



Host plant and field density of *Tetranychus phaselus* Ehara (Acari: Tetranychidae)

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ABSTRACT

Tetranychus phaselus (Acari: Tetranychidae) lives chiefly on the mulberry weed *Fatoua villosa* (Moraceae), and reaches high densities in the autumn. To test the prediction that *T. phaselus* is adapted to *F. villosa*, we investigated the suitability of six host plants available in this species' habitat. We supplementarily investigated the association between the density of *T. phaselus* and the density of an important predatory mite, *Neoseiulus womersleyi* (Acari: Phytoseiidae) on *F. villosa* leaves to infer the predation pressure. To know the characteristics of host plant use in *T. phaselus*, these characteristics were compared with the population of the common polyphagous species *T. kanzawai* on ramie (*Boehmeria nivea*, Urticaceae) leaf. With respect to host-plant suitability, *T. phaselus* had one of the highest egg production and the survival rate on *F. villosa* among all six host plants examined, which partly supports the prediction that *T. phaselus* is adapted to *F. villosa*. On the other hand, *T. phaselus* egg production and development time on *F. villosa* was similar to that of *T. kanzawai* reared on *F. villosa*. Nevertheless, *T. phaselus* was found only on *F. villosa* in the field, and the density per leaf area of *T. phaselus* on *F. villosa* was higher than that of *T. kanzawai* on ramie leaves. Considering that the density of *N. womersleyi* was not significantly associated with the density of *T. phaselus*, the high density of *T. phaselus* could be partly attributed to the scarcity of *N. womersleyi* on *F. villosa* leaves.

Key words: *Neoseiulus womersleyi*, phytoseiid mite, *Tetranychus kanzawai* Kishida

INTRODUCTION

Tetranychus phaselus (Acari: Tetranychidae) is a minor pest species distributed in East Russia, China, Japan, Korea, and Taiwan and appears on about ten plant species (Gotoh et al., 2015). In Japan, *T. phaselus* feeds on *Fatoua villosa* (Moraceae), ramie (*Boehmeria nivea* [L.] Gaudich.

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var. *concolor* Makino f. *nippononivea* [Koidz.] Kitam. ex H. Ohba, Urticaceae), cultivated bean plants (Ehara, 1960; Gotoh et al., 2015), and, in the Okinawa Prefecture, *Acalypha australis* L. (Euphorbiaceae) (Ohno and Kijima, 2012). Although the host range of *T. phaselus* has not been clarified in detail, it appears to be narrower than those of the common polyphagous pest species *T. kanzawai*, which is broadly distributed among Asian countries and feeds on various plants (Gomi and Gotoh, 1996; Ehara and Gotoh, 2009).

Tetranychus phaselus frequently lives on *F. villosa*, which other mite species do not often use as host plant. The population size of *T. phaselus* in the Kochi Prefecture, located in southwestern Japan, increases after the maturation of *F. villosa* leaves in July, and maintains a high density on that host until defoliation in December (K. Ito, personal observation). Thus, we predicted that *T. phaselus* is adapted to *F. villosa*. To test this prediction, the host-plant suitability in *T. phaselus* should be evaluated, but the knowledge of host plants in *Tetranychus* species is restricted to pest species such as *Tetranychus urticae* or *T. kanzawai* (e.g. Gomi and Gotoh, 1996).

In this study, we investigated the pattern of host-plant utilization in *T. phaselus*. We assessed the suitability of six potential host plants of *T. phaselus*, using developmental time, immature survival rate, and egg production. We predicted that its development time would be shortest, survival rate highest, and egg production highest, on *F. villosa* leaves among these host plants, because host adaptation often results in such changes to increase fitness on the host plant (Fry, 1989, 1990). We supplementally surveyed the field occurrence of *T. phaselus* and the predatory mite *Neoseiulus womersleyi* (Schicha) (Acari: Phytoseiidae) to infer predation pressure on *F. villosa*. Comparing the pattern of host plant utilisation with the pattern in well-studied species would be fruitful to know the characteristics of host plant use adaptation in *T. phaselus*. Therefore, we compare the results in *T. phaselus* with the results in *T. kanzawai*.

MATERIAL AND METHODS

Host-plant suitability

Tetranychus phaselus and *T. kanzawai* populations were collected from the Monobe campus of Kochi University, Kochi, Japan (33°33'N, 133°40'E) in early October 2010. *Tetranychus phaselus* were principally identified by microscopy ($\times 400$) based on the male aedeagus in the mounted specimen: *T. phaselus* males have a conspicuous sharp-ended aedeagus, whereas the *T. kanzawai* male has a door-nob shaped aedeagus (Ehara, 1960; Ehara and Gotoh, 2009). Body colour was supplementally used for identification; the adult female body colour of *T. phaselus* and *T. kanzawai* is bright red and dark red, respectively, and the quiescent deutonymph colour of *T. phaselus* is darker than that of *T. kanzawai* (Ehara and Gotoh, 2009).

These mites were maintained for two weeks (approximately one generation time) on detached kidney bean leaves (*Phaseolus vulgaris*, Fabaceae) at 25°C under a 16L:8D photoperiod (the same conditions were used for all experiments).

The host species *Mallotus japonicus* (Euphorbiaceae), *B. nivea*, *Clerodendrum trichotomum* (Lamiaceae), *F. villosa* and *Bidens pilosa* var. *pilosa* (Asteraceae), which were prevalent in the habitat of the two species of spider mites, were collected from October to November 2010.

Phaseolus vulgaris (bred at 25°C under 16L:8D), which is a favourable host for many *Tetranychus* species, was also provided. All leaves were washed in running tap water, and insects and mites were removed with a fine brush under stereomicroscopy.

To assess the egg production of *T. phaselus*, leaf squares of 1 × 1 cm of each host plant, including the midrib of the leaf, were placed on water-soaked cotton pads in an insect breeding dish (9-cm diameter, Insect Breeding Dish; SPL Life Sciences Inc., Korea). A teleiochrysalis (pre-imaginal) female and an adult male were introduced onto each leaf square (n = 12 for each plant) and observed daily. The number of eggs deposited within five days of adult emergence (including the day the adult emerged) was recorded for each host plant. Data for females that died within five days were excluded from the analysis.

To assess development time, one-day-old larvae hatched on *P. vulgaris* leaf squares from the egg production experiment were individually isolated and placed on new leaf squares from each of the six test plants. Adult emergence and survival was recorded by daily observation. Individuals trapped in water barriers around the leaf squares during development were ignored. Twelve larvae were examined for each plant.

The effect of host plants on egg production and on female development time were analysed separately using two-way ANOVA, in which host plant and mite species were independent variables. Tukey's HSD was used to conduct pairwise comparisons between host plants for each mite species. The differences in the survival rates during development among host plants were tested using a chi-square test for each mite species. Pairwise comparisons of survival rates between host plants in each mite species were conducted by Ryan's method (Ryan, 1960). The survival rates included both sexes, because sex identification was difficult for the immature stages. To compare performance, we compared the egg production, development time, and survival rate on *F. villosa* between the two mite species (t-test for egg production and development time, and the chi square test for survival). All statistical analyses were conducted using R ver. 2.15.2 (R Core Team, 2012).

Mite density in the field

Fifteen *B. nivea* shoots from three sites and 24 *F. villosa* shoots from four sites with mite-infested leaves were randomly selected at the Monobe campus on 6–22 October 2010. We randomly sampled 46 and 77 leaves with feeding scars from *B. nivea* and *F. villosa* shoots, respectively (3–6 leaves per shoot). The number of spider mites and predatory mites on each leaf was counted under a stereomicroscope (× 20). We calculated the average numbers per leaf. *Neoseiulus womersleyi* were identified as described by Ehara (2007). The density of other phytoseiid mites, fly larvae, thrips, rove beetles, and ladybird beetles was too low for analysis.

Because *B. nivea* leaves were larger than *F. villosa* leaves, we converted the densities per leaf into densities per leaf area (mm²). For this, leaf length of infested leaves was measured from the base to the leaf vein tip, excluding petioles, using a ruler (n = 28 and n = 6 for *F. villosa* and *B. nivea*, respectively). The association between leaf length and leaf area was obtained by scanning 50 and 53 undamaged mature leaves from *B. nivea* and *F. villosa*, respectively, using a document scanner DCP-750CN (HP Development Company) at 200 dpi on 20 September 2012. Leaf length (*straight line* in ImageJ 1.43u, <http://rsbweb.nih.gov/ij/>) and area (*particle analysis*) were measured for each leaf. The leaf area of the two plant species was compared using a two-sample

t-test. Leaf area for each host species was regressed onto leaf length by fitting a quadratic curve without intercept. Using the equations for the fitted lines, spider mite density per leaf area was calculated based on the lengths of the leaves sampled in October 2010. To decrease any bias resulting from differences in mite density, the leaves of each plant species were collected from sites at which both mite species occurred at a similar density (sites A and F; see Results). The densities on the two host plants were compared using the nonparametric Wilcoxon rank-sum test, because the data were not normally distributed.

The spider mite density per leaf was linearly regressed to the predatory mite density. Slope homogeneity was analysed using an analysis of covariance (ANCOVA), in which the species of spider mite was the independent variable and spider mite density per leaf was the covariate. The homogeneity of regression slopes was tested using the significance level of the interaction term (Sokal and Rohlf, 2012).

RESULTS

Host-plant suitability

All the females that emerged survived for five days, except for one *T. kanzawai* female on *F. villosa*. The oviposition pattern was different for the two mite species (Fig. 1), as indicated by the two-way ANOVA (all three terms were significant; Table 1). Of the six plant species examined, the median egg number for *T. phaselus* was highest on *F. villosa* (Fig. 1A). The average egg production of *T. phaselus* on *M. japonicas*, *F. villosa*, and *P. vulgaris* was significantly higher than that on *B. nivea*, *C. trichotomum*, and *B. pilosa* (no eggs were produced on *B. pilosa*; Tukey's HSD, $p < 0.05$). For *T. kanzawai*, the median egg number on *F. villosa* and *B. pilosa* was lower than that on the other four host plants (Fig. 1B). The average egg production of *T. kanzawai* on *F. villosa* was significantly lower than on *M. japonicas*, *C. trichotomum*, or *P. vulgaris* (Tukey's HSD, $p < 0.05$). The egg production of *T. phaselus* on *F. villosa* was not significantly different from that of *T. kanzawai* ($t_{19} = 1.910$, $p = 0.071$).

Of the adults that emerged, 83% and 94% were female for *T. phaselus* and *T. kanzawai*, respectively ($n = 36$ and 53 ; Table 2). The survival rate of *T. phaselus* varied significantly among host plants, with few surviving on *C. trichotomum* and *B. pilosa* ($\chi^2_5 = 41.454$, $p < 0.001$). On the other hand, more than 67% of *T. kanzawai* larvae survived until adult emergence on all host plants ($\chi^2_5 = 5.109$, $p = 0.403$). Pairwise comparison demonstrated that the *T. phaselus* survival rates on *F. villosa*, *B. nivea*, and *M. japonicas* were significantly higher than those on the other plant species. All *T. phaselus* larvae developed into adults on *F. villosa*, while 58% of *T. kanzawai* individuals developed ($\chi^2_1 = 0.015$, $p = 0.037$).

Female development time is shown in Fig. 2. The average development time of *T. phaselus* on *F. villosa* was not significantly different from that on *M. japonicas*, *B. nivea*, or *P. vulgaris* (Tukey's HSD, $p > 0.05$). The average development time of *T. kanzawai* on *F. villosa* was not significantly different from that on the other host plants, except for *C. trichotomum* (Tukey's HSD, $p < 0.05$). The two-way ANOVA showed that all factors and interactions were significant, suggesting that the pattern of development time was different between the two mite species (Table 3). The development time of *T. phaselus* on *F. villosa* was not significantly different from

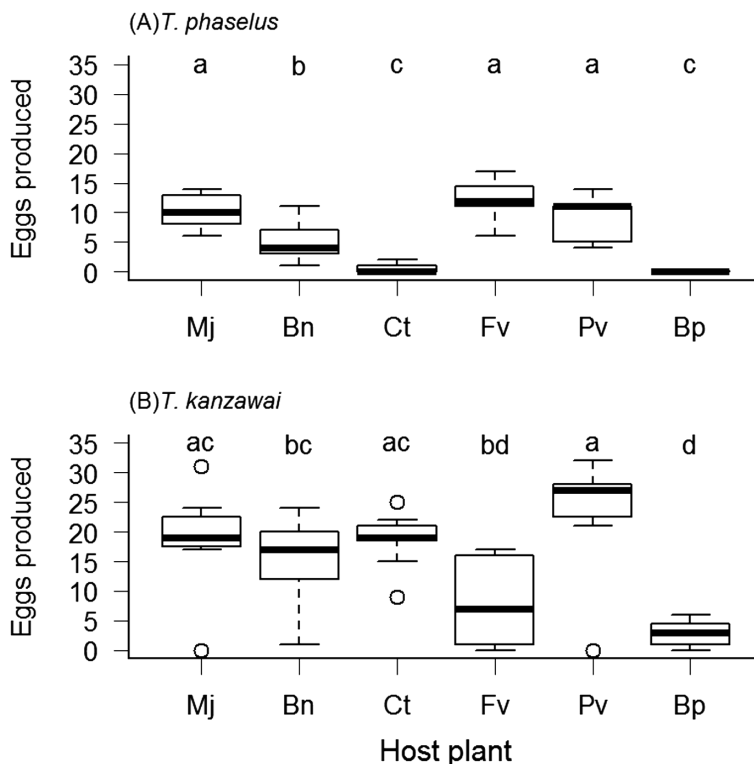


Fig. 1. Egg production in five days on six host plants at 25°C / 16L:8D. (A) *T. phaselus*; (B) *T. kanzawai*. Different letters indicate significant differences in each mite species, according to the Tukey's HSD test ($p < 0.05$). Mj, *Mallotus japonicas* (Euphorbiaceae); Bn, *Boehmeria nivea* (Urticaceae); Ct, *Clerodendrum trichotomum* (Lamiaceae); Fv, *Fatoua villosa* (Moraceae); Pv, *Phaseolus vulgaris* (Fabaceae); Bp, *Bidens pilosa* var. *pilosa* (Asteraceae).

that of *T. kanzawai* ($t_{14} = 1.151$, $p = 0.269$).

Mite density in the field

In the field, *T. phaselus* and *T. kanzawai* colonies were found only on *F. villosa* and *B. nivea*, respectively. Fig. 3 shows the density per leaf for both species on the host plants. There was no significant difference in the spider mite density per leaf (Table 4, species), although the density of each species varied among locations (Table 4, site within species). However, the average leaf area of *B. nivea* was 1.78 times larger than that of *F. villosa* (mean \pm SD, 3844 ± 3263 [n = 50] and 2156 ± 1871 mm² [n = 53], respectively; $t_{101} = 3.244$, $p < 0.001$). The leaf area (y) was fitted to the quadratic regression line on leaf length (x): $y = 0.365x^2 - 0.073x$, $r^2 = 0.96$ for *B. nivea* (n = 50) and $y = 0.521x^2 - 8.231x$, $r^2 = 0.97$ for *F. villosa* (n = 53). The density per area (mm²) of *T. phaselus* on *F. villosa* was significantly higher than that of *T. kanzawai* on *B. nivea* (Wilcoxon rank-sum test, $W = 36$, $p = 0.026$; Fig. 4).

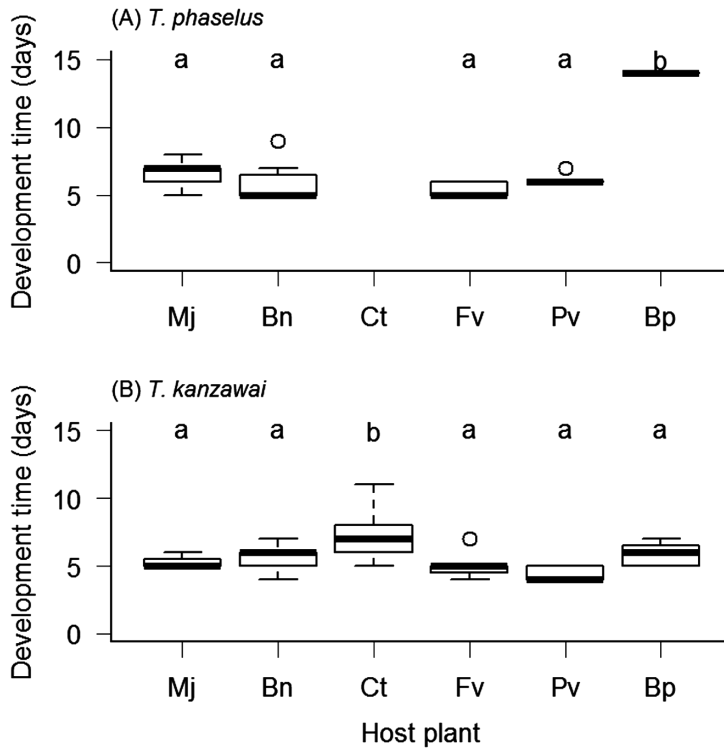


Fig. 2. Development time of females at 25°C / 16L:8D. (A) *T. phaselus*; (B) *T. kanzawai*. Different letters indicate significant differences in each mite species, according to the Tukey's HSD test ($p < 0.05$). Mj, *Mallotus japonicas* (Euphorbiaceae); Bn, *Boehmeria nivea* (Urticaceae); Ct, *Clerodendrum trichotomum* (Lamiaceae); Fv, *Fatoua villosa* (Moraceae); Pv, *Phaseolus vulgaris* (Fabaceae); Bp, *Bidens pilosa* var. *pilosa* (Asteraceae).

Table 1. Two-way ANOVA for egg production at 25°C / 16L:8D within 5 d for each host plant

	<i>Df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>p</i>
Species	1	2692	2692.1	107.11	< 0.001
Host	5	2638	527.6	20.99	< 0.001
Interaction	5	1792	358.4	14.26	< 0.001
Residuals	110	2765	25.1	-	-

Table 2. Survival rate during development on each host plant at 25°C / 16L:8D. Sexes are pooled. Mj, *Mallotus japonicas* (Euphorbiaceae); Bn, *Boehmeria nivea* (Urticaceae); Ct, *Clerodendrum trichotomum* (Lamiaceae); Fv, *Fatoua villosa* (Moraceae); Pv, *Phaseolus vulgaris* (Fabaceae); Bp, *Bidens pilosa* var. *pilosa* (Asteraceae). Different letters indicate significant differences in each mite species (Ryan's test, $p < 0.05$)

Host	<i>T. phaselus</i>			<i>T. kanzawai</i>		
	Dead	Survived	%	Dead	Survived	%
Mj	0	10	100 a	2	10	83 a
Bn	3	8	73 a	1	11	92 a
Ct	11	0	0 c	2	9	82 a
Fv	0	11	100 a	5	7	58 a
Pv	5	6	55 b	4	8	67 a
Bp	10	1	9 bc	4	8	67 a

Table 3. Two-way ANOVA for development time of females at 25°C / 16L:8D

	<i>Df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>p</i>
Species	1	6.16	6.163	5.891	0.018
Host	5	63.66	12.733	12.171	<0.001
Interaction	4	50.74	12.685	12.125	<0.001
Residuals	69	72.18	1.046	-	-

Fig. 5 shows the association between the density of each species of spider mite and of *N. womersleyi* per leaf. The ANCOVA revealed a significant interaction between species and density (Table 5), indicating that the regression lines of the two species were not parallel. The slope was significantly positive only for *T. kanzawai* (*T. phaselus*: $y = 0.319^* + 0.0004^{NS}x$, $r^2 = 0.001$, $n = 77$; *T. kanzawai*: $y = 1.410^{**} + 0.013^{**}x$, $r^2 = 0.217$, $n = 46$; ^{NS} non-significant; * $p < 0.05$; ** $p < 0.01$). Furthermore, the proportion of *F. villosa* leaves on which *N. womersleyi* individuals were found was significantly lower than the proportion for *B. nivea* (*B. nivea*: 50.0% [$n = 46$]; *F. villosa*: 16.9% [$n = 77$]; $\chi^2_1 = 13.680$, $p < 0.001$).

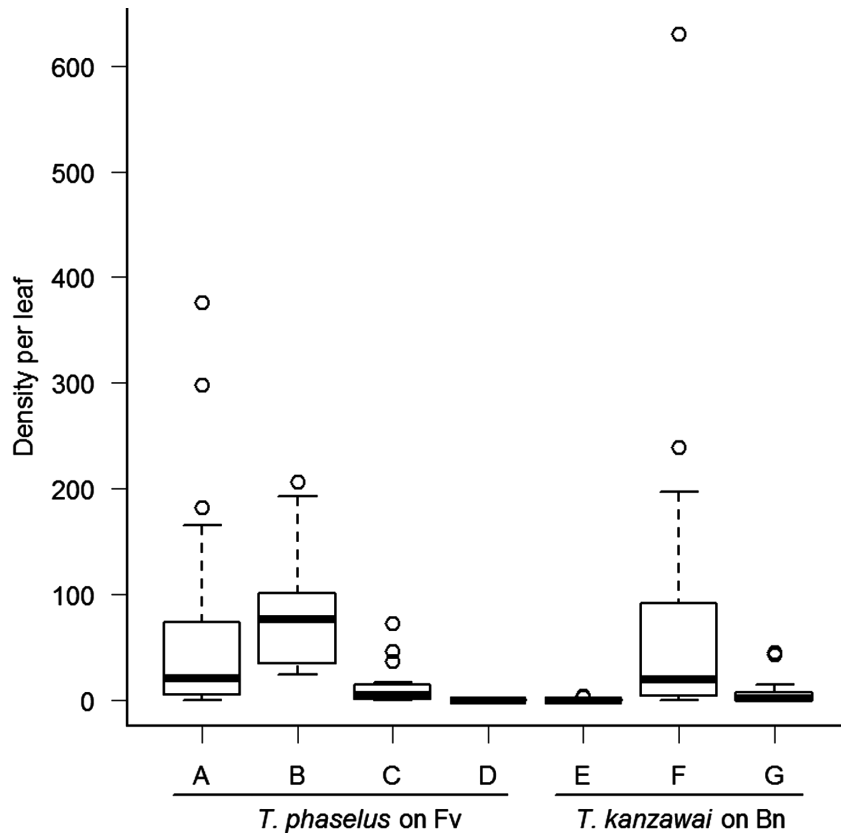


Fig. 3. Density of individuals at each site on the Monobe Campus of Kochi University. A–D: *T. phaseolus* populations on *F. villosa*; E–G: *T. kanzawai* populations on *B. nivea*.

DISCUSSION

The pattern of host use of *T. phaseolus* were different from that of *T. kanzawai*. *Tetranychus phaseolus* exhibited one of the highest egg production, and the highest survival rates on *F. villosa* among six plant species examined, though no significant difference was observed in development time except for *B. pilosa*. On the other hand, the average egg production, the average development time, and the survival rate of *T. kanzawai* on *F. villosa* tended to be lower than *B. nivea*, though a significant difference was not observed in either trait. Furthermore, *T. phaseolus* could not reproduce on *C. trichotomum* or *B. pilosa*, whereas *T. kanzawai* could reproduce well on both of these plants. These results suggest that *T. phaseolus* has a narrower host range and appears to be adapted to *F. villosa*. Therefore, the prediction that *T. phaseolus* are adapted to *F. villosa*, which is not often used by other mite species, was partly supported. However, we did not observe *T. phaseolus* to have a particularly high reproductive capacity on *F. villosa* leaves; the egg

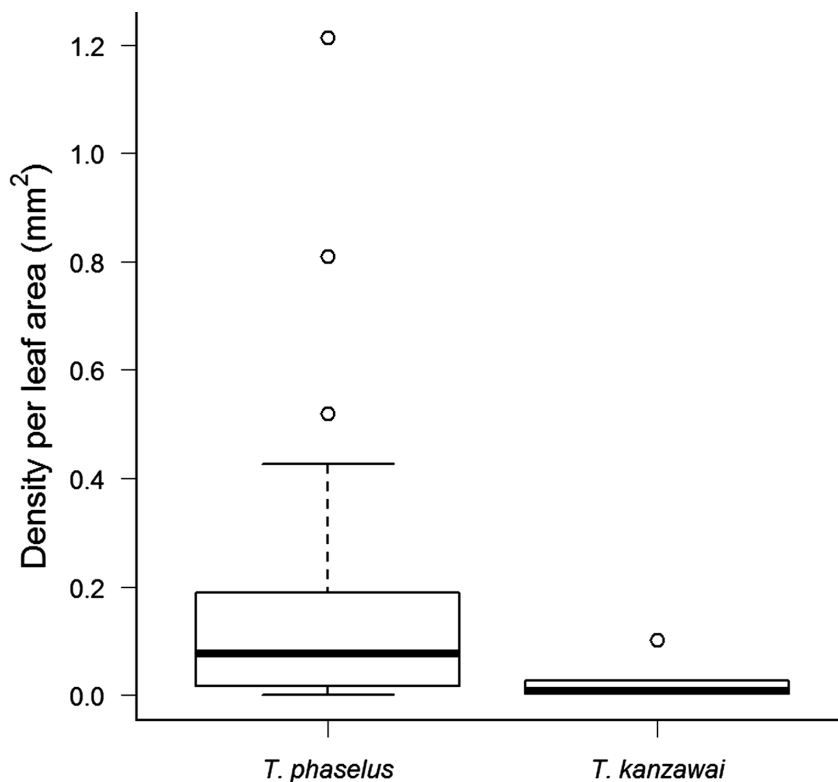


Fig. 4. Density of individuals for all instars per leaf area. $N = 28$ and $n = 6$ for *T. phaselus* (site A in Fig. 3) and *T. kanzawai* (site F), respectively. Zero data are excluded. $p = 0.026$ between species, according the Wilcoxon rank-sum test.

production and development time of *T. phaselus* were not significantly different from those of *T. kanzawai*, though the survival rate on *F. villosa* was higher than that of *T. kanzawai*.

Nevertheless, we found *T. kanzawai* exclusively on *B. nivea* and *T. phaselus* only on *F. villosa* in the field. Furthermore, although the density per leaf of *T. phaselus* was not different from that of *T. kanzawai*, the *T. phaselus* density per leaf area on the *F. villosa* leaves was much higher. This differences in the density of the spider mites may be associated with the density of their predators. On *T. phaselus*-infested leaves, the density of *N. womersleyi* was low, but where *T. kanzawai* occurred the density was much higher. If *N. womersleyi* feeds on spider mite individuals and reproduces, the density of *N. womersleyi* would increase alongside the spider mite. However, the density of *N. womersleyi* on *F. villosa* increased only slightly, as demonstrated by the nonsignificant slope of the predator density regression line in the *T. phaselus* habitat (Fig. 5A). The difference in the predator density was notable on leaves with > 100 spider mite individuals per leaf, on which only a few individuals of *N. womersleyi* were found on *F. villosa*, whereas more than five *N. womersleyi* individuals were found on *B. nivea*. Therefore, *T. phaselus*

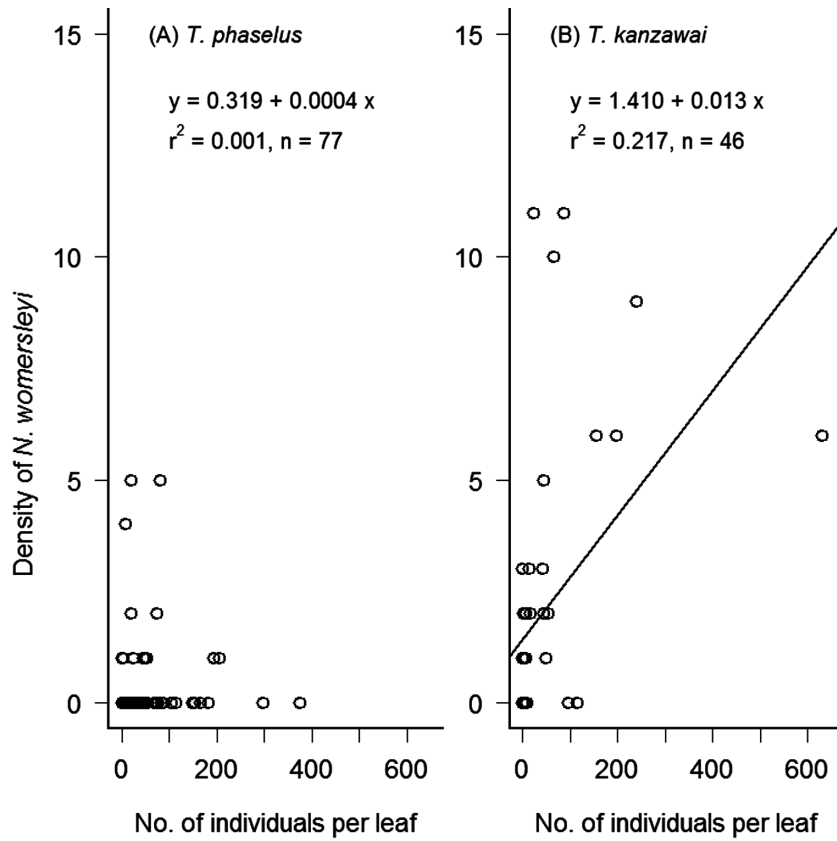


Fig. 5. Association between spider mite density (A, *T. phaselus*; B, *T. kanzawai*) and that of *N. womersleyi*. The slope of the regression line is significant only for *T. kanzawai*.

Table 4. Nested ANOVA for spider mite density per leaf

	<i>Df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>p</i>
Species	1	164	164	0.007	0.939
Site within species	5	126351	25270	4.052	0.002
Residuals	116	723372	6236	-	-

Table 5. ANCOVA of phytoseiid mite density

	<i>Df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>p</i>
Species	1	81.42	81.422	23.559	< 0.001
Density	1	53.81	53.813	15.571	< 0.001
Interaction	1	38.29	38.292	11.080	0.001
Residuals	119	411.27	3.456	-	-

might have evolved some predator-avoidance strategies on *F. villosa* leaves, though they need to be elucidated in future studies.

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摘要

サガミナミハダニの寄主植物と野外密度

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サガミナミハダニはクワクサを主な寄主植物とし、夏から秋にかけて高密度となる。サガミナミハダニがクワクサに適応しているという予測を検証するため、本種における寄主植物の適合性、および野外密度について調査を行い、それらの傾向を普通種のカンザワハダニ（カラムシ寄生）と比較した。葉の質について、サガミナミハダニの食性幅はカンザワハダニより狭く、クワクサでもっとも産卵数と生存率が高かったことから、クワクサに適応している可能性が示唆された。しかし、クワクサ上での産卵数や発育速度はカンザワハダニとは有意差がなかった。一方、野外ではサガミナミハダニとカンザワハダニはそれぞれクワクサとカラムシにしか見られず、サガミナミハダニの葉面積当たり密度はカンザワハダニよりも高かった。この傾向は、クワクサ上では捕食者のケナガカブリダニの密度が低いことと関係している可能性がある。