Experimental and Applied Acarology (2019) 77:27–41
https://doi.org/10.1007/s10493-018-0334-z

The response of three species of phytoseiid mite (Acari: Phytoseiidae) to synthetic pyrethroid pesticides in the laboratory and the field

Satoshi Kakoki[1,5] · Takeshi Kamimuro[2] · Yuki Ikenoue[3] · Mahomi Inokuchi[4] · Katsuo Tsuda[5] · Yositaka Sakamaki[5]

Received: 27 August 2018 / Accepted: 12 December 2018 / Published online: 19 December 2018

Abstract

The Kanzawa spider mite, *Tetranychus kanzawai* Kishida, is a major pest in tea fields [*Camellia sinensis* (L.) O. Kuntze] in Japan. However, recently, there have been some instances where acaricides are no longer applied as a result of the low occurrence of *T. kanzawai* in tea fields in Japan. In the period of 2015–2017, surveys of predatory mites in the study tea field detected *Amblyseius eharai* Amitai and Swirski, *Phytoseiulus persimilis* Athias-Henriot, *Euseius sojaensis* (Ehara), *Amblyseius obtuserellus* Wainstein and Begljarov, and *Typhlodromus vulgaris* Ehara in tea fields, but not *Neoseiulus womersleyi* (Schicha), indicating that a major change in the composition of the phytoseiid mite population had occurred. In laboratory studies, we confirmed the ability to avoid synthetic pyrethroid insecticides of the major beneficial mites in tea fields, *A. eharai* and *P. persimilis*, but not of *E. sojaensis*, a predatory mite whose population declined heavily after pesticide application. Attempts are made in this study to associate the decrease in *T. kanzawai* frequency in Japan with changes in pesticide used, method of spraying, and composition of the phytoseiid mite population. By continuing the method of pesticide spraying ('partial surface'), which leaves refugia in the leaf layer with sub-lethal dosages of pesticide, phytoseiid mites are aided to evade pesticides, resulting in maintenance of the composition of the phytoseiid mite populations in terms of diversity and abundance. Maintaining the diversity and abundance of Phytoseiidae may have contributed to the stabilization of the *T. kanzawai* population at low densities in Japanese tea fields.

Keywords Phytoseiid mite · Behavioral avoidance · Method of spraying · Natural enemies · Temporal variation · Pyrethroid pesticide

---

✉ Satoshi Kakoki
kakoki-satoshi@pref.kagoshima.lg.jp

Extended author information available on the last page of the article

Introduction

Tetranychus kanzawai Kishida is one of the important pests of tea [*Camellia sinensis* (L.) O. Kuntze] fields; when the damage is noticeable, it results in fallen leaves (Fukunaga and Ishiyama 2001). Acaricides are the main control strategy against *T. kanzawai* in tea fields. Because this mite inhabits the lower (abaxial) surface of tea leaves (Ehara 1993), application of higher volumes of pesticides is needed to ensure that sufficient acaricide reaches these lower surfaces. However, repeated application of pyrethroids (Osakabe 1986; Ikeda and Yamamoto 1989) and continuous application of the same pesticidal component (Osakabe 1973) has resulted in adaptation of the mite by selection for pesticide resistance populations. As a consequence, it is now considered to be difficult to control *T. kanzawai* in tea fields. On the other hand, in recent years, there have been increasing instances where the low densities of *T. kanzawai* have led to the curtailment of acaricide applications. There have been suggestions that the decrease in *T. kanzawai* frequency could be due, at least in part, to changes in the type of pesticide used, whereas it has also been attributed to biological control of the spider mite by its natural enemies (Ozawa et al. 2006). Masui et al. (2018) found that the species composition of natural enemies was affected by insecticide application in a citrus field. However, Mochizuki (1990) demonstrated that a strain of *Neoseiulus womersleyi* (Schicha), which is resistant to synthetic pyrethroids, controls *T. kanzawai* in a tea field. Therefore, if phytoseiid mites could survive in tea fields that had been sprayed with synthetic pyrethroids, they may reduce the abundance of *T. kanzawai*. Consequently, attention has been drawn to the phytoseiid predatory mites, which are the predominant natural enemies of *T. kanzawai*.

In the tea fields of Japan, pesticides to control pests other than spider mites—e.g., *Empoasca onukii* Matsuda (Hemiptera, Cicadellidae), *Scirtothrips dorsalis* Hood (Thysanoptera, Thripidae), *Caloptilia theivora* (Walsingham) (Lepidoptera, Gracillariidae), and *Homona magnanima* Diakonoff (Lepidoptera, Tortricidae)—are generally applied several times a year. There is no doubt that synthetic pyrethroid pesticides adversely affect natural enemies against the spider mite (Takafuji and Inoue 1993), especially the phytoseiid mite fauna in tea fields (Ozawa et al. 2006). Ullah et al. (2016) reported that two neonicotinoids (acetamiprid and imidacloprid) had severe effects on phytoseiid predators and, thus, could account for the observed change in predator species composition in Japanese orchards. Moreover, it is known that the composition of phytoseiid mites in Japanese pear orchards differs depending on the types of pesticides that are used by farmers (Kishimoto 2002). Therefore, it seems likely that the differences in pesticide susceptibility among phytoseiid mites in tea fields also lead to changes in their composition.

Conversely, Kakoki et al. (2018) found that when small amounts of a nonselective pesticide were sprayed onto the plucking surface of the tea plant using the ‘spotting spray method’, the leaf layer functioned as a protective shelter to spiders because not all of the leaf layer was covered with pesticide, and it will maintain some safety space for spiders and other natural enemies of tea pests in part of leaf layer. Furthermore, Hamamura (1986) showed that *N. womersleyi* was able to escape the effects of low concentrations of 12 synthetic pyrethroids, including fenpropathrin, cypermethrin, and permethrin. From these observations, we hypothesized that a partial spray method that does not completely cover the leaf layer with pesticide helps the predatory phytoseiid mites to escape damage from pesticides, and that the decline in the occurrence of *T. kanzawai* is affected, at least in part, by increasing numbers and diversity of phytoseiid mite populations.

To test this hypothesis, we examined whether the method of pesticide spraying used against many species of pest in tea fields and the difference in coverage area by the pesticide in different leaf layers affects the diversity and abundance of phytoseiid mite species. As part of this study, we investigated behavioral avoidance of three species of predatory phytoseiid mites, namely *Amblyseius eharai* Amitai and Swirski, *Phytoseiulus persimilis* Athias-Henriot, and *Euseius sojaensis* (Ehara), in response to the synthetic pyrethroids fenpropathrin, permethrin, bifenthrin, and cypermethrin.

Materials and methods

Nonselective pesticides with high activity against natural enemies of pests (Ozawa 2013) were selected as test compounds, and tests were conducted under circumstances (method and amount of spray applied) which maximized the likelihood of detecting any effects (Table 1). In addition, acaricide spraying for control of *T. kanzawai* was not carried out in order to confirm any suppressive effect of the phytoseiid mites, against *T. kanzawai*. Phytoseiid mites were identified as described previously (Toyoshima et al. 2015a).

Temporal variation in the abundance of *Tetranychus kanzawai* and Phytoseiidae in a tea field (field trial)

Study field and pesticide application

We conducted this study in a tea field of the Kagoshima Prefectural Institute for Agricultural Development, Tea Division (Minamikyushu City, Kagoshima Prefecture, Japan) in Japan (31°37N, 130°45E). The trial area in the tea field (cultivar ‘Kuritawase’) was 240 m^2 (14-m rows), with three treatments (including the control), and three replicates per treatment, each replicate plot being 7×1.7 m^2. In addition, a no-pesticide buffer row of tea plants was provided between rows in a treatment. The treatments were as follows: (1) an application rate of 40 L/1000 m^2 (subsequently referred to as ‘40-L treatment’ or ‘partial spraying’), using a lower-volume sprayer (Kagoshima-style MCS-KAGO3-2; Matsumoto Kiko, Kagoshima, Japan; spray pressure, 1.5 MPa; spray speed, 0.7 m/s); (2) an application rate of 200 L/1000 m^2 (subsequently referred to as ‘200-L treatment’ or ‘conventional spraying’), using a conventional sprayer (MCS8A; Matsumoto Kiko; spray pressure, 1.0 MPa; spray speed, 0.5 m/s); and (3) no spraying (control treatment). Pesticide spraying against *E. onukii*, *S. dorsalis*, *C. theivora*, *H. magnanima*, and *Adoxophyes honmai* Yasuda (Lepidoptera, Tortricidae) was carried out, and the effects of these sprayings on the populations of *T. kanzawai* and Phytoseiidae were monitored.

Monitoring of *Tetranychus kanzawai* and Phytoseiidae

The sampling of *T. kanzawai* was conducted from August 2015 (sampling of phytoseiid mites was started in May 2015) to October 2017, with survey intervals of about 7 days from May to October, and about 15–30 days from November to April. Leaves 10 cm from the plucking surface were collected. We randomly sampled 100 leaves from each treatment and put them into a polyethylene self-sealing bag to transport them. We counted the leaves that were infested by adult and immature stages of *T. kanzawai* and phytoseiid mites (not including the egg stage) under a stereoscopic microscope (SZ-61; Olympus, Tokyo, Japan)

Table 1 Timing, rates, and concentrations of pesticide applications

Application date	Tea season	Application rate (L/1000 m^2)		Trade name	Chemical name (% a.i.)	Acaricide properties	Conc. (ppm)
		Partial spraying	Conventional spraying				
2015							
May 8	Second	40	200	Rody EC	Fenpropathrin (10)	Yes	100
June 24	Third	40	200	Rody EC	Fenpropathrin (10)	Yes	100
Aug 5	Autumn	40	200	Adion EC	Permethrin (20)		100
Aug 19	Autumn	40	200	Rody EC	Fenpropathrin (10)	Yes	100
Sep 18	Autumn	40	200	Diana SC	Spinetoram (11.7)		23.4
2016							
May 13	Second	40	200	Rody EC	Fenpropathrin (10)	Yes	100
July 1	Third	40	200	Rody EC	Fenpropathrin (10)	Yes	100
Aug 8	Autumn	40	200	Rody EC	Fenpropathrin (10)	Yes	100
Aug 24	Autumn	40	200	Adion EC	Permethrin (20)		100
2017							
May 19	Second	40	200	Telstar WP	Bifenthrin (2)	Yes	20
July 3	Third	40	200	Agrosrin WP	Cypermethrin (6)		60
Aug 16	Autumn	40	200	Adion EC	Permethrin (20)		100
Aug 30	Autumn	40	200	Telstar WP	Bifenthrin (2)	Yes	20
Oct 2	Autumn	40	200	Phoenix FL	Flubendiamide (18)		90

in the laboratory. Phytoseiid mites were preserved in 70% (vol/vol) ethanol and were prepared as specimens for identification. We identified phytoseiid mite species under a phase-contrast microscope (DM750; Leica, Wetzlar, Germany) according to Toyoshima et al. (2015a) and Ehara (2007).

Behavioral avoidance of phytoseiid mite species against synthetic pyrethroid insecticide (laboratory trial)

We chose *A. eharai, P. persimilis*, and *E. sojaensis*, which had been confirmed as present in the tea field under study, as the test species of phytoseiid mites for this investigation. In 2017, we captured *A. eharai* in the study tea field from October to December, and *E. sojaensis* from the tea field of the Kagoshima University campus (31°57′N, 130°54′E) in November. *Amblyseius eharai* and *E. sojaensis* individuals (fed with tea pollen) were kept in a 24-well multiwell plate (BM Equipment, Tokyo, Japan) in an incubator (MLR-352H; Panasonic, Tokyo, Japan) at 25 ± 0.5 °C (16 h/8 h light/dark) for tests. In 2017, *P. persimilis* individuals were collected from the study tea field from May to December and they were raised together with *T. kanzawai* in an incubator under conditions of 25 °C and 16 h/8 h light/dark on kidney bean (*Phaseolus vulgaris* L. cv. 'Taisho-Kintoki') leaf disks (2 × 2 cm). *Tetranychus kanzawai* that is food for *P. persimilis* were also collected from the Kagoshima Prefectural Institute for Agricultural Development (Tea Division) from May to October 2017. We reared *T. kanzawai* that had been multiplied on kidney bean seedlings at 25 °C and under a 16 h/8 h light/dark photoperiod (Shinkaji 1991).

We selected fenpropathrin (100 ppm), permethrin (100 ppm), bifenthrin (20 ppm), and cypermethrin (60 ppm) as the test pesticides for use in the study tea field trial, with distilled water being used as the control. The test apparatus was prepared following the method of van den Boom et al. (2003), and each test was carried out by the following procedure: (1) a primary leaf of kidney bean was cut into a 2 × 2 cm square, and one side was immersed in one of the pesticide solutions and allowed to dry in air; (2) two leaf pieces (one treated with pesticide and one without) were connected via a strip of parafilm cut to a T shape (3.5 × 3 cm) with the treated leaf surface facing upwards. In a Petri dish (9 cm diameter), absorbent cotton was laid in the base, the leaf pieces and parafilm were placed on top, and the dish base was filled with water (Fig. 1); (3) one adult female of the phytoseiid mite species under investigation was placed on the parafilm, equidistant from the treated and control leaf pieces, using a fine face brush; after 60 min, the location of the phytoseiid mite was noted and the condition of the phytoseiid mite was recorded. The condition was classified as being healthy, distressed, or escaped. In 60 min after of test, the mites, showing the normal behavior as that before the test, were classified as 'healthy' and those showing

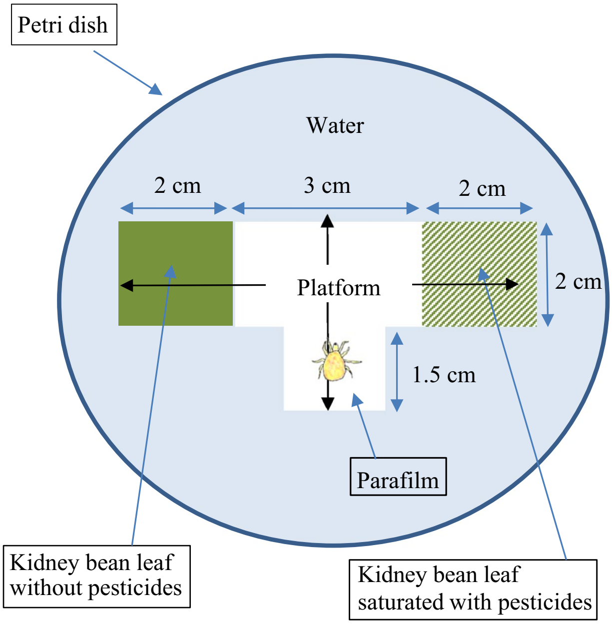

Fig. 1 The setup of the pesticide-avoidance (evasion) laboratory test with of phytoseiid mites

the status of phytoseiid poisoning with trembling legs were classified as ‘distressed’. Each test was terminated when submergence of the phytoseiid mite was confirmed. Submergence was classified as an avoiding behavior (‘escape’), and the test was terminated at that moment; therefore, the survival or death of the phytoseiid mite could not be confirmed following avoidance. Immediately after submergence was confirmed, we moved the individual from the Petri dish to a Munger cell (4 × 5 cm, 2.5 cm diameter) (Munger 1942), provided tea plant pollen as feed, and observed the condition of the phytoseiid mite for further 24 h following submergence. These classifications of condition (i.e., healthy, distressed, and escaped) have previously been used (e.g., Wakou and Odagiri 1985; Hamamura 1986; Mochizuki 1990; Hashimoto et al. 2000). Twenty-five individuals were tested for each pyrethroid–mite species combination.

Statistical analysis

In the avoidance test, the results were converted to ratios, then a χ^2 test was carried out on the results from each pyrethroid/control comparison; if a significant difference was observed ($\alpha = 0.05$), the Ryan multiple comparison test was performed. If zero was present in any of the cells (e.g., with distilled water, no distress should be observed), the χ^2 test would be inappropriate, so the Fisher’s exact probability test was used instead after dividing the result into avoidance and healthiness, followed by the Ryan multiple comparison test was used if a significant difference was detected. The statistical analysis of the field trial data used a repeated-measures analysis of variance, performed with $\log_{10}(N + 0.5)$-transformed data for mite abundance per leaf in each treatment. If a significant difference was observed, multiple comparisons were carried out using Tukey’s honestly significant difference (HSD) test. JMP v.7 software (SAS Institute 2007) was used to carry out all statistical analyses.

Results

Temporal variation in the abundance of *Tetranychus kanzawai* and Phytoseiidae in a tea field (field trial)

Throughout the study periods, the number of *T. kanzawai* did not differ significantly among the treatments ($F_{2,79} = 2.33$, $p = 0.10$). In 2015, the number of *T. kanzawai* in the 40-L treatment was significantly higher than in the 200-L treatment or the control, whereas there were no significant differences in numbers among treatments in 2016 and 2017. The maximum value of the ratio of leaf on which *T. kanzawai* fed was 17.3% in the area subjected to the 200-L treatment, 20% in the area subjected to the 40-L treatment, and 10% in the untreated (control) area (Fig. 2).

From May to December 2015, the total number of phytoseiid mites was not significantly different between the 40-L treatment and the control, nor between the 40-L and 200-L treatments, but that in the 200-L treatment was significantly lower than in the control ($F_{2,24} = 4.04$, $p = 0.024$). There was no significant difference in the number of phytoseiid mites among the different pesticide treatments (January to December 2016: $F_{2,33} = 0.40$, $p = 0.67$; January to October 2017: $F_{2,32} = 1.71$, $p = 0.19$) (Figs. 2, 3). The species of phytoseiid mite collected after pesticide spraying were *A. eharai, P. persimilis, Amblyseius obtuserellus* Wainstein and Begljarov, *E. sojaensis*, and *Typhlodromus vulgaris* Ehara.

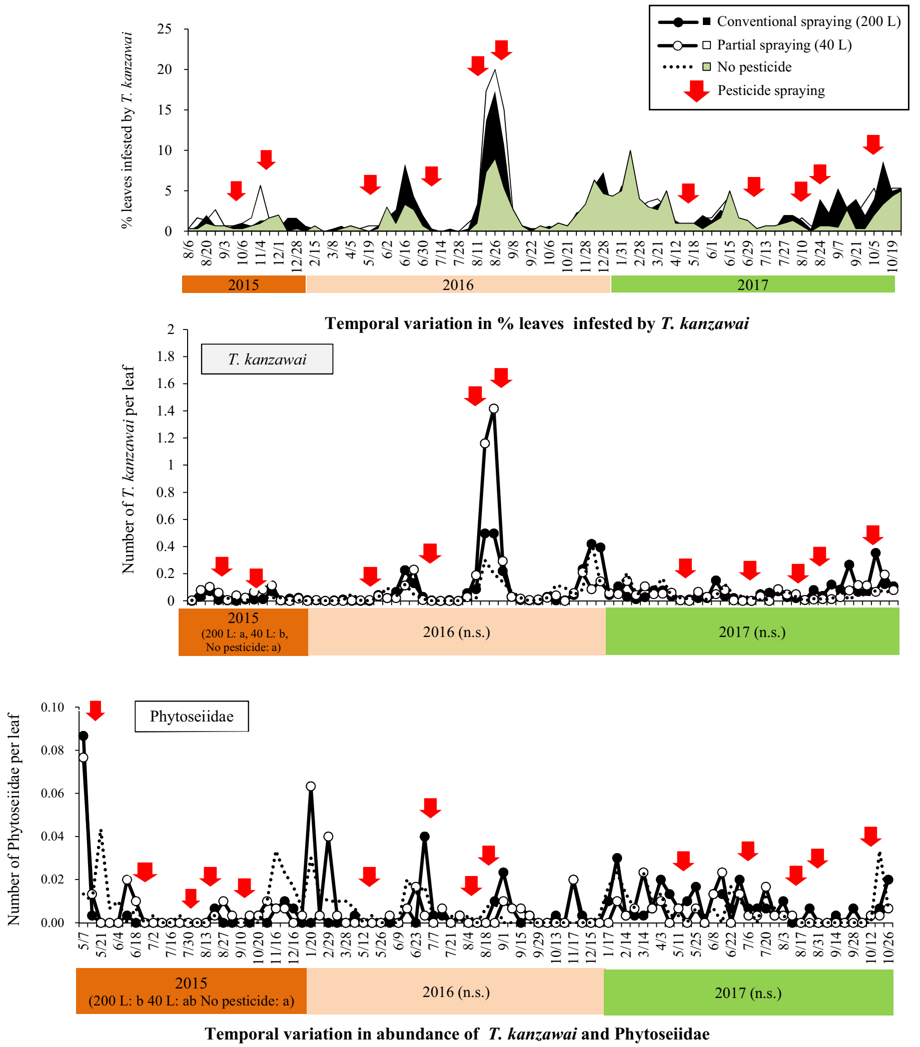

Fig. 2 Temporal variation in the percentage of leaves infested by *Tetranychus kanzawai* and abundance of *T. kanzawai* and Phytoseiidae. Treatments within a tea season followed by the same letter are not significantly different (ANOVA followed by Tukey's HSD test: $p > 0.05$; *n.s.* not significant). Arrows in the figure indicate the dates of pesticide application

Euseius sojaensis, which was a dominant species before the pesticide application, was barely detectable after, and the composition of the phytoseiid mite population had markedly changed as a result of the pesticide treatment (Fig. 3). Throughout the study period, the number of *A. obtuserellus* was significantly higher ($F_{2,91} = 3.20$, $p = 0.043$) in the control than in the 200-L treatment, whereas the number in the 40-L treatment was similar to that in the control (Table 2). However, there was no significant difference in the number of *A. eharai* ($F_{2,91} = 1.07$, $p = 0.35$), *P. persimilis* ($F_{2,91} = 1.22$, $p = 0.30$), *E. sojaensis* ($F_{2,91} = 1.35$, $p = 0.26$), or *T. vulgaris* ($F_{2,91} = 1.00$, $p = 0.37$) among treatments (Table 2).

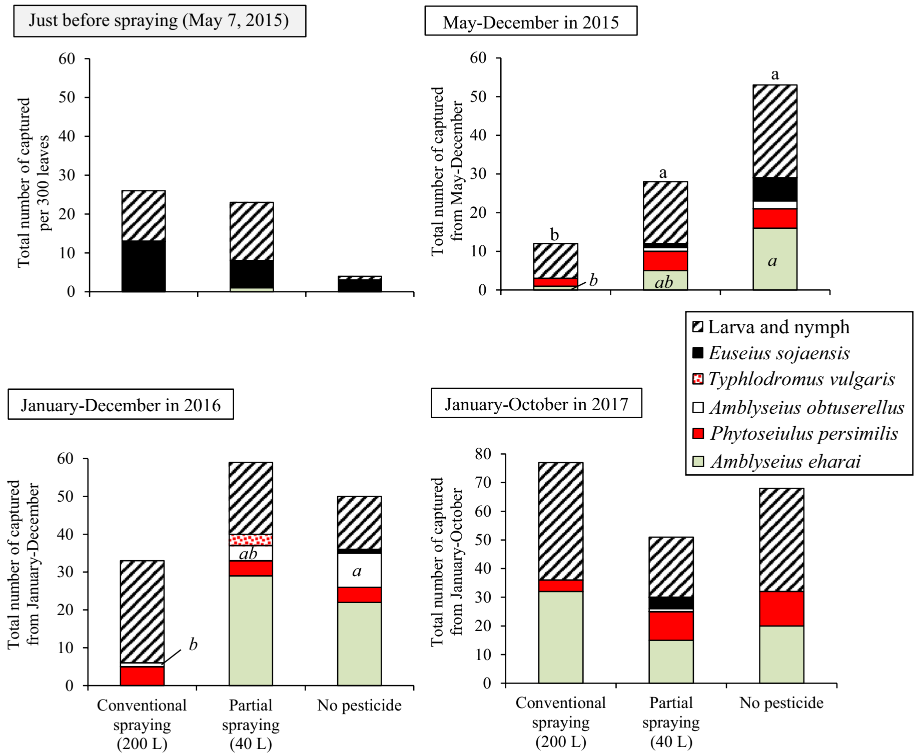

Fig. 3 Differences in the composition of the Phytoseiidae. Study periods were May to December 2015, January to December 2016, and January to October 2017. Means within a tea season capped with different letters are significantly different (ANOVA followed by Tukey's HSD test: $p < 0.05$). Italicized letters denote significant differences for separate phytoseiid mite species

Table 2 Mean (± SE) total numbers of Phytoseiidae collected during the study period (May 2015–October 2017)

Species	Conventional spraying (200 L)	Partial spraying (40 L)	No pesticide
Amblyseius eharai	0.43 ± 0.11	0.53 ± 0.19	0.66 ± 0.13
Phytoseiulus persimilis	0.12 ± 0.04	0.21 ± 0.06	0.23 ± 0.06
Amblyseius obtuserellus	0.01 ± 0.01b	0.04 ± 0.02ab	0.12 ± 0.05a
Euseius sojaensis	0	0.05 ± 0.04	0.08 ± 0.06
Typhlodromus vulgaris	0	0.03 ± 0.03	0
Total number of mites	133	128	174

Means within a row followed by a different letter are significantly different (Tukey's HSD test: $p < 0.05$)

There were also no significant differences among the treatments in the total numbers of the five species of phytoseiid mites that were confirmed in the study tea field ($F_{2,91} = 1.14$, $p = 0.32$; Table 2).

Figure 3 shows the species composition of the mite population in each year. In 2015, the numbers of Phytoseiidae ($F_{2,24}=4.04$, $p=0.024$) and *A. eharai* ($F_{2,24}=4.49$, $p=0.016$) were similar in the 40-L and control treatments, but the numbers in the 200-L treatment were significantly lower than those in the control treatment. There were no significant differences in the number of the other phytoseiid mites among the treatments. In 2016, the number of *A. obtuserellus* ($F_{2,33}=3.08$, $p=0.053$) was similar between the 40-L treatment and the control, whereas the number in the 200-L treatment was significantly lower than that in the control. Apart from *A. obtuserellus*, no significant differences in the number of phytoseiid mites were observed among the treatments. In 2017, the numbers of four (*T. vulgaris* were not detectable) species of phytoseiid mites were not significantly different among the treatments. After the spraying of pesticides, *P. persimilis* and *A. eharai* became dominant.

Behavioral avoidance of phytoseiid mite species against synthetic pyrethroid insecticide (laboratory trial)

For *P. persimilis* and *E. sojaensis*, the avoidance scores were not significantly different between the control and each of the pyrethroid treatments, but *A. eharai* exhibited significantly more escape and avoidance in response to permethrin or bifenthrin compared to the control (Fig. 4). The number of healthy individuals of *A. eharai* was significantly lower in all the pyrethroid treatments than in the control plot (Fig. 5). *Phytoseiulus persimilis* acted normally in response to most of the synthetic pyrethroids, and no significant differences compared with the control were observed (Fig. 5). By contrast, there were few healthy individuals of *E. sojaensis* remaining after the pyrethroid treatments, and many distressed individuals were also observed. Table 3 shows the destiny of the *A. eharai* individuals 24 h after escaping from the platform. The *A. eharai* individuals were transferred to individual rearing containers (Munger cells) immediately after their submergence (avoidance). In the permethrin and bifenthrin treatments, most *A. eharai* individuals that escaped from the platform and became submerged in the water were acting normally. On the other hand, one out of nine individuals in the fenpropathrin treatment and one out of eight in the cypermethrin treatment showed abnormal behavior, with one *A. eharai* individual from the cypermethrin treatment ($n=8$) being dead within 24 h.

Discussion

Ikeda and Yamamoto (1989) reported that if synthetic pyrethroids were sprayed in the summer, there would be a very high probability that a resurgence of *T. kanzawai* would occur in the autumn and continue to the second tea season of the following year, as these phenomena were mainly caused by a pyrethroid-based decrease in the numbers of *N. womersleyi*, a natural enemy of *T. kanzawai*. In the tea field of the current study, the temporal abundance growth of *T. kanzawai* in 40-L and 200-L treatments in August 2016 were confirmed; however, the densities of *T. kanzawai* tended to decrease as time passed. In addition, *N. womersleyi* was not detected, and *A. eharai*, which also plays a role in preventing increases in spider mite population size (McMurtry 1992), and *P. persimilis*, which can suppress an outbreak of spider mites (Takafuji and Chant 1976; Takahashi et al. 1998), were the dominant species in the field trial of the current study.

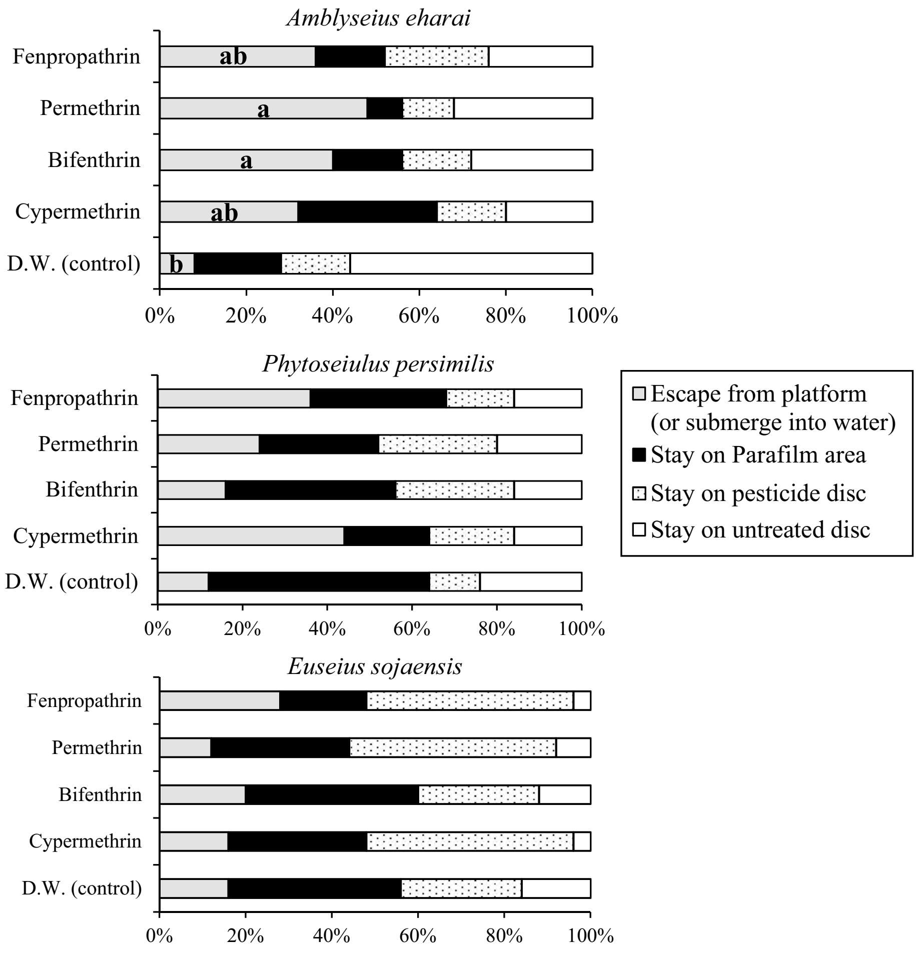

Fig. 4 Results of the avoidance test of three phytoseiid mite species, testing leaf discs treated with four pyrethroid pesticides (n = 25). Bar sections labelled with different letters indicate significant differences among pyrethroids (significant χ^2 tests between pyrethroids vs. control plot, followed by Ryan multiple comparison procedure: $p < 0.05$)

Nagatomo et al. (1991) reported that the presence of *N. womersleyi* only was confirmed in a tea field adjacent to our study tea field in the 1980s, whereas in 2015–2017, *A. eharai, P. persimilis, A. obtuserellus, E. sojaensis*, and *T. vulgaris* were all confirmed, and the species diversity of phytoseiid mites had become quite rich. Ullah et al. (2016) indicated that ‘displacement in predator complexes of fruit-tree orchards could be due to different degrees of pesticide susceptibility’. Moreover, it is known that the composition of the phytoseiid mite population differs depending on the management of the tea fields (Santoso et al. 2004). As a general trend in the Japanese tea fields, the species diversity of phytoseiid mites has changed drastically since the 1960s (Toyoshima et al. 2015b), such that acaricide spraying to control *T. kanzawai* is occasionally curtailed (Ozawa et al. 2006). Thus, the phytoseiid species richness may contribute to the relatively rare occurrence of *T. kanzawai*, and, even then, at low densities (Toyoshima 2014).

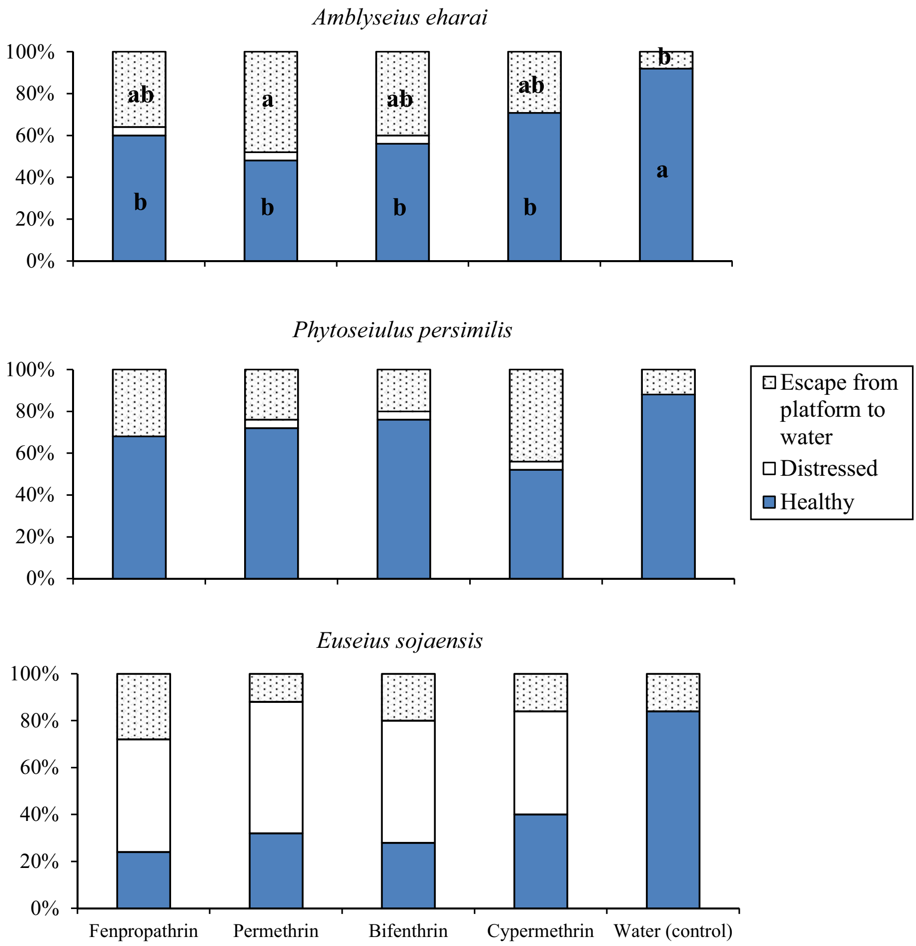

Fig. 5 Results of the avoidance test (classified into healthy, distressed, or escaped) of three phytoseiid species (n = 25; 60 min after application). Column sections labelled with different letters indicate significant differences among categories (Fisher's exact probability test followed by Ryan multiple comparison procedure: $p < 0.05$)

Table 3 Fate of *Amblyseius eharai* individuals 24 h after being rescued from water

Chemical name (% a.i.)	Conc. (ppm)	No. mites escaping from test platform into water (n)	Fate after 24 h		
			Healthy	Distressed	Dead
Fenpropathrin (10)	100	9	8	1	0
Permethrin (20)	100	12	12	0	0
Bifenthrin (2)	20	10	10	0	0
Cypermethrin (6)	60	8	6	1	1
Water (control)	–	2	2	0	0

In the indoor trials, most of the *P. persimilis* individuals continued their normal behavior even after contact with leaves that were saturated with fenpropathrin, permethrin, bifenthrin, or cypermethrin. In addition, about 40% of the submerged (escaped) *P. persimilis* individuals were also found in the cypermethrin treatment. According to previous studies (Wakou and Odagiri 1985; Hamamura 1986), escape from the platform and subsequent submergence of phytoseiid mites was regarded as a behavioral avoidance from pesticides on the platform, so we believe that *P. persimilis* in our indoor experiments also showed behavioral avoidance from the pesticide. In addition, Hamamura and Isobe (1998) discovered that *P. persimilis* has an extremely high resistance to fenpropathrin, permethrin, and cypermethrin at practical (used in fields) concentrations. Moreover, in our indoor experiments, 96% of *P. persimilis* individuals exposed to bifenthrin remained healthy. This result supported the finding that the number of individuals in the pesticide spraying area was not significantly different from that in the control treatment in the field experiments. Therefore, it was considered that *P. persimilis* tends to survive due to resistance and behavioral avoidance when fenpropathrin, permethrin, bifenthrin, or cypermethrin were sprayed on tea fields under conditions similar to those used in this test. There are also similar findings from previous reports on tea fields (Ozawa et al. 2014) and pear gardens (Yamazaki and Itoyama 2015).

Kishimoto et al. (2018) reported that synthetic pyrethroid insecticides have a strong influence on *A. eharai*. In the indoor experiments of the current study, however, the mortality rate of *A. eharai* was lower than that reported by Kishimoto et al. (2018), though that discrepancy may have been caused by shorter pesticide contact time and also to the existence of refugia without pesticide. In the laboratory tests, many of the *A. eharai* individuals submerged, following which most of the rescued individuals recovered, showing a normal behavior after 24 h. In addition, in the tea field of this current study, no significant differences were observed in the number of *A. eharai* in the sprayed treatments. When pesticides are sprayed (200 L/1000 m^2) on the plucking surface, only about 20% of pesticide would reach inside the leaf layer (15 cm below the plucking surface) (Kawai et al. 1999), so that the number of *N. womersleyi* individuals in the sprayed and the control areas did not differ (Kawai 2001). However, when the spraying volume was high enough to reach inside the leaf layer (400 L/1000 m^2), the population size of the predatory mite decreased (Kawai 2001). Consequently, in the spray treatment of our study (200 L/1000 m^2), the ability to resist and be repelled by the pesticide might enable *A. eharai* to survive; alternatively, in the leaf layer, not enough of the pesticide might cover to achieve the lethal dosage for *A. eharai*. In the current study, control of *T. kanzawai*, which is conventionally achieved by spraying acaricide into the inside of the tea bushes (400 L/1000 m^2), had not been carried out, but this may also have led to the maintenance of high predatory phytoseiid mite populations. In the 200-L treatment, the *A. eharai* incidence was significantly lower than that in the control plots in 2015, but not in 2016 and 2017. It seemed either that the growth of the tea bush provided refugia for *A. eharai* to shelter from pesticides and/or that the sensitivity of *A. eharai* to pesticides had changed. To distinguish between these phenomena, further research is needed.

In contrast, *E. sojaensis*, which was a dominant species before pesticide spraying, proved to be particularly susceptible to pyrethroids. The moment *E. sojaensis* individuals came into contact with pesticide-treated leaves, they began to act distressed and were unable to walk normally. This finding is in agreement with a previous report (Shibao et al. 2006), which showed that the mortality rate of *E. sojaensis* in response to permethrin at concentrations of 200, 100, and 50 ppm were 100%, whereas at 25 ppm the mortality was 63%. *Euseius sojaensis* showed marked susceptibility to the nonselective pesticides. Also, in the fruit orchard fields, where nonselective pesticides are frequently used, *E. sojaensis* is only rarely observed (Kishimoto et al. 2018). In the current study, *E. sojaensis* was not detected in the 200-L treatment until the end of the study, about 2.5 years after the pesticide was applied, whereas in the 40-L and the control areas only a few individuals were confirmed. When the volume of pesticide applied was 200 or 40 L/1000 m^2, the pesticide coverage at 5 cm below the plucking surface was estimated to be > 90% or > 40%, respectively, whereas at 25 cm below the plucking surface it was estimated to be > 20% or > 0.1%, respectively (Kakoki et al. 2018). Thus, the pesticide coverage in the leaf layer was significantly higher in the 200-L treatment (Kakoki et al. 2015). In addition, the pesticide coverage on the lower surface of the leaf (5 cm below the plucking surface) was estimated to be 0.2% and 11.3% in the 40-L and 200-L treatments, respectively (Kakoki et al. 2015, 2018). It is possible that the side effect on *E. sojaensis* of the 40-L treatment was alleviated to an extent in a manner dependent on the degree of pesticide coverage in the leaf layer. Incidentally, the number of individuals of *A. obtuserellus* in the control and 40-L treatments tended to be more than that in the 200-L treatment.

In conclusion, by continuing the method of pesticide spraying that leaves refugia in the leaf layer free (in part) from pesticides, our research suggests that adequate populations of various species of phytoseiid mites can be maintained. In addition, it is known that a combination of phytoseiid mite species [*Neoseiulus californicus* (McGregor) and *P. persimilis*] can effectively control *T. urticae* (Shibao and Ioku 2016). Conserving the diversity and abundance of Phytoseiidae in this way may contribute to the stabilization of *T. kanzawai* at low densities.

Acknowledgements We thank Dr. H. Kishimoto (Division of Apple Research, Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organization, NARO), Dr. S. Toyoshima (Hokkaido Agricultural Research Center, NARO), and Dr. S. Yano (Laboratory of Ecological Information, Graduate School of Agriculture, Kyoto University) for their support. We thank Mr. S. Nakakihara and other staff of the Kagoshima Prefectural Institute for Agricultural Development and students of the Faculty of Agriculture Kagoshima University for their help with the research. Our special thanks go to Dr. A. Fujiie, Mr. S. Sato, Ms. M. Uchino, and staff of the Agriculture, Forestry and Fisheries Research Council for their continuous encouragement throughout the study. We also thank the anonymous reviewers for their useful and constructive comments that helped to improve our manuscript.

Funding This research was funded by the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry (27019C).

References

Ehara S (1993) Plant mites of Japan in color. Zennokyo Shuppan, Tokyo (**in Japanese**)

Ehara S (2007) A guide to the phytoseiid mites of Japan (Acari: Phytoseiidae). Plant Prot 10:78–120 (**in Japanese**)

Fukunaga K, Ishiyama M (2001) Acaricide resistance of Kanzawa spider mite, *Tetranychus kanzawai* on tea in Kyoto prefecture. Proc Kansai Pl Prot 43:17–18. https://doi.org/10.4165/kapps1958.43.0_17 (**in Japanese**)

Hamamura T (1986) Studies on the biological control of Kanzawa spider mite, *Tetranychus kanzawai* Kishida by the chemical resistant predacious mite, *Amblyseius longispinosus* (Evans) in tea fields (Acarina: Tetranychidae, Phytoseiidae). Bull Natl Res Inst Tea 21:121–201 (**in Japanese**)

Hamamura T, Isobe K (1998) The resistance against synthetic pyrethroid pesticides of *Phytoseiulus persimilis* Athias-Henriot. J Acarol Soc Jpn 7:57. https://doi.org/10.2300/acari.7.55 (**in Japanese**)

Hashimoto T, Motoyama N, Mizutani K, Shinjo G (2000) Repellent and lethal effects of seven chemicals on house dust mites, *Dermatophagoides farinae* Hughes (Acari: Pyroglyphidae) and *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae). Med Entomol Zool 51:275–281. https://doi.org/10.7601/mez.51.275

Ikeda F, Yamamoto A (1989) Resurgence of tetranychid mites after spraying of synthetic pyrethroid in tea fields. Annu Rep Kanto Tosan Plant Prot Soc 36:187–188. https://doi.org/10.11337/ktpps1954.1989.187 (**in Japanese**)

Kakoki S, Tokuda A, Kamimuro T (2015) Pesticide reduction by a riding-type pesticide sprayer in tea fields that makes use of a small fog nozzle and a ventilator. Tea Res J 120:37–45. https://doi.org/10.5979/cha.2015.37 (**in Japanese**)

Kakoki S, Kamimuro T, Tsuda K, Sakamaki Y (2018) Use of a lower-volume, surface pesticide spray conserves spider assemblages in a tea field. J Econ Entomol 111:1595–1604

Kawai A (2001) Effects of chemicals on the population of *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae) and *Tetranychus kanzawai* Kishida (Acari: Tetranychidae) when the tops and the inside of tea bushes were sprayed. Tea Res J 92:9–15. https://doi.org/10.5979/cha.2001.92_9

Kawai A, Kohata K, Yamaguchi Y (1999) Deposition of chemicals on various parts of tea bushes sprayed on the plucking surface. Appl Entomol Zool 34:387–389. https://doi.org/10.1303/aez.34.387

Kishimoto H (2002) Species composition and seasonal occurrence of spider mites (Acari: Tetranychidae) and their predators in Japanese pear orchards with different agrochemical spraying programs. Appl Entomol Zool 37:603–615. https://doi.org/10.1303/aez.2002.603

Kishimoto H, Yaginuma K, Toyama M (2018) Effects of pesticides on four native generalist phytoseiid species (Acari: Phytoseiidae). Jpn J Appl Entomol Zool 62:29–39. https://doi.org/10.1303/jjaez.2018.29 (**in Japanese**)

Masui S, Katayama H, Tsuchiya M (2018) Occurrence of *Panonychus citri* (Acari: Tetranychidae) and natural enemies in citrus fields under conventional pesticide application in Shizuoka Prefecture. Jpn J Appl Entomol Zool 62:47–53. https://doi.org/10.1303/jjaez.2018.47 (**in Japanese**)

McMurtry JA (1992) Dynamics and potential impact of 'generalist' phytoseiids in agroecosystems and possibilities for establishment of exotic species. Exp Appl Acarol 14:371–382. https://doi.org/10.1007/BF01200574

Mochizuki M (1990) A strain of the predatory mite *Amblyseius longispinosus* (Evans) resistant to permethrin, developing in the tea plantation of Shizuoka Prefecture (Acarina: Phytoseiidae). Jpn J Appl Entomol Zool 34:171–174. https://doi.org/10.1303/jjaez.34.171 (**in Japanese**)

Munger F (1942) A method for rearing citrus thrips in the laboratory. J Econ Entomol 35:373–375. https://doi.org/10.1093/jee/35.3.373

Nagatomo S, Atari N, Onimaru T, Nonaka T (1991) Studies on the control of Kanzawa spider mite, *Tetranychus kanzawai* Kishida by the predacious mite, *Amblyseius longispinosus* (Evans) in tea fields. Bull Kagoshima Tea Exp Stn 7:76 pp (**in Japanese**)

Osakabe M (1973) Studies on acaricide resistance of the Kanzawa spider mite, *Tetranychus kanzawai* Kishida, parasitic on tea plant. Bull Tea Res Stn Minist Agric For 8:1–95 (**in Japanese**)

Osakabe M (1986) Problems on the control of tea insect pests in Japan. Tea Res J 64:1–6. https://doi.org/10.5979/cha.1986.64_1 (**in Japanese**)

Ozawa A (2013) Effects of insecticide application on natural enemy complex in tea fields. Bull Shizuoka Res Inst Agric For 6:19–24 (**in Japanese**)

Ozawa A, Oishi T, Furuki T, Ishigami S, Yoshida R, Takafuji A (2006) Long-term fluctuation of populations of Kanzawa spider mite, *Tetranychus kanzawai* Kishida, in commercial tea fields for last thirty years in Shizuoka Prefecture, Japan. Tea Res J 102:31–40. https://doi.org/10.5979/cha.2006.102_31 (**in Japanese**)

Ozawa A, Uchiyama T, Yoshizaki M (2014) Selection of biodiversity indicators for environmentally friendly agriculture in tea fields of Tokai area of mild climate. In: Nakasuji F (ed) Selection of functional biodiversity indicators and development of assessment methods. Agriculture, Forestry and Fisheries Research Council, Tokyo, pp 113–121 (**in Japanese**)

Santoso S, Takafuji A, Amano H, Ozawa A (2004) Species composition of Phytoseiid mites (Acari: Phytoseiidae) in tea fields with different management practices in Shizuoka Prefecture, Japan. J Acarol Soc Jpn 13:77–82. https://doi.org/10.2300/acari.13.77

SAS Institute (2007) JMP user's guide. SAS Institute, Cary

Shibao M, Ioku Y (2016) Control of two-spotted spider mite, *Tetranychus urticae* Koch in greenhouse strawberries by releasing *Neoseiulus californicus* (McGregor) and *Phytoseiulus persimilis* Athias-Henriot. Ann Rept Kansai Pl Prot 58:73–76 (**in Japanese**)

Shibao M, Ehara S, Hosomi A, Tanaka H (2006) Effect of insecticide application on the population density of yellow tea thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) and *Euseius sojaensis* (Ehara) (Acari: Phytoseiidae) on grapes. Jpn J Appl Entomol Zool 50:247–252. https://doi.org/10.1303/jjaez.2006.247 (**in Japanese**)

Shinkaji N (1991) *Tetranychus urticae* Koch, *Tetranychus cinnabarinus* (Boisduval). In: Yushima T, Kamano S, Tamaki Y (eds) Rearing methods of insects. Japan Plant Protection Association, Tokyo, pp 372–373 (**in Japanese**)

Takafuji A, Chant DA (1976) Comparative studies of two species of predacious phytoseiid mites (Acarina: Phytoseiidae), with special reference to their responses to the density of their prey. Res Popul Ecol 17:255–310. https://doi.org/10.1007/BF02530777

Takafuji A, Inoue M (1993) The effect of permethrin spray on the dispersal of *Tetranychus kanzawai* Kishida and the abundance of its predators. Proc Kansai Pl Prot 35:19–25. https://doi.org/10.4165/kapps1958.35.0_19 (**in Japanese**)

Takahashi F, Inoue M, Takafuji A (1998) Management of the spider-mite population in a vinylhouse vinery by releasing *Phytoseiulus persimilis* Athias-Henriot onto the ground cover. Jpn J Appl Entomol Zool 42:71–76. https://doi.org/10.1303/jjaez.42.71 (**in Japanese**)

Toyoshima S (2014) Comparison of four techniques to estimate phytoseiid mite density in tea plantations with low density of *Tetanychus urticae*. Tea Res J 117:15–25. https://doi.org/10.5979/cha.2014.117_15 (**in Japanese**)

Toyoshima S, Kishimoto H, Goto C (2015a) Manual of discrimination of Phytoseiid species for beginners (1st edition). NARO, Tokyo, 18 pp. (**in Japanese**)

Toyoshima S, Yorozuya H, Sato Y (2015b) Estimation of the density of phytoseiid mites by leaf-sampling and 'Phyto-traps' in Japanese tea plantations. Tea Res J 120:17–23. https://doi.org/10.5979/cha.2015.17 (**in Japanese**)

Ullah MS, Hanawa M, Gotoh T (2016) Pesticide-mediated displacement of a phytoseiid predator, *Neoseiulus womersleyi*, by another phytoseiid predator, *N. californicus* (Acari: Phytoseiidae). Exp Appl Acarol 69:453–464. https://doi.org/10.1007/s10493-016-0053-2

Van den Boom CEM, Van Beek TA, Dicke M (2003) Differences among plant species in acceptance by the spider mite *Tetranychus urticae* Koch. J Appl Ent 127:177–183. https://doi.org/10.1046/j.1439-0418.2003.00726.x

Wakou M, Odagiri M (1985) Effect of some acaricides on the two spotted spider mite and on field population of predacious mite. Ann Rept Plant Prot N Jpn 36:90–93. https://doi.org/10.11455/kitanihon1966.1985.90 (**in Japanese**)

Yamazaki D, Itoyama K (2015) Seasonal occurrence of Phytoseiid mites in urbanized Japanese pear orchards. Annu Rep Kanto Tosan Plant Prot Soc 62:153–155. https://doi.org/10.11337/ktpps.2015.153 (**in Japanese**)

Affiliations

Satoshi Kakoki[1,5] · Takeshi Kamimuro[2] · Yuki Ikenoue[3] · Mahomi Inokuchi[4] · Katsuo Tsuda[5] · Yositaka Sakamaki[5]

[1] Tea Division, Kagoshima Prefectural Institute for Agricultural Development, 3964, Nagasato, Chiran-cho, Minamikyushu 897-0303, Japan

[2] Kagoshima Prefectural Institute for Agricultural Development, Osumi Branch, 4938, Hosoyamada, Kushira-cho, Kanoya 893-1601, Japan

[3] Agricultural Promotion and Advisory Division, Kagoshima Prefectural Osumi Regional Promotion Bureau, 2-16-6, Utsuma, Kanoya 893-0011, Japan

[4] Entomological Laboratory, The Graduate School of Agricultural Sciences Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan

[5] Entomological Laboratory, The United Graduate School of Agricultural Sciences Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan