A comparison of the effects of gravity and the nutritional advantage of leaf surfaces on fecundity in the two-spotted spider mite (Acari: Tetranychidae)

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(Received 20 October 2011; Accepted 14 December 2011)

ABSTRACT

The tendency of the two-spotted spider mite, *Tetranychus urticae*, to lay eggs on the abaxial (lower) side of host plant leaves has been explained as an adaptation to avoid rainfall and solar ultraviolet radiation. However, differences in the nutrition and effects of gravity between the abaxial and adaxial (upper) leaf surface could affect mite fitness. We investigated the fecundity of mites using kidney bean leaf discs with their adaxial or abaxial sides facing upward or downward. Fecundity was greater on adaxial leaf surfaces and on leaf discs facing downward but did not differ between adaxial surfaces facing upward and abaxial surfaces facing downward, suggesting that the effects of gravity and nutrition compensated for each other. Consequently, although leaf surface nutrition and gravity direction affected fecundity, these factors do not explain the biased distribution of *T. urticae*.

Key words: Spatial distribution, habitat heterogeneity, bottom-up effects, *Tetranychus urticae*, Kidney bean

INTRODUCTION

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a cosmopolitan, polyphagous herbivore (Helle and Sabelis, 1985; Bolland et al., 1998) that is one of the worst agricultural pests worldwide. Adult *T. urticae* usually inhabit the lower (abaxial) side of host plant leaves (Foott, 1963; Osakabe et al., 2006). They infest and oviposit on the upper (adaxial) side of leaves only when the abaxial population reaches saturation (Jeppson, 1975). This bias has been explained as an adaptation to avoid rainfall (Jeppson, 1975) and solar ultraviolet B (UVB) radiation (Barcelo, 1981; Ohtsuka and Osakabe, 2009). Ohtsuka and Osakabe (2009) and Sakai and Osakabe (2010) concluded that UVB was responsible for the *T. urticae* concentrations on the abaxial side of kidney bean *Phaseolus vulgaris* L. (Fabaceae) leaves.

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This study was partially supported by Grant-in-Aid for JSPS Fellows No. 23.2696 to SM from Japan Society for the Promotion of Science (JSPS).

DOI: 10.2300/acari.21.1

However, studies have not considered the effect of gravity, which differs between the upper and lower surfaces of plant leaves (Sudo and Osakabe, 2011). While some studies have determined that the behavior of minute animals, including tetranychids, is not affected by the direction of gravitational pull on host leaves (e.g., Yanagida et al., 2001; Kanazawa et al., 2011), Li and Margolies (1991) reported that the banks grass mite, *Oligonychus pratensis* (Banks), exhibited positive geotaxis, which contributed to the concentration of mites on the lower side of corn leaves. No studies have examined the effect of gravity on *T. urticae* fitness on host plant leaf surfaces.

To investigate how differences in the gravitational pull and other traits (e.g. nutrition) on the different sides of leaves affect fecundity, we conducted a laboratory experiment on *T. urticae* with kidney bean leaves as their host plant.

MATERIALS AND METHODS

Mite

The *T. urticae* used in this study were cultured on potted kidney bean plants continuously illuminated with fluorescent lights in the laboratory at a temperature between 25°C and 28°C for at least 8 years. They were a mixture of populations from several different localities in Japan.

Quiescent deutonymphal females were transferred from the stock culture to previously uninfested kidney bean leaves on water-soaked cotton in 9-cm-wide Petri dishes. The Petri dishes were kept in a laboratory at 23°C with a 16:8 h light to dark regimen (light period: 0700–2300). They were covered with transparent plastic lids to increase the relative humidity (RH) on the leaves, as quiescent tetranychid mites delay molting under humid conditions (Ikegami et al., 2000). At 0900 the following morning, the lids were removed and most of the virgin adult females emerged within 30 min. The females were immediately used for the experiment.

Experimental design

The effects of gravity direction (upward or downward pull, from the mite's perspective) and leaf surface (adaxial or abaxial) on *T. urticae* fecundity were tested using 1×1 cm kidney bean leaf discs placed in 10-cm-wide, 4-cm-deep plastic cups (Clean Cup 200B, Risupack, Japan) with a 7×15 cm cotton band secured to the bottom of the cup with staples (Fig. 1). A 4×4 cm hole was cut in the bottom of the cup so that the leaf discs could contact the cotton, with four leaves per apparatus, all with either the adaxial or abaxial surface facing upward.

Then, two cups were placed upward and two downward inside a 33.5-cm-long, 25-cm-wide, 5-cm-deep plastic vat. The cotton bands were dipped in distilled water to supply the leaves with water (Fig. 1). Of the two cups facing in each direction, one contained adaxial-facing leaf discs; the other had abaxial-facing leaf discs. Therefore, the experiment had a 2×2 treatment design: gravity × leaf surface. Leaves were irradiated by 40 W fluorescent tubes (16L8D photoperiod) that were fixed perpendicularly at 40 cm interval on a side wall 30 cm apart from the center of the vats.

On the 1st day of the experiment, one adult virgin female *T. urticae* was introduced onto each leaf disc within 2 hours of the adult emergence described above. Adult mites were removed

Fig. 1. The experimental equipment used to test *T. urticae* fecundity on the upper and lower sides of leaves facing upward or downward. Using staples, a cotton band was fixed to the bottom of the cup, where a square hole was cut. The cup had two air vents on the side.

6 days later, and the number of eggs laid on each leaf disc was counted. We prepared 128 *T. urticae* mothers in eight vats, 12 of which died or escaped from the leaf discs. Data from these 12 mothers were excluded from the analysis. Consequently, the analysis included 32 females for both the adaxial leaf surfaces facing upward and downward and 27 and 25 for the abaxial leaf surfaces facing upward and downward, respectively. We used four mothers for each treatment in a batch (Fig. 1).

To assess the effects of the microclimate on the experimental equipment facing either upward or downward, we constructed equipment similar to that for the mite fecundity test with temperature/humidity data loggers (Hygrochron, KN Laboratories, Japan) instead of leaves. Temperature and RH were logged every hour for 20 h.

We evaluated the effects of gravity and leaf side on *T. urticae* fecundity, i.e., the total number of eggs laid during the 6 days after adult emergence, with a generalized linear model (GLM) assuming a Poisson distribution (log-link) and model-selection with Akaike's information criterion (AIC). We constructed the models using the "glm" module in R (version 2.10.1; R Development Core Team 2009).

Fig. 2. The 6-day *T. urticae* fecundity on kidney bean leaf discs facing upward (open bars) or downward (gray bars). The vertical line on the top of the bars indicates the standard error.

RESULTS AND DISCUSSION

The 6-day *T. urticae* fecundity was 52.9 ± 1.37 and 57.7 ± 1.29 eggs (mean $\pm$ SE) on the adaxial leaf surfaces facing upward and downward, respectively, and 42.3 ± 2.37 and 51.0 ± 1.58 eggs on the abaxial leaf surfaces facing upward and downward, respectively (Fig. 2). The Poisson GLM and subsequent model selection supported the effects of gravity, leaf surface, and their two-way interaction on *T. urticae* fecundity (Table 1). The results indicate that fecundity was greater on the adaxial than abaxial leaf surfaces, concurring with the results of upward-facing leaf disk experiments conducted by Sakai and Osakabe (2010). This nutritional difference between leaf surfaces might result from difference in the thickness of epidermis (Vásquez et al., 2008) and/or the adaxial-biased distribution of palisade mesophyll in a leaf (Tateishi, 1987). Fecundity was greater on downward-facing leaf discs than upward-facing ones. There was no temperature difference between the cups facing upward ($23.2 \pm 0.3^\circ\text{C}$: mean $\pm$ SD) and downward ($23.2 \pm 0.2^\circ\text{C}$). The relative humidity (RH) was lower on the cups facing upward ($68.7 \pm 2.9\%$: mean $\pm$ SD) than downward ($75.4 \pm 2.6\%$).

Between 20 – 25°C , daily spider mite fecundity is reduced only in arid ($< 30\%$ RH) or humid (> 85 – 90% RH) climates (Boudreaux, 1958; Nickel, 1960; Boyne and Hain, 1983; Perring et al. 1984). To the best of our knowledge, there are no specific descriptions of mite fecundity variation between 60 – 75% RH. Nickel (1960) reported that *Tetranychus desertorum* Banks fecundity did not change between 30% and 85% RH; likewise, Boyne and Hain (1983) found that the daily *Oligonychus ununguis* (Jacobi) fecundity did not change between low (30 – 40% RH) and moderate (50 – 60% RH) humidity. Therefore, the difference in *T. urticae* fecundity between the upward- and downward-facing kidney bean leaf discs was unlikely to have been caused by the humidity difference. Instead, gravity direction affected the oviposition rates.

However, when the leaf surface effect was considered, *T. urticae* fecundity on the adaxial leaf surfaces facing upward competed with that on the abaxial leaf surfaces facing downward.

Table 1. The GLM (log-link, Poisson errors) results for *T. urticae* fecundity on kidney bean leaf discs. AIC = 875.43 (the selected model) and 876.91 (the model lacking interactions).

	Estimate	Std. Error	z value	Pr (> z)
Intercept	3.93	0.0280	140.0	$< 2.00 \times 10^{-16}$
Gravity (upside)	-0.186	0.0407	-4.57	4.82×10^{-6}
Surface (adaxial)	0.124	0.0364	3.40	6.77×10^{-4}
Gravity $\times$ surface	0.0986	0.0530	1.87	0.0620

Because adaxial leaf surfaces have a nutritional advantage over abaxial surfaces, this advantage could counteract the negative effects of the upward-facing condition on the leaves of normal kidney bean plants. Therefore, despite their respective effects, gravity and nutritional quality between leaf surfaces do not explain the concentration of *T. urticae* on the lower side of kidney bean leaves under normal conditions.

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摘要

葉面の表裏における栄養条件の差がナミハダニの産卵数に与える影響は重力により補償される

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ナミハダニが寄主葉の上面を産卵場所として利用しないことは、風雨や太陽光紫外線による卵への悪影響を回避するための適応だと考えられている。一方で葉面の表裏における栄養条件の違い、および上下の葉面において虫体に掛かる重力方向の違いが、ナミハダニの適応度に与える影響は十分に検討されていなかった。本研究ではインゲンマメのリーフディスク（単一葉面）を用い、その表裏および上下がナミハダニの産卵数に与える影響を評価した。葉表では葉裏よりも、下面では上面よりもそれぞれ産卵数が増加する傾向が支持された。しかし「葉表かつ上面」と「葉裏かつ下面」のリーフディスク間では、これら2因子の影響が打ち消し合い産卵数は拮抗した。すなわち葉面の栄養条件ないし重力方向について、単独でのボトムアップ効果は認められるものの、ナミハダニがインゲンマメ葉の上面（葉表）に卵を産まない理由にはならないと考えられた。