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Effect of Plant Nectars and Sugars on the Predatory Thrips *Scolothrips takahashii*

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Abstract: *Scolothrips takahashii* (Priesner) is a specialist predator of many spider mite species and is known from East Asia and Hawaii. *S. takahashii* can control spider mites effectively because of its high predating ability even when the pest density is low. To understand why *S. takahashii* can work when the prey is scarce, we investigated the importance of plant nectars and sugar foods on its survival and reproduction. Laboratory experiments demonstrated that *S. takahashii* detects and feeds on both plant nectar and sugar-rich food sources. Female *S. takahashii* were significantly attracted to the odors from soba and phacelia flowers whereas they did not respond to faba bean flowers. The flowers and extrafloral nectar significantly prolonged the longevity of female *S. takahashii*. Moreover, sugar-rich foods such as honey, sucrose and fructose also enhanced *S. takahashii*'s survival, but not its reproduction. The present study suggests that manipulating flowers or providing sugar solutions within agroecosystems may increase the control effectiveness of *S. takahashii* against spider mite pests such as *Tetranychus urticae*.

Keywords: Biocontrol, Natural enemies, *Tetranychus*, Alternative food, Spider mite , Non-prey plant food

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Introduction

Scolothrips takahashii (Priesner) is a native and widespread specialist predator belonging to the family Thripidae and attacks many spider mite species in Tetranychidae and Tenuipalpidae (Kishimoto, 2003; Gotoh *et al*., 2004a; Masumoto *et al*., 2012). In the field, *S. takahashii* density is abundant from early summer to fall (Kishimoto, 2002; Shimoda *et al*., 2016) and is easily found in strawberry seedling farms (Yanagita *et al*., 2012). The predatory thrips has a highly predatory ability and is an effective predator of spider mite pests (Gotoh *et al*., 2004b; Ding-Xu *et al*., 2007). They

are attracted to and stay longer on plants where the spider mite silk occurs (Kishimoto and Adachi, 2008) and use the volatiles from mite-infested leaves to locate their prey (Takahashi *et al*., 2001; Shimoda *et al*., 2002). Because of its high predatory ability, *S. takahashii* can effectively control target pest spider mites such as *Tetranychus urticae* Koch on strawberry, which are often difficult to manage solely with chemical pesticides (Yanagita *et al*., 2014).

Although *S. takahashii* is a promising specialist natural enemy for biological control of spider mite pests, limitation occurs in the mass-rearing by high labor costs. Effective cost-effective mass-rearing is often key in biological control with natural enemies (Leppla *et al*., 2014; Riddick, 2009). Therefore, to overcome the limitation, it is ideal to produce a large population of *S. takahashii* on alternative prey or foods. Use of alternative food or laboratory prey that can easily be provided is widely used to produce beneficial insects (De Clercq *et al*., 2005; Zou *et al*., 2013; Cohen, 2015). Moreover, supplying alternative foods may enhance biocontrol effectiveness because the supply can help the establishment and increase biocontrol agents when normal food (host or prey) is at low density (Muñoz-Cárdenas *et al*., 2017). Indeed, a number of studies have shown evidence that the introduction of flowering plants can increase the control effectiveness of natural enemies including insect predators (Berndt *et al*., 2005; Wäckers *et al*., 2005; Begum *et al*., 2006; Lee and Heimpel, 2008; Lundgren, 2009). Likewise, alternative foods or flowering plants can be used to facilitate biological control of spider mites with *S. takahashii;* the establishment and maintenance of *S. takahashii* populations before target pests begin to increase can be promoted with them, which should enhance the control efficiency.

Although the above-mentioned subjects should be addressed in order to use this predator effectively for spider mite control, no previous studies have examined the feasibility of the use of alternative foods and/or flowering plants for mass-rearing systems of *S. takahashii* and biological control using the predatory thrips. In the present study, we investigated: (i) the response of female *S. takahashii* to odors of flowers and sugar sources, and (ii) the impact of such food sources on the survival and reproduction of *S. takahashii*, in order to explore the feasibility of the use of alternative foods in improving the rearing and control efficacy.

Materials and Methods

Predatory thrips and spider mites:

The predator thrips *S. takahashii* and its natural prey, the two-spotted mite *Tetranychus urticae*, were originally obtained from the kudzu plant at the Ito campus of Kyushu University (KU), Fukuoka, Japan. The predator thrips and prey spider mites were reared on lima bean leaves (*Phacelia lunatus* L.) (Kishimoto, 2003; Gotoh *et al*., 2004a). One bean leaf was placed on the top of a cotton pad saturated with water in an insect breeding disc (100x40 mm) with a ventilation cap (40 mm). Several spider mites were placed on a leaf and kept in an incubator at 25±0.5°C and a photoperiod of 16 h of light and 8 h of dark (16L:8D). Some of the discs were used to maintain spider mite colonies in the laboratory, while others were used to rear *S. takahashii*. A disc with a sufficient number of spider mites was selected and some individuals of *S. takahashii* were gently placed on the lima bean leaf to maintain the predatory thrips population. The stock colony of predator thrips had been kept in the incubator for several generations in the laboratory of Insect Natural Enemies (KU) before being used in the experiments.

Plant nectars were obtained from some widely grown conservation biological control programs species such as sweet alyssum (*Lobularia maritima* L.), buckwheat (*Fagopyrum esculentum*), phacelia (*Phacelia tanacetifolia*) (Fiedler *et al*., 2008) and faba bean (*Vicia faba* L.). These plant species were grown from seed and maintained in the laboratory or field conditions, depending on the weather conditions.

Flower attractiveness on Scolothrips takahashii:

A modified version of Takabayashi and Dickes (1992) olfactometer system was used to investigate the response of *S. takahashii* toward flower odor versus clean air. The olfactometer consisted of a Y-shaped glass tube with two arms. Each arm was connected to an odor source. Cutting-flowers or extrafloral nectaries particles for testing were put in an Eppendorf tube and were used as an odor source. The Eppendorf tube with an odor source was placed in one arm and another arm contained a wet cotton pad. An airflow was generated by a small pump and flew through charcoal to prevent the contamination odor. Then, the airflow was led through an odor tube. Subsequently, the two odor flows were gone through the two arms of a glass Y-tube olfactometer. The airflow through each olfactometer arm was 4 litres per minute. Starving females of the predatory thrips was individually released into the olfactometer on an iron wire that was located in the middle of the olfactometer tube and parallel to the tube wall, and observations were initiated to examine the thrips response to the odors. The observation was ended when the *S. takahashii* moved or went to the end of one of the olfactometer arms. If the predator did not respond within five minutes, it was defined as no response to the odor and was discarded from the following statistical analysis. The experiment was conducted once as a completely random design (CRD) with 60 replications per treatment.

Effect of the plant nectars on the survival of Scolothrips takahashii:

The experiment was conducted to determine the role of plant nectar on the survival of female *S. takahashii*. A newly moulting adult female from the stock culture was collected and was then released in an experimental cage, modified by van Rijn and Wäckers (2016). The cage was an insect breeding dish (100 x 20 mm) with a ventilation cap (40 mm in diameter) (Cat. No. 310201, SPL Life Sciences, Korea). Cutting flowers of buckwheat or phacelia or extrafloral particles of faba bean were used in this experiment. The flowers or extrafloral nectary particles were cut and put in an Eppendorf tube as the food source and were given to each test female in the breeding dish. The tube with a test food was exchanged every three days to give fresh food to the thrips. A wet cotton pad was provided to maintain the moisture in the dish. The dish was kept at 25 ± 0.5°C under 16 h of light and 8 h of dark (16L:8D) photoperiod. The thrips' survival was recorded every 24h using the dissecting microscope until the thrips died. Test individuals that had escaped or died because of accidents were excluded from the following analyses.

Feeding preference on alternative foods bioassay:

The feeding behavior of *S. takahashii* females on alternative foods was observed using a modified version of the method applied by Provost *et al.* (2006). The feeding preference was observed in the arena, which contained a parafilm (3x3 cm) placed on the top of a pad of cotton saturated with water in a Petri dish. The alternative foods, *i.e.*, honey, glucose, sucrose, fructose, all in 50% solutions (Wako Pure Chem Inc.), and bee pollen (suspension) were sequentially placed on the parafilm, while the leaf discs with surplus spider mite eggs (3x3 cm) were used in the control treatment. A starving adult female (5–7 days after moulting) was individually released into the arena for observation. The handling time from release, until they finished the first feeding, was noted. When they did not feed on the foods within 30 min, it was defined as no choice; and those that flew away from the arena were discarded from the statistical procedure. The experiment was conducted once as a CRD with 20 – 30 replications for each treatment.

The effect of alternative foods on the survival of Scolothrips takahashii:

This experiment was conducted to compare the effectiveness of some alternative foods on the survival of *S. takahashii* females, in order to find the method to increase the rearing efficiency. The method proposed by Kishimoto and Adachi (2010) was applied with some modifications; one nymph was transferred into a 1.5 ml Eppendorf tip

containing different food sources. Each tube contained 200 µl of one of the following sugar sources; honey (10% solution), sucrose (10% solution), glucose (10% solution), fructose (10% solution), or bee pollen (suspension), at the bottom of each tube. A small hole was made in the tube's cap for ventilation. A surplus spider mite-infested leaf disc (3 x 3 cm) and a water-only tube were used as the positive and negative controls, respectively. Foods and leaf discs were renewed every 3–5 days. The experiment was conducted once as a CRD with 20–30 replications per treatment experiment. The effectiveness of sugar types (sucrose, glucose, and fructose), and their concentrations (10%, 20%, and 40%) on the survival of *S. takahashii* adult females were also observed, using the same method as described in the previous section. This experiment was conducted once as a CRD with 20 replications for each treatment. All of the tubes were kept in the incubator at 25±0.5°C under 16 h of light and 8 h of dark (16L:8D) photoperiod. The survival time was estimated as the time from moulting into an adult to death. Test thrips were checked every 24 h using a dissecting microscope.

Data analysis:

We used the Shapiro-Wilk test to check the normality of data while the Levene test was used to analyze the homogeneity of variances. In the Y-tube experiments, data had a 50:50 distribution and were analyzed with the Chi-square (χ^2) goodness of fit test. The female survival and handling time data were analyzed with One-way ANOVA, while the effects of sugars and their concentrations were analyzed with two-way ANOVA. Tukey's HSD test was used to compare the mean values. All survival and handling time data were naturally log-transformed before being analyzed to improve normality and homogeneity of variances and then back-transformed for reporting. The statistical tests and procedures were conducted with the R (version 4.3.3) software (The R Foundation for Statistical Computing).

Results

The olfactory experiment showed that female *S. takahashii* strongly preferred the soba flower's odor over clean air ($\chi^2 = 7.14$, $p < 0.01$); it was observed that they moved immediately to the odor source after being released into the Y-tube. In contrast, they had a slow reaction to the phacelia or faba flowers and did not distinguish between phacelia odor ($\chi^2 = 2.4$, $p > 0.05$) or faba bean ($\chi^2 = 0.53$, $p > 0.05$) and clean air (Fig. 1). When flowers or extrafloral nectaries were provided, female *S. takahashii* survived longer than the control (Fig. 2) ($F = 46.23$, $df = 3$, $p < 0.0001$), indicating that they fed on the plant nectar provided. Female *S. takahashii* survived about 7–9 days when soba, phacelia, or faba bean were provided in the rearing dish. There was no statistical difference among these plant nectar treatments (Fig. 2).

When the feeding preference of the *S. takahashii* experiment was performed, the results showed that the predatory thrips females spent most of their time crawling around in the parafilm area to search for food after being released in the tested arena. All of the tested *S. takahashii* were found to feed on spider mite eggs ($\chi^2 = 20$, $p < 0.0001$). In addition, it was observed that *S. takahashii* consumed sugar-rich foods but they did not show any interest in pollens and did not feed on them ($\chi^2 = 20$, $df = 1$, $p < 0.0001$) (Fig. 3). The feeding preference for sugar-rich foods depended on sugar type (Fig. 3). The feeding preference was high for sucrose ($\chi^2 = 22.53$, $p < 0.0001$), fructose ($\chi^2 = 26.13$, $p < 0.0001$) and honey ($\chi^2 = 26.13$, $p < 0.0001$) with more than 90% of *S. takahashii* fed on them, while it was low on glucose ($\chi^2 = 13.33$, $df = 1$, $p < 0.0001$) with only 17% of test females fed on it.

When *S. takahashii* found a suitable food source, they sucked out the liquid of the food solution or material inside the spider mite eggshell. There was a significant difference in the handling time and it corresponded with the degree of preference (Fig. 4; $F = 2.98$, $df = 4$, $P = 0.02$). They spent more time (793.4 ± 477.5 sec) to

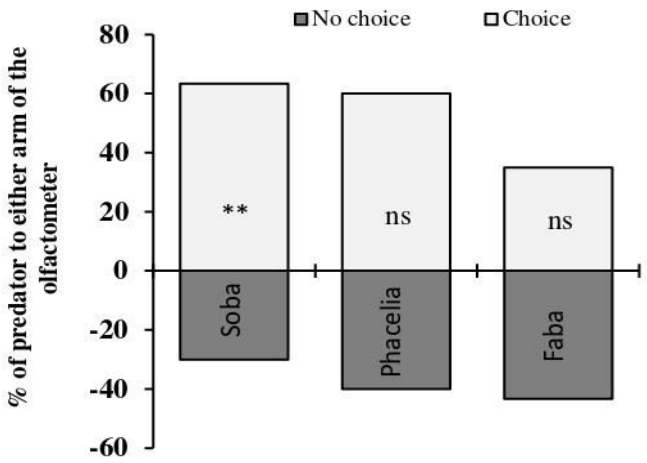

Fig. 1: The responses of *S. takahashii* to the odor of flower plants *vs.* clean air under the olfactometer system. Asterisks indicate a significant preference for the odor or the clean air (Chi-squared of the goodness of fit test: **p< 0.01, ns: p> 0.05).

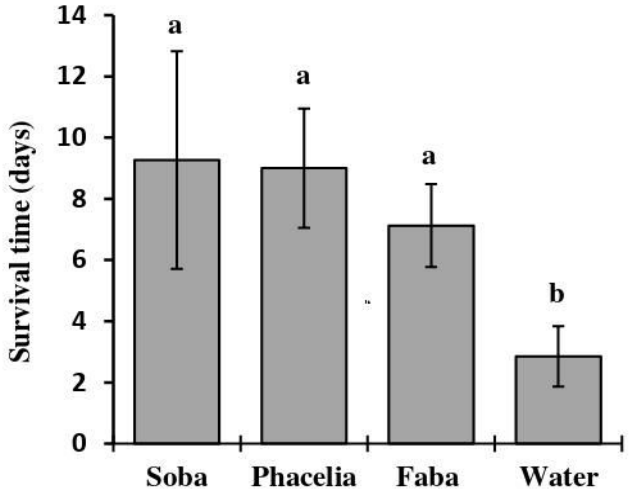

Fig. 2: The survival in the day (mean± SD) of *S. takahashii* when feeding on the plant's nectars. Mean shares the same letter are not significantly different (Tukey's HSD test, p < 0.05).

finish their "hunt" on glucose solutions while they took around 300 sec to search and complete feeding on the other test foods, including their native prey, *T. urtica*e mite's eggs (Fig. 4). *S. takahashii* fed continuously on the sugar-rich solution but only fed on one mite egg per attack before leaving.

Alternative foods significantly affected the survival of *S. takahashii* (df = 6, F = 82.72, p< 0.0001) female adults (Fig. 5A). They survived the longest when feeding on their natural prey, mite eggs (21.0 ± 3.9 days), and their survival was the shortest when water only (2.8 ± 1.1 days) or pollen (2.9 ± 0.9 days) was provided. Sugar-rich foods increased the survival but not as much as the mite eggs did. The longevity varied from 7.1 ± 2.8 days (honey 50% solution) to 10.2 ± 4.9 days (sucrose 10% solution). There were no significant differences among sugar-rich foods on the survival of *S. takahashii* (Fig. 5A). The sugar type was not

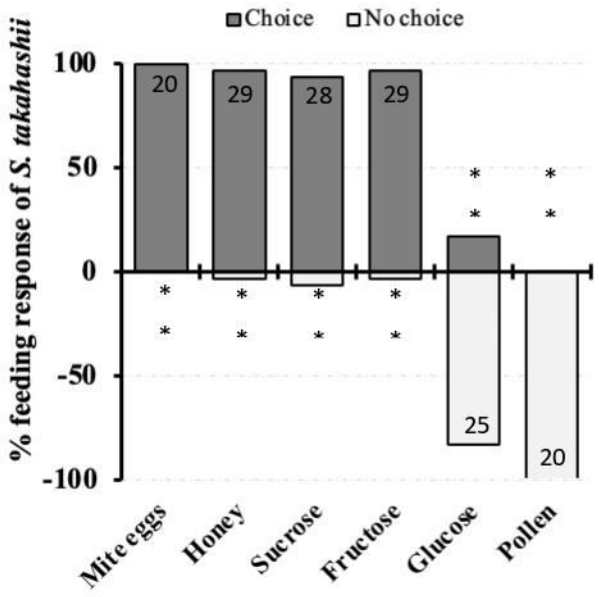

Fig. 3: The feeding references of *S. takahashii* female adults when feeding on alternative foods. The numbers on the bars show the number of the tested predators. Asterisks indicate the significant reference for feeding or not on a specific food (Chi-squared of the goodness of fit test: ***p< 0.001).

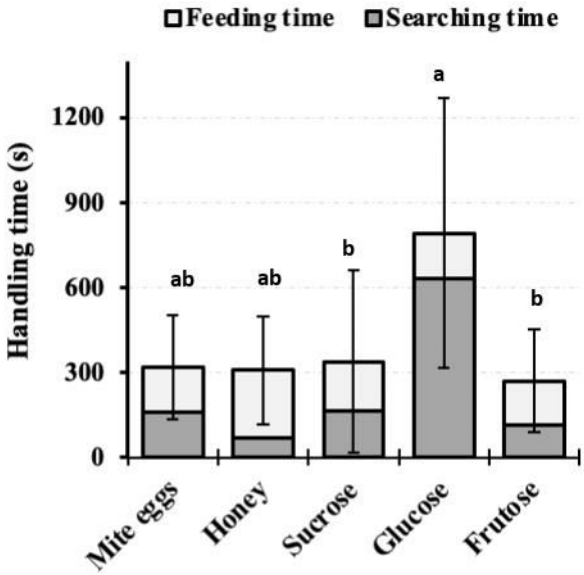

Fig. 4: The handling time (mean ± SD, handling time = searching + feeding time) of *S. takahashii* when preferring to feed on alternative foods. Means sharing the same letter are not significantly different (Tukey's HSD test, $p < 0.05$).

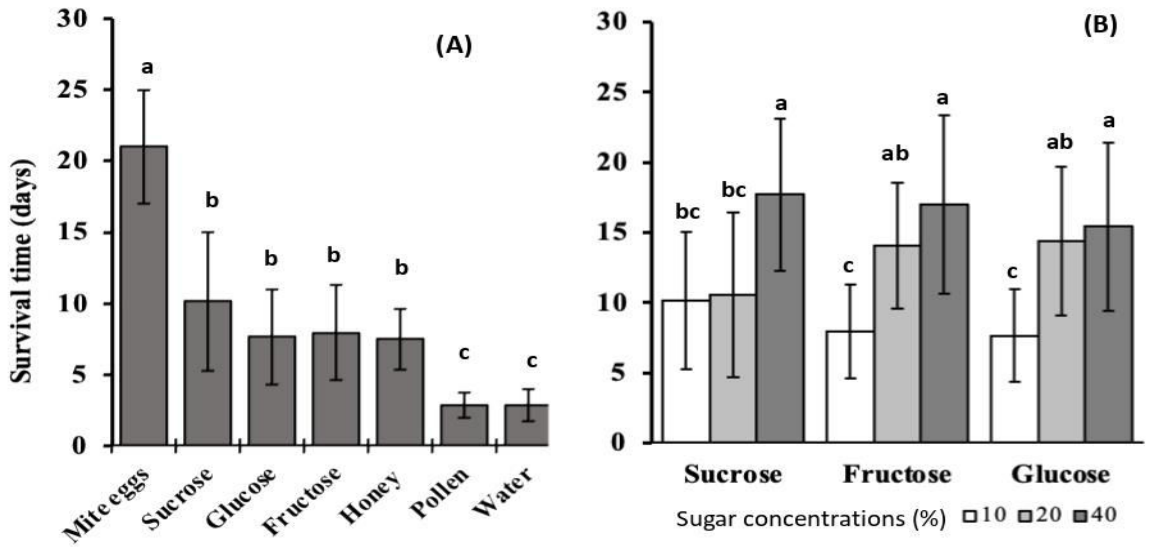

Fig. 5: The survival in the day (mean± SD) of *S. takahashii* when feeding on alternative foods (A), and different sugar concentrations (B). Means sharing the same letter are not significantly different (Tukey's HSD test, $p < 0.05$).

an important factor affecting the survival of the thrips (F_t = 4, df_t = 2, p_t = 0.76) while sugar concentrations significantly affected on the survival (F_c =36.62, df_c= 2,p_c < 0.0001). There was a significant interaction effect between sugar types and concentrations on the survival of *S. takahashii* female adults(F_{t*c} =2.49, df_{t*c} = 4, p_{t*c} = 0.045). When the sugar concentrations increased, *S. takahashii* survived longer (Fig. 5B).

Discussion

Flowering plants and plants with extrafloral glands are planted to attract the natural enemies of agricultural pests and to increase their diversity in habitat management conservation biocontrol programs (Kopta *et al*., 2012; Lu *et al*., 2014; Hatt *et al*., 2017; Zhao *et al*., 2017; Chen *et al*., 2020; Barley *et al*., 2022). Our study showed that buckwheat flower odor was highly attractive to female *S. takahashii* and they strongly preferred the odor in the olfactometer system. However, they did not show significant responses to faba and phacelia flower odors. Hence, buckwheat is useful in attracting *S. takahashii*. In addition, buckwheat flowers had a positive influence on the predator survival. Buckwheat is thus a candidate plant in conservation biological control with *S. takahashii.* Curiously, alyssum and faba bean also prolonged the longevity of the predatory thrips, whereas these plants did not attract them. This result may demonstrate that *S. takahashii* cannot recognize the odor sources as a potential sugar source, at least, innately. Also, *S. takahashii* females survived longer when allowed to feed on sugar solutions. Taken together, the present results indicate that *S. takahashii* uses carbohydrate sources as an alternative food when the prey is scarce. The use of such foods should allow them to increase their reproductive success under a variable prey abundance. It is known that insect predators such as *Phytoseiulus persimilis* also use carbohydrates as an important energy source (Fontellas and Zucoloto, 2003), helping them to maintain their movement and physical activities (Limburg and Rosenheim, 2001; Wäckers *et al*., 2007; Rojas and Morales-Ramos, 2008; Gonzalez *et al*., 2016; Reis *et al*., 2019).

In the present study and study of Kishimoto and Adachi (2010), even when natural prey is absent, adult female *Scolothrips takahashii* maintained their survival when feeding on sucrose. Also, in our results, *S. takahashii* were attracted to and consumed other sugar-rich

sources such as honey, glucose, and fructose which prolonged their longevity. Among tested sugar foods, glucose appears less attractive to *S. takahashii* because it took the longest time for searching glucose solution, despite the survival of *S. takahashii* being the same as when feeding on other sugar-rich sources. We do not know what causes the difference in their responses to different food sources.

When feeding on the mite eggs, *S. takahashii* has difficulty directly accessing the eggs because of the fine silk web covering them (Fasulo and Denmark, 2012). The predator thrips, therefore, should first enter into the web channel or eliminate the web before feeding on the eggs; such behavior is observed in *Scolothrips sexmaculatus* (Nickle and Bauchan, 2012). Unlike the natural prey, which is protective, liquid foods such as plant nectar should be easier to access and feed on for *S. takahashii*, though such liquid foods have a limited influence on their reproduction. Therefore, we suggest that *S. takahashii* should use liquid sugar foods in natural conditions, particularly when spider mites are at low density. When the sugar concentration increases, it becomes stickier, especially on honey and the consequence is that *S. takahashii* may be entrapped in the food solution though the higher sugar concentration is better for the survival of *S. takahashii*.

The present study confirms that alternative sugar foods do not enhance the reproduction of *S. takahashii;* they are required to feed on spider mites to sustain oviposition. Kishimoto and Adachi (2010) also found that sugar foods cannot maintain the oviposition of *S. takahashii*. It may be because they need to feed on a protein-rich diet (Wang *et al.*, 2018). It means that sugar-rich foods cannot replace their natural food. Even so, we suggest that sugar-rich foods are useful to enhance the activities or populations of *S. takahashii* in the crop field. The provision of sugar-rich food in the field or the greenhouse may help the early establishment of *S. takahashii*'s population before the first occurrence. A similar practice has been applied to enhance the lacewings' populations and reduce pest aphid populations in the maize field (Carlson, 1973), and also was used to maximize the effectiveness of the biocontrol program using the *Aphytis melinus* parasitoid in the citrus agroecosystems (Tena *et al.*, 2015). Besides sugar-rich materials, many beneficial predatory insects can use pollen as the main food source when the prey is scarce or in a rearing system condition (Van Rijn and Tangoshi, 1999; Skirvin *et al.*, 2006; Kishimoto *et al.*, 2014; Delisle *et al.*, 2015; Riahi *et al.*, 2017; Xie *et al.*, 2017), but our results demonstrated that pollen cannot be used to increase the survival of *S. takahashii* or the life expectancy. This may be because *S. takahashii* is a spider mite specialist predator so this predator may not be attracted to or feed on pollen.

Finally, the present study suggests that manipulating flowers or providing high-concentration sugar solutions within agroecosystems may increase the control effectiveness of *S. takahashii* against pest mite *T. urticae*. Thus, when this predator is to be used in greenhouse conditions, for example, farmers can enhance the control efficacy by adding flowering plants to their greenhouse. This would be the case in the field, and making flower stripes may also improve biological control with this predator. However, it is needed to choose the right plant species for planting, because some species may enhance the development of pests (Nuessly *et al*., 2004; Romeis *et al*., 2005), which may reduce the control effectiveness.

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