INVERTED-gravity

Table 1 GLMM (logit-link, binomial error) for leaf-surface distribution (upper versus lower surface) of *P. nipponicus* adults. AIC = 271.0 (the selected model) or 287.5 (model with intercept only)

	Coefficient	SE	z value	Pr (> z)
(Intercept)	-1.535	0.196	-7.828	5.00×10^{-15}
Gravity direction (INVERSE)	1.358	0.258	5.269	1.37×10^{-7}

Table 2 GLMM (logit-link, binomial error) for leaf-surface distribution (upper versus lower surface) of *B. obovatus* adults. AIC = 557.6 (the selected model) or 558.7 (second lowest AIC value: model lacking the interaction term)

	Coefficient	SE	z value	Pr (> z)
(Intercept)	0.546	0.294	1.859	0.063
Gravity direction (INVERSE)	-0.681	0.405	-1.682	0.093
Predator (present)	0.247	0.407	0.607	0.544
Gravity direction (INVERSE): predator (present)	-1.040	0.576	-1.805	0.071

Table 3 Binomial GLM (logit-link) for the effect of predator distribution on leaf-surface distribution (upper surface versus lower) of *B. obovatus* adults in predator-present INVERTED-gravity treatments. The explanatory variable of the model is the total observed frequency of *P. nipponicus* on the upper side of each VEP leaf over 10 days.

	Coefficient	SE	z value	Pr (> z)
(Intercept)	0.285	0.391	0.728	0.467
Predator frequency on the upper side	-0.129	0.047	-2.740	0.006 **

Table 4 GLMM (log-link, Poisson error) for 10-day fecundity of *B. obovatus* on each surface of VEP leaves offset by the observed frequencies of the mothers on each surface. AIC = 180.9 (the selected model) and 181.7 (the second lowest AIC value: model lacking the surface \times predator interaction term)

	Coefficient	SE	z value	Pr ($> z $)
(Intercept)	-0.392	0.118	-3.334	8.57×10^{-4}
Side (upward)	0.123	0.071	1.738	0.082
Surface (adaxial)	0.102	0.098	1.048	0.295
Predator (present)	0.009	0.158	0.059	0.953
Surface (adaxial): predator (present)	-0.243	0.144	-1.686	0.092

treatments, respectively (Fig. 2). In the predator-present treatments, these medians changed to 76.7 and 28.3% in the TRUE- and INVERTED-gravity treatments, respectively. Model selection for the intra-leaf distribution of *B. obovatus* adults supported a version containing gravity direction, predator (absence/presence), and their interaction term as explanatory variables (Table 2). The coefficient of the intercept was positive, whereas the coefficient of gravity direction was negative, overriding the effect of the intercept, indicating a preference for adaxial over abaxial surfaces and for the upper over the lower side of VEP leaves (i.e., negative geotaxis) in *B. obovatus* females. The positive coefficient of predator and the negative coefficient of the interaction term (gravity direction \times predator) suggest that the presence of *P. nipponicus* motivates *B. obovatus* females to move to adaxial leaf surfaces (Table 2).

Avoidance of predator-abundant leaf surfaces by *B. obovatus*

Although *P. nipponicus* adults were concentrated on lower sides of VEP leaves under the TRUE-gravity configuration, the distribution of predators was divided between upper and lower sides (Fig. 2) on inverted leaves. GLM analysis followed by Wald testing demonstrated that *P. nipponicus* frequency had negative effects on the frequency of *B. obovatus* females present on the same leaf side; the coefficient was -0.12942 (Table 3). This suggests that *B. obovatus* females actively avoided predator-abundant leaf sides regardless of leaf surface type (adaxial/abaxial).

Indirect effects of predators on the fecundity of *B. obovatus*

The numbers of eggs per leaf laid by three *B. obovatus*

females over 10 days were 24.0 ± 3.31 (mean \pm SE), 21.1 ± 2.64 , 24.6 ± 2.86 , and 20.1 ± 3.05 in the predator-absent TRUE-gravity, the predator-present TRUE-gravity the predator-absent INVERTED-gravity and the predator-present INVERTED-gravity treatments, respectively. Model selection supported the GLM containing only predator (AIC = 360.8) as an explanatory variable over a version containing both gravity direction and predator but lacking the interaction term (the second lowest AIC: AIC = 362.8). This suggests that the fecundity of *B. obovatus* over both leaf surfaces was reduced by the presence of predators (indirect effects) but was not affected by direction of gravity.

Factors affecting oviposition site choice by *B. obovatus*

Distribution of *B. obovatus* eggs was obviously biased toward adaxial leaf surfaces (Fig. 3), and the upper side tended to be preferred over the lower as an oviposition site. The

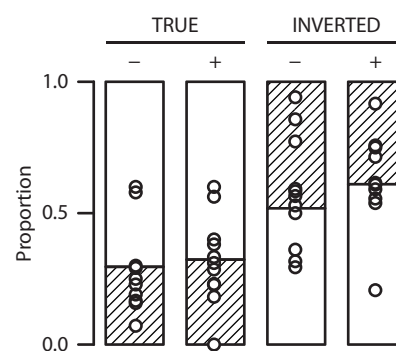


Fig. 3 Distributions of *B. obovatus* eggs on leaves in the predator-absent/present TRUE/INVERTED-gravity treatments. Open bars indicate the proportions of eggs oviposited on adaxial surfaces, and shaded bars indicate proportions on abaxial sides. Minus and plus signs above the bars indicate predator-absent and predator-present treatments, respectively. Circles in the bars represent the proportions of eggs laid on the lower surfaces of each leaf.

proportion of eggs laid on adaxial surfaces was 0.704 (n = 240, 95% CI: 0.642–0.761) in the predator-absent TRUE-gravity treatments, 0.677 (n = 232, 95% CI: 0.612–0.736) in the predator-present TRUE-gravity treatments, 0.518 (n = 272, 95% CI: 0.457–0.579) in the predator-absent INVERTED-gravity treatments, and 0.610 (n = 200, 95% CI: 0.539–0.678) in the predator-present INVERTED-gravity treatments. Though these distributions of eggs (Fig. 3) resembled the upper–lower frequency ratios of *B. obovatus* mothers (Fig. 2), environmental factors also affected the egg-production rate in individual females. Poisson GLMMs and subsequent model selection supported the selection of side, surface, predator, and the two-way surface × predator interaction as explanatory variables for 10-day fecundity on the two leaf surfaces offset by observed frequencies of *B. obovatus* mothers (Table 4). When *P. nipponicus* was absent, the coefficients of the models when mothers were on the leaf sides facing upward were 0.123 units higher than coefficients for models when mothers were on surfaces facing downward (signifying a fecundity increase of 13% in the natural logarithm-linked model). When mothers were on adaxial surfaces, coefficients were 0.102 units higher than when they were on abaxial surfaces (an increase of 11%). On the adaxial leaf surfaces, the introduction of *P. nipponicus* decreased fecundity by 0.233 units (a decrease of 21%). In contrast, the presence of predators scarcely affected fecundity (0.009 units; 1% increase) on abaxial surfaces.

Effects of leaf surfaces and direction of gravity on *B. obovatus* egg hatchability

When predators were absent, almost all *B. obovatus* eggs hatched regardless of leaf surface. In predator-absent TRUE-gravity treatments, the hatchability ratios were 0.975 (n = 159, 95% CI: 0.937–0.993) and 0.943 (n = 70, 95% CI: 0.860–0.984) on adaxial (upper) and abaxial (lower) leaf surfaces, respectively. In predator-absent

Table 5 GLMM (logit-link, binomial error) of predation risk (preyed upon vs. not preyed upon) for each *B. obovatus* egg on a VEP leaf with the predator. Eggs that died (but were not preyed upon) were excluded from the analysis. AIC = 531.1 (the selected model) and 532.9 (second lowest model, which contains the interaction term)

	Coefficient	SE	z value	Pr (> z)
(Intercept)	0.252	0.318	0.794	0.427
Side (upper)	-0.491	0.236	-2.084	0.037
Gravity direction (INVERSE)	-0.664	0.413	-1.608	0.108

INVERTED-gravity treatments, hatchabilities were 1.000 (n = 129, 95% CI: 0.972–1.000) and 0.984 (n = 124, 95% CI: 0.943–0.998) on abaxial (upper) and adaxial (lower) leaf surfaces, respectively.

However, egg hatchability decreased in the presence of predators, and the magnitude of the reduction was greater on the lower sides of leaves (Fig. 4). In predator-present TRUE-gravity treatments, the hatchabilities were 0.538 (n = 145, 95% CI: 0.453–0.621) and 0.440 (n = 75, 95% CI: 0.325–0.559) on upper (adaxial) and lower (abaxial) sides of leaves, respectively. In the predator-present INVERTED-gravity treatments, hatchabilities were 0.744 (n = 78, 95% CI: 0.632–0.836) and 0.569 (n = 116, 95% CI: 0.474–0.661) on upper (abaxial) and lower (adaxial) sides of leaves, respectively. The model selection supported the selection of gravity direction (TRUE/INVERTED) and side (upper/lower) as explanatory variables for egg predation risk (Table 5). This suggests that predation rate was lower on the upper than on the lower sides of VEP leaves; the INVERTED-gravity treatment moderated the risk of predation by *P. nipponicus* for *B. obovatus* eggs.

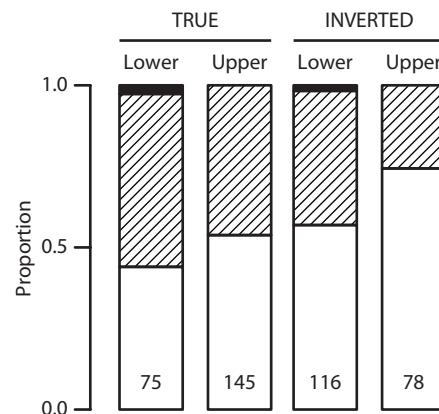


Fig. 4 Proportion of *B. obovatus* eggs that hatched (open bars), died from predation (shaded bars), and died due to other factors (filled bars) on upper and lower surfaces of VEP leaves in the predator-present TRUE- and INVERTED-gravity treatments. Numbers in the bars are total numbers of eggs observed in each treatment.

Most *P. nipponicus* predation on individual *B. obovatus* eggs occurred within the first half of the egg period. The mean incubation period for *B. obovatus* eggs was 11.66 days, and this was unaffected by gravity direction, leaf surface (adaxial or abaxial), and absence/presence of predators (Poisson GLMM analysis with log-link). The 95th percentiles of ages of eggs eaten by the predator were 5.7 and 6 days on predator-present leaves with TRUE- or INVERTED-gravity treatments, respectively.

DISCUSSION

Abaxial surfaces of VEP leaves have more stellate hairs than do adaxial surfaces. Dense leaf hairs protect eggs of *B. obovatus* from *P. nipponicus* predation (Sudo and Osakabe unpublished data). Nevertheless, *B. obovatus* adults occur more frequently and oviposit more often on adaxial leaf surfaces than on the obverse surfaces. On first consideration, this behavior may seem inappropriate in the face of losses to predation, but it may be explained by the effects of gravity and leaf-surface architecture on local predator density. Positive geotaxis and a preference for surface environments on the abaxial surfaces of VEP leaves accounted for the concentration of *P. nipponicus* population on lower abaxial leaf surfaces. Conversely, *B. obovatus* had negative geotaxis and a preference for the adaxial surface over the abaxial surface. Both the direction of gravity pull and leaf-surface architecture likely explain the occurrences of *P. nipponicus* and *B. obovatus* on different surfaces of the same leaf, though gravity had less effect on the distribution of *B. obovatus* in comparison with leaf-surface architecture. This spatial segregation would clearly reduce opportunities for encounters between predator and prey. In this study, the mites were confined to a single leaf. However, the prey might move off of the leaf in the presence of the predator. Future studies will show how the prey mites respond to the predator presence under the circumstance that they are accessible to the adjoining leaves.

The daily fecundity of *B. obovatus* on predator-present leaves was approximately 5/6 that on predator-free leaves. We interpret this reduction as an indirect, negative predator effect. Although the extent of fecundity reduction was greater than in previous experiments using VEP leaf discs, *B. obovatus* oviposition rates on adaxial and abaxial leaf surfaces in the presence of predators decreased by 1/2 and 2/3, respectively, in comparison with predator-free surfaces

(Sudo and Osakabe unpublished data; three *B. obovatus* mothers versus two predators per leaf disc). In addition to having reversed preferences for gravity direction and leaf surfaces in comparison with *P. nipponicus*, *B. obovatus* adults actively changed their leaf-surface distribution to avoid the predator. This change in distribution indicates that the decrease in *B. obovatus* fecundity was mitigated when both leaf surfaces were available, paralleling circumstances in nature. Thus, the presence of the predator is a significant determinant of intra-leaf distribution of *B. obovatus*.

The egg-predation rate was lower on the upper sides of VEP leaves than on lower sides. Reduced predation risk may compensate for the reduced frequency of sheltered sites for oviposition on the upper adaxial surface of a VEP leaf. Effects of leaf-surface microstructures (e.g., hairs, glandular trichomes, and domatia) on loss rates of plant mites and their eggs to predation have been investigated previously (van Haren et al. 1987; Krips et al. 1999; Norton et al. 2001; Roda et al. 2001; Kasai et al. 2005), but the effects of leaf side (upper or lower) were scarcely considered in the past. Our findings demonstrate that gravity direction detected by mites on upper and lower leaf surfaces affects prey–predator interactions between *B. obovatus* and *P. nipponicus*.

Many mite herbivores lay eggs on upper leaf surfaces only when population densities reach a saturation point on lower-leaf surface (Jeppson 1975). In contrast, the oviposition rate of *B. obovatus* was slightly higher on the upper side than on the lower side of VEP leaves when predators were absent. The hatchability of *B. obovatus* eggs was nearly 90% on both sides of VEP leaves in the absence of predators. Together with the upper–lower frequency ratio of eggs on wild VEP leaves (35% of eggs on upper leaf surfaces; Sudo and Osakabe 2011), combined use of upper and lower leaf surfaces of VEP as oviposition sites by *B. obovatus* might represent a strategy by which the species mitigates indirect effects of phytoseiids on both its fecundity and the predation risks on its eggs.

It is possible that *B. obovatus* adults on wild hosts might switch oviposition sites between upper and lower sides of leaves depending on predator presence or density. Although *B. obovatus* experiences lower levels of regulation by predatory mites on upper sides of leaves than on lower sides, its eggs will be exposed to greater environmental stresses, such as rainfall (Foott 1963) and solar ultraviolet B radiation (Ohtsuka and Osakabe 2009; Onzo et al. 2010),

on the upper leaf side. The break-even point of declining predation risk and increasing environmental stresses on leaf upper sides will vary with spatio-temporal changes in stressors and the magnitude of environmental tolerance intrinsic to each mite species.

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