Response of the predatory mite, *Amblyseius womersleyi* (Acari: Phytoseiidae), toward herbivore-induced plant volatiles: Variation in response between two local populations

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Abstract

We studied the response of two populations of the predatory mite, *Amblyseius womersleyi*, toward volatiles of kidney bean leaf infested by *Tetranychus urticae* in a Y-tube olfactometer. The predators collected in Kyoto (*A. womersleyi*-Kyoto) showed a significant preference for infested leaf volatiles to uninfested leaf volatiles, whereas the predators collected in Shizuoka (*A. womersleyi*-Shizuoka) did not. This difference was not due to a difference in their feeding experiences but to a genetic difference, since both populations were tested after being reared under the same laboratory conditions for more than three months (more than 10 generations). We then compared the performance and behavior of the two populations. The fecundity and oviposition periods of *A. womersleyi*-Kyoto were not significantly different from those of *A. womersleyi*-Shizuoka. However, the dispersal of *A. womersleyi*-Kyoto from a *T. urticae*-infested bean leaf culture occurred earlier than that of *A. womersleyi*-Shizuoka. The lower dispersal ability in *A. womersleyi*-Shizuoka compared that of *A. womersleyi*-Kyoto is attributed to a difference in ability to distinguish infested plant volatiles from uninfested ones.

Key words: *Amblyseius womersleyi*, *Tetranychus urticae*, herbivore-induced plant volatiles, genetic variation, dispersal behavior

INTRODUCTION

Several studies on tritrophic systems consisting of plants, herbivores and carnivorous natural enemies showed that the herbivores' damage induced plants to produce specific blends of volatiles that attract carnivorous natural enemies of the herbivores (Dicke, 1994; Takabayashi and Dicke, 1996, for review). The blend of herbivore-induced volatiles is affected by plant- and herbivore-factors, i.e. plant species, plant cultivar, developmental stage of the plants and the herbivores, and herbivore species (Takabayashi and Dicke, 1996, for review). Characteristic olfactory responses of carnivores corresponding to the above specificities have been reported in several systems (e.g. Sabelis and Van de Baan, 1983; Dicke et al., 1990a, b; Turlings et al., 1993; Takabayashi et al., 1994, 1995; Moraes et al., 1998).

In addition to the above plant- and herbi-

vore-factors, carnivore-factors also affect tri-trophic interactions. Previous feeding experience, starvation and dietary needs are factors reported to affect the response of predatory mites and parasitic wasps (Sabelis and Dicke, 1985; Dicke et al., 1986, 1989, 1990a; Dicke and Groeneveld, 1986; Turlings et al., 1993). In addition, Margolies et al. (1997) reported the presence of genetic components that affect the olfactory response of the predatory mite, *Phytoseiulus persimilis*, from Koppert BV (The Netherlands). It is of interest to elucidate whether native natural enemies of different local populations show different olfactory responses toward herbivore-induced plant volatiles.

A. womersleyi is a predatory mite native to Japan. It suppresses populations of dominant spider mite species, such as *T. urticae* and *T. kanzawai* (Hamamura, 1986). Many studies on *A. womersleyi* have focused on the life history and possibility for biological control of this

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species (e.g. Mori and Saito, 1979, 1981; Hamamura, 1986; Kishimoto and Takafuji, 1994). However, nothing is known about the response of *A. womersleyi* toward herbivore-induced plant volatiles. We report here the response of *A. womersleyi* toward *Tetranychus urticae*-infested kidney bean leaf volatiles. We used *A. womersleyi* collected from two different environments in Japan, to study whether there is any difference in the olfactory response between the two local populations. The local populations of predatory mites may also show different performance (e.g. insecticide susceptibility, Hamamura, 1986; Mochizuki, 1994). In this study, therefore, we measured fecundity, the oviposition period and dispersal rate in the two populations. The correlation between the olfactory responses and the above performance of the two populations is discussed.

MATERIALS AND METHODS

Plant. We used kidney bean plant (*Phaseolus vulgaris*) as food for *T. urticae*. The plants were reared in plastic pots (12 cm diameter, 10 cm depth) in a climate room ($25 \pm 2^\circ\text{C}$, 50–70 R.H., 16L–8D). Two to three weeks after germination, the plants were offered to the spider mites. The plants were also used for the olfactometer experiments.

Mites. We used *T. urticae* from a laboratory-maintained culture for the experiments. The culture had been reared on kidney bean plants in a climate room ($25 \pm 2^\circ\text{C}$, 50–70 R.H., 16L–8D) for more than 3 years.

The predatory mite, *A. womersleyi*, was collected on potted chrysanthemum plants infested by *T. urticae* in an experimental greenhouse at the Kyoto University campus in August 1995. These predatory mites were named the *A. womersleyi*-Kyoto population. Predatory mites were also collected on tea plants infested by *T. kanzawai* in a tea plantation in Kanaya, Shizuoka Prefecture in June 1994. These predatory mites were named the *A. womersleyi*-Shizuoka population.

We reared the predators separately on a plastic board (10 × 15 cm, 0.5 cm thickness) placed on a foam plastic saturated with water (10 × 15 cm, 5 cm thickness). The foam plastic was placed in a plastic tray (13 × 18 cm) filled

with water in a growth chamber ($25 \pm 2^\circ\text{C}$, 50–70 R.H., 16L–8D). The predatory mites were offered two to three bean leaves infested by *T. urticae* (30 to 50 adult females and other stages of *T. urticae* were on each leaf) every two days. To exclude previous feeding experiences at the collection sites, the predators used for the experiments were reared in a laboratory for at least three months (ca. 10 generations) prior to the first experiment.

Response of female predatory mites toward leaves infested by *T. urticae* in a Y-tube olfactometer. We used a Y-tube olfactometer (Takabayashi and Dicke, 1992) to test the olfactory response of *A. womersleyi*. Air (3 l/min) was first cleaned through granular activated charcoal (1 l in a glass bottle) and then sent to the odor source bottles (a sample odour bottle and a control odour bottle). The air containing each odour was sent separately to either arm of the olfactometer setup. Duration of starvation in two predator species affected their olfactory responses to the infested kidney bean leaf volatiles (unpublished data). Therefore, in this experiment, satiated females of the predatory mites were individually introduced at a start point (downwind end) on a Y-shaped iron wire set in the tube, and their behavior on the wire was observed for 5 min. When the predator walked across the finish line set on the upwind ends of the Y-shaped wire, this was classified as a final choice. When the predator did not make a final choice within 5 min, it was classified as no choice. We switched the arm containing the sample odour and the control odour in the olfactometer after every 5 bioassays. The bioassay was performed in a climate room ($25 \pm 2^\circ\text{C}$, 50–70 R.H.).

Odour sources for the Y-tube olfactometer bioassay were prepared in a climate room ($25 \pm 2^\circ\text{C}$, 50–70 R.H.) as follows.

1. Uninfested leaves: We used newly detached leaves.

2. Infested leaves: Leaves infested with 30–50 adult female *T. urticae* together with other stages were detached from heavily infested plants and were immediately used for the bioassay.

3. Artificially damaged bean leaves: We damaged the leaves mechanically by slightly rubbing

the surface with fine sandpaper. We used the leaves for the bioassay immediately after damaging them.

4. Infested bean leaves without *T. urticae* and their products: Leaves infested with 30–50 adult female *T. urticae* together with other stages were detached from the heavily infested plants. The leaves, from which all spider mites and their visible products had been removed, were immediately used for the bioassay.

5. *T. urticae* and their products: We detached leaves infested with 30–50 adult female *T. urticae* together with other stages from the heavily infested plants. We collected all living spider mites and their visible products such as feces, exuviae, eggs, egg shells, dead bodies and webs from infested leaves with a fine brush. The collected mites and their products were immediately used for the bioassay.

We used ten leaves for each sample. The results were subjected to a one-tailed binomial test to determine whether the predatory mites significantly preferred the sample odour over the control odour in the olfactometer.

Performance and dispersal behavior of female predatory mites on a leaf culture. Leaf cultures (2 cm × 2 cm) were separately placed on moist cotton wool in petri dishes (9 cm diameter). We inoculated 15 female *T. urticae* on each culture. After one day, we placed one newly molted female and male adult *A. womersleyi* on

each culture. After one week, the males were removed. To prevent a shortage of prey mites, we exchanged the old infested cultures with new ones every three days. We counted the number of predator eggs laid on the cultures when exchanging the cultures. When we did not find newly-laid eggs on a culture, we judged that the oviposition of the mites was over. The duration of the oviposition period and the oviposition rates of the two populations were analyzed by a Student's *t*-test. The predatory mites that escaped from a leaf culture were judged to have dispersed. The dispersal functions of the two populations were compared with a generalized Wilcoxon test.

RESULTS AND DISCUSSION

When uninfested leaf volatiles and infested leaf volatiles were compared in the olfactometer, *A. womersleyi*-Kyoto showed a significant preference toward the infested leaf volatiles ($p < 0.001$, Fig. 1). The predators were not attracted to either uninfested leaves or spider mites and their products over clean air. They did not show a preference for either artificially damaged leaf volatiles or uninfested leaf volatiles. Finally, the predators preferred the volatiles of the infested leaves without spider mites and their products over uninfested leaves ($p < 0.05$). These results suggest that volatiles that attracted *A. womersleyi*-Kyoto are emitted

Fig. 1. The responses of *A. womersleyi*-Kyoto to kidney bean leaves with different treatments in a Y-tube olfactometer. The number of predators that made a final choice is shown in each bar. NC: no choice. *** $p < 0.001$, * $0.01 < p < 0.05$ by a one-tailed binomial test.

by leaves in response to the spider-mites' damage but not by mechanically damaged leaves or by *T. urticae* themselves or their associated products. We reported that several terpenoids and methyl salicylate were emitted from *T. urticae*-infested kidney bean leaves but not from uninfested leaves (Maeda et al., 1998). The response of predators toward volatiles emitted from leaves infested by *T. urticae* was also reported in tritrophic systems consisting of the predatory mite, *Phytoseiulus persimilis*, and Lima bean leaves (Sabelis et al., 1984), and of the predatory thrips, *Scolothrips takahashii*, and Lima bean leaves (Shimoda et al., 1997).

In contrast to *A. womersleyi*-Kyoto, *A. womersleyi*-Shizuoka did not show a preference for either uninfested or infested leaves (Fig. 2). *A. womersleyi*-Shizuoka, however, preferred the infested leaves when compared with clean air ($p < 0.001$). The predators showed a significant preference for uninfested leaf volatiles over clean air ($p < 0.05$). Thus, the lack of preference in *A. womersleyi*-Shizuoka toward infested leaf volatiles vs. uninfested ones can not be explained by the absence of a response toward infested leaf volatiles. However, it can be explained by a preference not only for the infested leaf volatiles but also for uninfested leaf volatiles over clean air. Namely, the attraction of uninfested leaf offsets that of infested leaf, rendering it insignificant. Both *A. womersleyi*-Shizuoka and *A. womersleyi*-Kyoto were used after being reared for ca. 3 months with *T. urticae* on kidney bean leaves under the same laboratory conditions. Therefore, the different responses between the two

populations toward uninfested vs. infested leaf volatiles is not due to their predation experiences within one generation but to a genetic difference originating at their respective local sites.

The numbers of eggs laid for three days by *A. womersleyi*-Kyoto and *A. womersleyi*-Shizuoka were not significantly different (2.88 ± 0.16 ; $N=36$ and 2.68 ± 0.11 ; $N=36$, respectively) by a *t*-test. The duration of the oviposition periods of *A. womersleyi*-Kyoto and *A. womersleyi*-Shizuoka were also not significantly different (13.1 ± 1.0 ; $N=13$ and 14.3 ± 0.9 ; $N=20$, respectively) by a *t*-test. The results suggest that there is no correlation between olfactory response and performance (oviposition rate and oviposition period). For dispersal behavior from a leaf culture, the Kyoto population dispersed significantly earlier than the Shizuoka population ($p < 0.05$, generalized Wilcoxon test) (Fig. 3). It is interesting to note that the predators which could distinguish between infested- and uninfested-plant volatiles dispersed earlier than those which could not distinguish the two volatiles. The ability to distinguish the two volatiles may diminish the travel cost, because the predator could find a new prey colony easier by being attracted to the mites-infested plants. These results support the prediction of a model of Bernstein et al.'s (1991): as travel cost is increased, predators that maximize foraging efficiency become more sedentary.

In this study, we found variation in the olfactory response toward herbivore-induced plant volatiles and dispersal behavior in two local populations of the predatory mite, *A.*

Fig. 2. The responses of *A. womersleyi*-Shizuoka to kidney bean leaves with different treatments in the Y-tube olfactometer. The number of the predators that made a final choice is shown in each bar. NC: no choice. *** $p < 0.001$, * $0.01 < p < 0.05$ by a one-tailed binomial test.

Fig. 3. Dispersal functions of *A. womersleyi*-Kyoto and *A. womersleyi*-Shizuoka. The predatory mites were placed on each leaf culture and those that escaped from the culture were judged to have dispersed. The two dispersal functions were significantly different by a generalized Wilcoxon test ($p < 0.05$).

womersleyi. Such variation may be the result of plant-carnivore co-evolution in different environmental conditions: environmental differences in their original habitats may have affected their olfactory responses and behaviors. To test this possibility, a comparison of the predatory mites collected from several sites in Japan needs to be carried out.

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