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## Use of a Lower-Volume, Surface Pesticide Spray Conserves Spider Assemblages in a Tea Field

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### Abstract

Since spiders are sensitive to pesticides, the use of lower-volume pesticide sprays that specifically target the plucking surface may help to preserve their assemblages. In this study, we investigated the effect of four pyrethroid pesticides on spider populations in tea (*Camellia sinensis*) fields when applied using a lower-volume sprayer. Abundance and composition at family level of spiders were assessed before and after treatments. We found that fewer spiders were eliminated when we used a lower-volume sprayer (40 liters/10 ares) rather than a conventional sprayer (200 liters/10 ares) due to the lower-volume treatment only covering the plucking surface (top layer) of the tea plants. These findings indicate that the tea leaf layer plays a good role in sheltering spiders during pesticide application and that the lower-volume treatment that specifically targets the plucking surface can enhance this protection. Therefore, to successfully maintain predatory spiders that prey on tea pests, tea farmers should reduce the volume of pesticides they use and try to restrict the spray to the plucking surface of the plants.

**Key words:** hunting spider, web-weaving spider, ecological selectivity, temporal variation

The standard practice of pruning and maintaining tea (*Camellia sinensis*) plants in a bush form provides habitat for natural enemies of tea pests (e.g., spiders), with the maintenance of a thick leaf layer being particularly important (Kawai 1997). To further encourage these natural enemies, it is important the use of selective pesticides (physiology selectivity) and, in addition, the form of application using a spray device that minimizes their impact on beneficial species and in a way that reduces pesticide infiltration to areas below the leaf layer as much as possible (Yoshioka and Takeda 2006), can provide ecological selectivity. Pesticides are conventionally sprayed in Japanese tea fields at a rate of 200 liters/10 ares (Kawai et al. 1999), which can reduce the density of natural enemies to below that which occurs in organic tea fields (where no pesticides are used) (Tatara 1997). Thus, we hypothesized that conventional spraying methods cannot sufficiently protect natural enemies because pesticides that are sprayed by the conventional method penetrate the tea leaf layer which usually provides a refuge for these species.

A lower-volume sprayer (Kagoshima-style MCS-KAGO3-2; Matsumoto Kiko Co., Ltd., Kagoshima, Japan) that makes use of a small fog nozzle and a ventilator was developed in 2013 for use in tea fields. Kakoki et al. (2015) found that this lower-volume sprayer had equivalent effects to the conventional sprayer in terms of pest control, but reduced both pesticide and water use by 1/3–1/5. Moreover,

Kakoki et al. (2015) suggested that the application of lower-volume spraying to the plucking surface may help to protect natural enemies seeking refuge inside the leaf layer. For example, it has been shown that the walking behavior of Linyphiidae spiders changes depending on whether the leaf they are moving along has been applied with deltamethrin (Jagers op Akkerhuis 1994).

Spiders are one of the most abundant predator groups in agroecosystem, playing an important role as natural enemies of various pests (Pekár 2012). Although spiders are commonly considered as polyphagous predators and not all species are useful against a determinate pest, preserving their diversity could be important for controlling different pests (Maloney et al. 2003, Wang et al. 2016). In tea fields, Kosugi (2003) has reported the predatory quality of *Trachelas japonicus* Bösenberg & Strand (Araneae: Corinnidae) and *Philodromus subaureolus* Bösenberg & Strand (Araneae: Philodromidae) on *Empoasca onukii* Matsuda (Hemiptera: Cicadellidae), keeping their populations under control. Spiders can control the density of leafhoppers in tea fields (Liu et al. 2015), with larger spider populations effectively reducing populations of *E. onukii* and *Scritothrips dorsalis* Hood (Thysanoptera: Thripidae) (Goto et al. 1995). Shiraki and Ohashi (1992) have shown that the population density of spiders and other natural enemies are higher in pesticide-free tea fields than in those sprayed with pesticides. Moreover, spiders prey on major pests of tea

(Kosugi 2003), such as *E. onukii*, keeping their populations under control. However, spiders are generally sensitive to insecticides and acaricides (Ohtaishi and Hamamura 1986; Goto et al. 1995; Pekár 1999, 2012). Therefore, if the effects of pesticides on spiders could be reduced, it is expected that the surviving spiders could suppress these pests. Furthermore, Riechert and Lockley (1984) reported that if the extent of pesticide dispersion is restricted to defined areas, spiders can repopulate those areas to a similar density by emigrating from other areas where the pesticide was not sprayed. Therefore, the objective of this study was to examine whether the use of a lower-volume pesticide sprayer would allow spider assemblages in tea fields to be maintained. To do this, we compared the abundance and family composition of spiders inhabiting tea plants where pesticides were sprayed using a lower-volume spray treatment and a conventional treatment. Generally, selective pesticides such as insect growth regulators, neonicotinoid, and diamide are used in the tea fields of Japan. However, in this study, to more readily detect the effect of pesticide use on spiders, we chose nonselective pesticides that show high activity toward indigenous natural enemies (Ozawa 2013).

## Materials and Methods

### Study Field and Pesticide Application

This study was conducted in a tea field (cultivar ‘Kuritawase’) at the Kagoshima Prefectural Institute for Agricultural Development, Tea Division (Minamikyushu City, Kagoshima Prefecture, Japan) located in the southwestern region of the mainland of Japan (31°37'N, 130°45'E). Tea plants were pruned to a height of 74 cm in May 2015 to allow the riding-type harvest and spray machine to pass over the tea plants. There were few weeds on the soil surface because of the pressure of the machine, and shadow of tea plants. Spider population surveys were conducted from May to July in 2015, and from May to September in 2016 and 2017. We applied two pesticide treatments to the field: 1) pesticide application using a lower-volume sprayer (spray pressure, 1.5 MPa; spray speed, 0.7 m/s) at an application rate of 40 liters/10 ares (hereafter referred to as the 40-liter treatment); 2) pesticide application using a conventional sprayer (MCS8A; Matsumoto Kiko Co., Ltd., Kagoshima, Japan; spray pressure, 1.0 MPa; spray speed, 0.5 m/s) at an application rate of 200 liters/10 ares (hereafter the 200-liter treatment); and 3) no application (hereafter the control treatment). The

distance between the plucking surface and spray nozzle of each type of spray was adjusted to 10 cm (Fig. 1). There were three replicates per treatment (each covering 12.6 m<sup>2</sup>, minimum distance of 1 m between treatments) that were spatially arranged randomly throughout the tea field. To distinguish more distinctly the difference between spray treatments, we applied four nonselective pesticides of the class pyrethroids, including fenpropathrin, permethrin, bifenthrin, and cypermethrin, which were authorized for use in the tea fields of Japan during a range of tea seasons (Table 1). They were sprayed at the optimum time for controlling pests, which equated to leaf stage 1 (average number of opened leaves = one) of the tea growing period in the second and third seasons, when the major pests include *E. onukii*, *S. dorsalis*, and *Caloptilia theivora* Walsingham (Lepidoptera, Gracillariidae), and leaf stages 1 and 3 in the autumn season, when the major pests are *E. onukii* and *S. dorsalis*. The survey in 2015 was completed in the end of July, since three tea seasons were passed. However, to determine the effect of lower-volume sprayer on spider populations including residual effects after tea seasons, the survey period was extended to autumn in 2016 and 2017. Tea yield per 10 ares were recorded. Data on average temperature, precipitation, and humidity recorded at the Makurazaki weather station of Japan Meteorological Agency situated at a distance of 18 km from the research field was used in this study.

### Measurement of Pesticide Coverage Inside the Tea Leaf Layer

In this report, we use the terms ‘plucking surface’, which refers to newly flushed leaves at the top of a tea branch, and ‘leaf layer’, which is the space between the underside of the plucking surface and the bottom leaf in the tea bush (Fig. 1). These definitions are consistent with those given in the ‘Terminology of Tea Science’ (Japanese Society of Tea Science and Technology 2007).

To evaluate the spray coverage through the tea bush, we placed water-sensitive paper (WSP; 52 × 76 mm; Syngenta, Basel, Switzerland) on 7-mm-diameter plastic props 5 cm below the plucking surface ( $n = 6$ ) and 25 cm below the plucking surface at the bottom of the leaf layer ( $n = 6$ ) in each tea plant just before spraying the pesticide (13 May 2016; 1 July 2016; 8 August 2016; and 24 August 2016). We then collected the WSPs immediately after spraying, scanned them with an image scanner (DCP-J952N; Brother Industries Ltd., Aichi, Japan), and used image-processing software

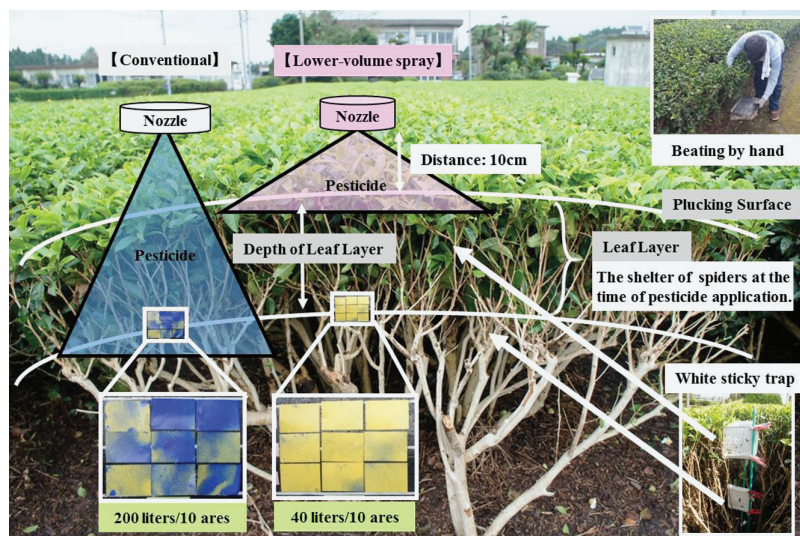


Fig. 1. Schematic representation of the conventional and lower-volume sprayers. The beating method and white sticky traps used to collect spiders are shown in insets.

to measure the cover-area ratio on water-sensitive paper (National Agricultural Research Organization [NARO]) to determine the percentage of the WSP that was coated with pesticide.

### Spider Survey

Spider sampling was carried out in May–July in 2015, and May–September in 2016 and 2017. We collected spiders just before spraying the pesticides, and approximately 7, 14, and 21 d after spraying. We also collected spiders 2–3 d after spraying in 2016 and 2017. Spiders were collected from four plants chosen randomly from within each of the 12.6 m<sup>2</sup> replicate areas (including approximately 30 plants). A 21 × 30 cm tray covered with a transparent plastic sheet and sprayed sticky glue (Kinryu; SDS Biotech K.K., Tokyo, Japan) was placed under the plant such that the distance between the ground and the lowermost branches of the plant was 50 cm, and the plant was beaten 10 times by hand (Fig. 1). All of the spiders that fell onto the tray were taken to the laboratory for identification to family level (note: spiders in the families Theridiidae and Linyphiidae are difficult to distinguish and so were simply identified as belonging to the superfamily Theridioidea; however, this is referred to as a ‘family’ hereafter for simplicity). Spiders were identified as described previously (Chikuni 2008, Shinkai 2010). Each of the sticky trays on which the spiders were wrapped with food wrap film (Hitachi Chemical Company, Ltd., Tokyo, Japan) and placed in a refrigerator for preservation.

### Survey of Synchrony Between Spiders and *S. dorsalis*

We installed two 10 × 10 cm white sticky traps (Sankei Chemical Co., Ltd., Kagoshima, Japan) at the plucking surface and also at the

bottom of the leaf layer at a depth of 25 cm from the surface (Fig. 1). These sticky traps were replaced weekly. Sampling was carried out from May to October in 2016 and May to November in 2017. All spiders and *S. dorsalis* collected on sticky traps were brought to the laboratory, and spiders were identified and assigned to the respective families. All sticky traps were wrapped with cling wrap and placed in the refrigerator for preservation.

### Statistical Analyses

To characterize pesticide coverage inside the leaf layer after spraying, we analyzed the WSP pesticide contact data using a paired *t*-test to compare row by row. To assess temporal variation in the abundance of spiders and *S. dorsalis*, we conducted a repeated measures analysis of variance (ANOVA) followed by Tukey’s honest significant difference test. The Spearman rank correlation analysis was used to analyze correlation between the number of spiders and that of *S. dorsalis* caught by white sticky traps. For all statistical analyses, we used log<sub>10</sub> (N+0.5)-transformed data for *S. dorsalis* and spider abundance (i.e., for captured in each examination period) and arcsine-transformed data for the percentage of WSP that was coated with pesticide. Yield data of tea plants (kg/10 ares) was analyzed by ANOVA. All statistical tests were conducted using JMP 7 software (SAS Institute 2007).

## Results

### Pesticide Coverage Inside the Leaf Layer of Tea Plants

Table 2 compares the pesticide coverage on WSP at 5 cm below the plucking surface and at the bottom of the leaf layer between the two

**Table 1.** Timing, rates, and concentrations of pesticide applications used in this study

Application date	Tea season	Application rate (liters/10 ares)		Trade name	Chemical name (active ingredient %)	Acaricide properties	Concn (ppm)
		Lower-volume spraying	Conventional spraying				
2015							
May 8	Second	40	200	Rody EC	Fenprothrin (10)	Present	100
June 24	Third	40	200	Rody EC	Fenprothrin (10)	Present	100
2016							
May 13	Second	40	200	RodyEC	Fenprothrin (10)	Present	100
July 1	Third	40	200	Rody EC	Fenprothrin (10)	Present	100
Aug. 8	Autumn	40	200	Rody EC	Fenprothrin (10)	Present	100
Aug. 24	Autumn	40	200	Adion EC	Permethrin (20)		200
2017							
May 19	Second	40	200	Telstar WP	Bifenthrin (2)	Present	20
July 3	Third	40	200	Agrosrin WP	Cypermethrin (6)		60
Aug. 16	Autumn	40	200	Adion EC	Permethrin (20)		200
Aug. 30	Autumn	40	200	Telstar WP	Bifenthrin (2)	Present	20

**Table 2.** Comparison of the pesticide coverage rate achieved using conventional and lower-volume sprayers

Treatments (liters/10 ares)	n	5 cm below plucking surface				Bottom of leaf layer (25 cm depth)			
		Second	Third	Autumn 1	Autumn 2	Second	Third	Autumn 1	Autumn 2
Conventional 200 liters	6	90.9 ± 2.30	97.7 ± 0.63	95.8 ± 0.93	91.3 ± 2.04	48.5 ± 2.64	36.3 ± 3.01	35.8 ± 3.89	18.7 ± 3.99
Lower volume 40 liters	6	59.7 ± 3.97	70.1 ± 3.99	58.9 ± 4.37	41.5 ± 5.47	0.1 ± 0.01	8.6 ± 5.48	0.1 ± 0.03	1.0 ± 0.22
Statistical test		**	**	**	**	**	**	**	**

Data represent mean ± SE. Statistical significance of the differences between treatments was tested using the paired *t*-test. Statistically significant differences are indicated using asterisks (\*\**P* < 0.01).



pesticide spray treatments. At 5 cm below the leaf-plucking surface, pesticide coverage exceeded 90% ( $n = 6$ ) in the 200-liter treatment in all seasons, which was significantly greater than the coverage with the 40-liter treatment ( $P < 0.01$ ). At the bottom of the leaf layer, the coverage varied between  $18.7 \pm 3.99\%$  and  $48.5 \pm 2.64\%$  ( $n = 6$ ) with the 200-liter treatment and between  $0.1 \pm 0.01\%$  and  $8.6 \pm 5.48\%$  ( $n = 6$ ) with the 40-liter treatment. Pesticide coverage at the bottom of the leaf layer with the 200-liter treatment was lower in autumn but was significantly higher than with the 40-liter treatment in all seasons.

### Spider Survey: Temporal Variation in Spider Abundance

Figure 2 shows the temporal variation in the abundance of spiders following the pesticide treatments in 2015–2017. The average climatological conditions during 2015, 2016, and 2017 were as follows: temperature = 22.9, 25.5, and 24.9°C, relative humidity = 85.9, 83.0, and 81.8%, and precipitation = 24.4, 9.7, and 8.2 mm, respectively.

The total spider abundance was similar between the 40-liter and control treatments, but was significantly lower with the 200-liter treatment in all 3 yr (2015:  $F = 8.07$ ,  $df = 2, 6$ ,  $P = 0.0060$ ; 2016:  $F = 22.72$ ,  $df = 2, 15$ ,  $P < 0.0001$ ; 2017:  $F = 15.04$ ,  $df = 2, 14$ ,  $P < 0.0001$ ) (Fig. 2).

During the second tea season in all 3 yr, spider abundance was low following pesticide application and did not show significant differences among the different years (2015:  $F = 4.15$ ,  $df = 2, 2$ ,  $P = 0.1059$ ; 2016:  $F = 1.82$ ,  $df = 2, 3$ ,  $P = 0.2412$ ; 2017:  $F = 1.28$ ,  $df = 2, 3$ ,  $P = 0.3434$ ) (Fig. 2).

In the third tea season, the spider abundance tended to be lower following the 200-liter treatment in 2015 ( $F = 4.00$ ,  $df = 2, 3$ ,  $P = 0.0787$ ) and decreased to 20% ( $n = 10$ ) of the pre-pesticide abundance 6 d after the 200-liter treatment in 2016, with fewer spiders being retained than with the other treatments (Fig. 2). By contrast, the abundance of spiders increased 6 d after the 40-liter treatment (Fig. 2). With both pesticide treatments, the abundance of spiders increased 2 wk after pesticide application and approached control levels after 3 wk, at which time the total abundance of spiders was

56% ( $n = 100$ ) lower with the 200-liter treatment than the 40-liter treatment, but identical between the 40-liter treatment and the control (Fig. 2). In 2017, the abundance of spiders was significantly lower with the 200-liter treatment than with the 40-liter and control treatments ( $F = 15.88$ ,  $df = 2, 3$ ,  $P = 0.040$ ). The abundance of spiders was lower in 2015 than in 2016 and 2017.

In autumn tea season 1 (leaf stage 1) of 2016, the spider abundance increased 2 d after the 40-liter treatment but decreased after the 200-liter treatment (Fig. 2). The number of spiders declined 1 wk after the 200-liter treatment, despite increasing with the other treatments (Fig. 2). The cumulative abundance data showed that the abundance of spiders was similar between the 40-liter and control treatments, whereas there were significantly fewer spiders following the 200-liter treatment ( $F = 23.85$ ,  $df = 2, 3$ ,  $P = 0.0014$ ) (Fig. 2, Table 3). There were no significant differences in the total number of spiders ( $F = 2.84$ ,  $df = 2, 2$ ,  $P = 0.1705$ ) and hunting spiders ( $F = 0.89$ ,  $df = 2, 2$ ,  $P = 0.4780$ ) among treatments in 2017, however.

In autumn tea season 2 (leaf stage 3) of 2016, spiders were significantly more abundant in 40-liter and control treatments compared with the 200-liter treatment ( $F = 14.81$ ,  $df = 2, 3$ ,  $P = 0.0048$ ) (Fig. 2). In 2017, the total number of spiders was significantly higher 1 wk after the 40-liter and control treatments compared with the 200-liter treatment ( $F = 6.45$ ,  $df = 2, 2$ ,  $P = 0.0320$ ; Fig. 2). The abundance of spiders particularly decreased within the first week of applying the 200-liter treatment. The change in the abundance of spiders was similar between the 40-liter and control treatments. The total number of spiders was significantly lower with the 200-liter treatment than with the 40-liter and control treatments ( $F = 16.55$ ,  $df = 2, 3$ ,  $P = 0.0036$ ).

### Spider Survey: Regarding the Representation at the Family Level

Table 3 shows the cumulative spider composition at the family level over a 3-wk period following pesticide application. Four spider families (Salticidae, Theridiidae, Thomisidae, and Clubionidae) represented the majority of spiders inhabiting the study field, among which the most abundant was Salticidae, and the second most abundant

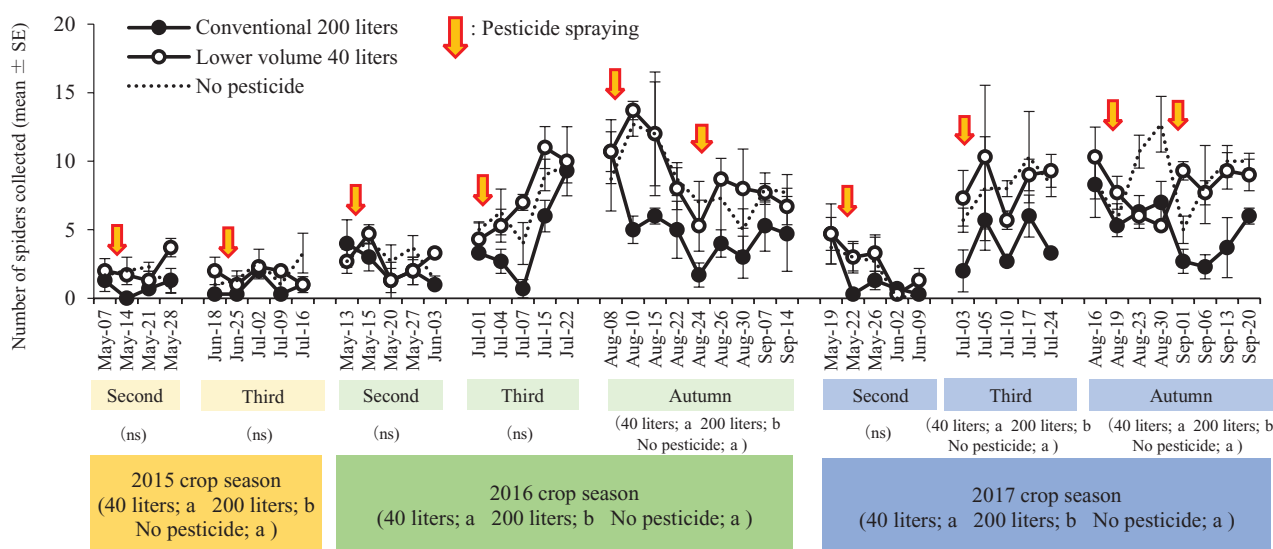


Fig. 2. Temporal variation in spider abundance following pesticide treatment. Data plotted represent mean  $\pm$  SE. Statistically significant differences among treatments within the same tea season were detected using Tukey's honest significant difference test, and indicated using different letters ( $P < 0.05$ ; ns, not significant). The arrows in the figure indicate timing of pesticide spraying.

**Table 3.** Cumulative abundance of spiders over 3 wk after pesticide spraying

Tea season	Spray treatments (liters/10 acres)	Spider abundance <sup>a</sup>											Total	
		Hunting type						Hunting and web-weaving type				Web-weaving type		
		Salticidae	Clubionidae	Thomisidae	Pisauridae	Oxyopidae	Sparassidae	Subtotal	Theridioidae	Pholcidae	Tetragnathidae	Araneidae		Agelenidae
2015 Second	Conventional 200 liters	0.7 ± 0.33	0.3 ± 0.33	0	0	0	0	1.0 ± 0.58	1.0 ± 0.58	0	0	0	0	2.0 ± 1.15
	Lower volume 40 liters	2.0 ± 0.58	2.3 ± 0.88	0.3 ± 0.33	0	0.3 ± 0.33	0	5.0 ± 0.58	1.7 ± 1.67	0	0	0	0	6.7 ± 2.19
2015 Third	No pesticide Conventional 200 liters	1.3 ± 0.88	1.7 ± 0.33	0.3 ± 0.33	0.3 ± 0.33	0.3 ± 0.33	0	4.0 ± 1.00	1.0 ± 0.58	0	0	0	0	5.0 ± 1.53
	Lower volume 40 liters	1.0 ± 0.71	1.0 ± 0.75	1.0 ± 0.48	0	0	0	3.0 ± 1.19	0.3 ± 0.25	0	0	0	0	3.3 ± 1.18
2015 Total <sup>b</sup> (No. of spiders collected)	No pesticide Conventional 200 liters	1.5 ± 0.50	2.3 ± 0.63	1.5 ± 1.19	0	0	0	5.3 ± 1.31	0.8 ± 0.48	0	0	0	0	6.3 ± 1.65
	Lower volume 40 liters	14 (35.9)	11 (28.2)a	2 (5.1)	0	3 (7.7)	0	30 (76.9)a	7 (17.9)	1 (2.6)	0	0	1 (2.6)	39a
2016 Second	No pesticide Conventional 200 liters	10 (25.0)	14 (35.0)a	7 (17.5)	1 (2.5)	1 (2.5)	0	33 (82.5)a	6 (15.0)	0	0	0	1 (2.5)	40a
	Lower volume 40 liters	1.5 ± 0.29	0.3 ± 0.25	0.3 ± 0.25	0	0	0	2.0 ± 0.71	3.3 ± 1.31	0.3 ± 0.25	0	0	0	5.5 ± 1.32
2016 Third	No pesticide Conventional 200 liters	3.8 ± 1.11	0.8 ± 0.48	0.5 ± 0.29	0	0.3 ± 0.25	0	5.3 ± 1.11	3.0 ± 1.29	0	0.3 ± 0.25	0	0	8.5 ± 2.22
	Lower volume 40 liters	3.0 ± 1.08	1.5 ± 0.50	0.3 ± 0.25	0	0	0	4.8 ± 1.49	4.0 ± 1.00	0	0.3 ± 0.25	0	0	9.0 ± 1.96
2016 Autumn 1	No pesticide Conventional 200 liters	4.8 ± 1.60	0.3 ± 0.25	2.8 ± 1.11	0.3 ± 0.25	0.3 ± 0.25	0	8.3 ± 2.69	5.5 ± 2.96	0.3 ± 0.25	0	0	0	14.0 ± 5.72
	Lower volume 40 liters	7.3 ± 1.38	1.0 ± 0.00	5.8 ± 0.63	0.5 ± 0.50	0	0	14.5 ± 2.06	9.8 ± 3.45	0.5 ± 0.50	0.3 ± 0.25	0	0	25.0 ± 3.94
2016 Autumn 2	No pesticide Conventional 200 liters	4.5 ± 1.44	1.0 ± 0.41	7.5 ± 0.87	0	0	0	13.0 ± 2.12	8.3 ± 3.97	0.3 ± 0.25	0	0	0	21.5 ± 3.75
	Lower volume 40 liters	6.0 ± 1.35	0	2.0 ± 0.71	0.5 ± 0.50b	1.0 ± 0.58	0	9.5 ± 1.94b	3.8 ± 1.89b	0	0	0	0	13.3 ± 2.84b
2016 Autumn 2	No pesticide Conventional 200 liters	12.0 ± 1.91	0.3 ± 0.25	3.8 ± 0.85	3.5 ± 1.66a	0.8 ± 0.75	0	20.3 ± 3.17a	8.8 ± 2.78a	0.3 ± 0.25	0	0	0	29.3 ± 5.68a
	Lower volume 40 liters	12.3 ± 1.70	0	3.8 ± 1.11	0.8 ± 0.48b	1.3 ± 0.95	0	18.0 ± 1.78a	12.3 ± 3.90a	0	0	0	0	30.3 ± 4.05a
2016 Autumn 2	No pesticide Conventional 200 liters	6.8 ± 1.97	0	1.3 ± 0.25	0.3 ± 0.25	0.3 ± 0.25b	0	8.5 ± 2.06b	3.8 ± 1.11	0.5 ± 0.29	0	0	0	12.8 ± 1.49b
	Lower volume 40 liters	11.8 ± 1.89	2.0 ± 0.91	3.0 ± 0.71	1.5 ± 1.50	1.8 ± 1.11 a	0	20.0 ± 0.71a	2.8 ± 1.31	0.5 ± 0.29	0	0	0	23.3 ± 1.25a
2016 Total <sup>b</sup> (No. of spiders collected)	No pesticide Conventional 200 liters	11.0 ± 2.27	3.3 ± 1.18	0.8 ± 0.75	0.8 ± 0.48	0.5 ± 0.50b	0	16.3 ± 2.43a	4.3 ± 0.75	0.5 ± 0.29	0	0	0	21.0 ± 2.04a
	Lower volume 40 liters	76 (42.0) b	2 (1.1) b	25 (13.7)	4 (2.2) b	6 (3.3)	0	113 (62.1) a	65 (35.7)	4 (2.2)	0	0	0	182b
2017 Second	No pesticide Conventional 200 liters	139 (40.4)a	16 (4.7)a	52 (15.1)	22 (6.4) a	11 (3.2)	0	240 (69.8)a	97 (28.2)	5 (1.7)	2 (0.6)	0	0	344a
	Lower volume 40 liters	123 (37.6)ab	23 (7.0)a	49 (15.0)	6 (1.8)ab	7 (2.1)	0	208 (63.6)a	115 (35.2)	3 (0.9)	1 (0.3)	0	0	327a
2017 Second	No pesticide Conventional 200 liters	1.3 ± 0.25	0.3 ± 0.25	0	0	0	0	1.5 ± 0.50	0.5 ± 0.29	0	0	0	0	2.0 ± 0.71
	Lower volume 40 liters	2.5 ± 1.32	1.0 ± 0.58	0.8 ± 0.48	0	0.3 ± 0.25	0	4.5 ± 2.22	1.5 ± 0.65	0	0	0	0	6.0 ± 2.12
2017 Second	No pesticide Conventional 200 liters	1.5 ± 0.65	1.0 ± 0.71	0.5 ± 0.29	0	0.5 ± 0.29	0	3.5 ± 1.76	1.0 ± 0.58	0	0	0	0	4.5 ± 2.33

Table 3. Continued

Tea season	Spray treatments (liters/10 ares)	Spider abundance <sup>a</sup>													
		Hunting type					Subtotal				Hunting and web-weaving type		Web-weaving type		Total
		Salticidae	Clubionidae	Thomisidae	Pisauridae	Oxyopidae	Sparassidae	Theridiidae	Pholcidae	Tetragnathidae	Araneidae	Agelenidae			
2017 Third	Conventional 200 liters	3.5 ± 0.50	1.5 ± 0.65	6.8 ± 2.50b	0	0.3 ± 0.25	0	12.0 ± 2.94b	1.3 ± 0.48	0	0	0	0	13.3 ± 2.50	
	Lower volume 40 liters	6.0 ± 1.78	2.3 ± 0.48	14.5 ± 3.33a	0	0	22.8 ± 3.35a	2.8 ± 0.85	0	0	0	0.3 ± 0.25	0	25.8 ± 3.04	
2017 Autumn 1	No pesticide	4.8 ± 1.55	2.5 ± 0.65	15.3 ± 3.33a	0.3 ± 0.25	1.0 ± 1.00	23.8 ± 1.80a	2.0 ± 0.71	0	0.3 ± 0.25	0	0	0	26.0 ± 1.68	
	Conventional 200 liters	10.0 ± 0.58	0.7 ± 0.67	5.3 ± 0.33	0.3 ± 0.33	0.3 ± 0.33	16.7 ± 0.88	1.7 ± 0.67	0.3 ± 0.33	0	0	0	0	18.7 ± 1.45	
2017 Autumn 2	Lower volume 40 liters	9.0 ± 2.31	1.3 ± 0.67	3.7 ± 1.20	0.7 ± 0.67	0.7 ± 0.33	15.3 ± 3.53	3.7 ± 1.45	0	0	0	0	0	19.0 ± 2.08	
	No pesticide	15.3 ± 4.18	2.0 ± 1.15	4.0 ± 1.15	3.0 ± 1.00	0.7 ± 0.33	25.0 ± 6.03	4.0 ± 1.15	0	0	0	0	0	29.0 ± 6.24	
	Conventional 200 liters	6.5 ± 1.19b	0.3 ± 0.25	3.8 ± 1.25	0.3 ± 0.25b	0	10.8 ± 2.25b	0.3 ± 0.25b	0	0	0	0	0	11.0 ± 2.48b	
2017 Total <sup>b</sup> (No. of spiders collected)	Lower volume 40 liters	13.3 ± 1.11a	1.0 ± 0.71	5.0 ± 0.82	4.5 ± 1.32a	0	23.8 ± 0.63a	2.5 ± 0.87ab	0.3 ± 0.25	0	0	0	0	26.5 ± 1.19a	
	No pesticide	12.0 ± 1.29ab	1.0 ± 1.00	4.8 ± 0.48	1.8 ± 0.48a	0.5 ± 0.29	20.3 ± 2.10a	4.8 ± 1.65a	0	0	0	0	0	25.0 ± 3.54a	
	Conventional 200 liters	75 (46.8)	10 (6.2)	58 (36.0)	2 (1.2)b	2 (1.2)	147 (91.3)b	13 (8.1) b	1 (0.6)	0	0	0	0	161b	
	Lower volume 40 liters	114 (39.3)	21 (7.2)	92 (31.7)	20 (6.9)ab	3 (1.0)	250 (86.2)a	38 (13.1)a	1 (0.3)	0	0	1 (0.3)	0	290a	
	No pesticide	119 (38.5)	24 (7.8)	94 (30.4)	17 (5.5)a	10 (3.2)	265 (85.7)a	43 (13.9)a	0	1 (0.3)	0	0	0	309a	

<sup>a</sup>Data represent mean ± SE. Statistical significance of the differences among treatments within a tea season was tested using Tukey's honest significant difference test. Different letters statistically significant differences ( $P < 0.05$ ).

<sup>b</sup>Values within parentheses are the percentage representation of the spider family across all families.

was Theridioidea in 2016 and Thomisidae in 2017. The cumulative percentages of these four major families that were collected following the 40-liter, 200-liter, and control treatments, respectively, were 88.4% ( $n = 344$ ), 92.5% ( $n = 182$ ), and 94.8% ( $n = 327$ ) in 2016, and 91.3% ( $n = 290$ ), 97.1% ( $n = 161$ ), and 90.6% ( $n = 309$ ) in 2017. Few spiders were collected from the families Pisauridae, Oxyopidae, Sparassidae, Pholcidae, Tetragnathidae, Araneidae, and Agelenidae. The abundances of Thomisidae and Theridioidea tended to be lower with the 200-liter treatment than with the 40-liter and control treatments in 2016 (Thomisidae:  $F = 3.07$ ,  $df = 2, 15$ ,  $P = 0.0614$ ; Theridioidea:  $F = 2.78$ ,  $df = 2, 15$ ,  $P = 0.0780$ ) and were significantly lower with the 200-liter treatment than with the other two treatments in 2017 (Thomisidae:  $F = 3.64$ ,  $df = 2, 14$ ,  $P = 0.0394$ ; Theridioidea:  $F = 6.97$ ,  $df = 2, 14$ ,  $P = 0.0035$ ).

In the second tea season, the cumulative abundance data showed that there were fewer Salticidae and Clubionidae spiders following the 200-liter treatment than following the other treatments, whereas no such decline was observed for Theridioidea spiders (Table 3).

In the third tea season, the abundance of Thomisidae spiders tended to show a greater decline following the 200-liter treatment than the 40-liter and control treatments, while the abundances of Salticidae and Theridioidea spiders did not significantly differ among the treatments ( $P > 0.05$ ). The abundances of Clubionidae, Pisauridae, Oxyopidae, Pholcidae, and Tetragnathidae spiders were low with all treatments, making the differences among treatments statistically indiscernible (Table 3). In 2017, hunting spiders were clearly more abundant following the 40-liter and control treatments than the 200-liter treatment ( $F = 12.2$ ,  $df = 2, 3$ ,  $P = 0.0078$ ). Notably, twice as many Thomisidae spiders were captured following the 40-liter and control treatments compared with the 200-liter treatment ( $F = 8.79$ ,  $df = 2, 3$ ,  $P = 0.0165$ ). There was no significant difference among treatments in the number of Theridioidea spiders captured (2015:  $F = 0.39$ ,  $df = 2, 3$ ,  $P = 0.6951$ ; 2016:  $F = 1.67$ ,  $df = 2, 3$ ,  $P = 0.2660$ ; 2017:  $F = 1.94$ ,  $df = 2, 3$ ,  $P = 0.2246$ ) or the total abundance of spiders (2015:  $F = 4.00$ ,  $df = 2, 3$ ,  $P = 0.0787$ ; 2016:  $F = 3.76$ ,  $df = 2, 3$ ,  $P = 0.0873$ ; 2017:  $F = 4.65$ ,  $df = 2, 3$ ,  $P = 0.0603$ ).

In the autumn tea season, the abundances of Salticidae, Clubionidae, Thomisidae, Pisauridae, Oxyopidae, and Theridioidea spiders tended to be higher following the 40-liter treatment than the 200-liter treatment, and the total number of spiders tended to be lower following the 200-liter treatment compared with the other treatments (Table 3).

### Abundances of Spiders and *S. dorsalis* at Plucking Surface and Bottom of Leaf Layer

Table 4 shows cumulative abundance of spiders and *S. dorsalis* caught using white sticky traps. At the plucking surface, the total number of spiders were higher following 40-liter and control treatments than with the 200-liter treatment (2016:  $F = 20.74$ ,  $df = 2, 22$ ,  $P < 0.0001$ ; 2017:  $F = 4.28$ ,  $df = 2, 23$ ,  $P = 0.0193$ ); similar tendency was observed at the bottom of leaf layer (2016:  $F = 3.44$ ,  $df = 2, 22$ ,  $P = 0.00409$ ; 2017:  $F = 3.39$ ,  $df = 2, 23$ ,  $P = 0.0425$ ). However, the number of Thomisidae and Pisauridae spiders caught using white sticky traps was less than those caught using beating (Table 4 vs Table 3). Theridioidea spiders were more at the bottom of the leaf layer than at the plucking surface. The total number of spiders, especially those belonging to the Salticidae family, and the number of *S. dorsalis* were more abundant at the plucking surface than at the bottom. Similar trends were not observed in other spider families. No significant differences were detected in the number of *S. dorsalis*

Table 4. Cumulative abundance of spiders and *Scritiothrips dorsalis* Hood caught using white sticky trap

Year	Placement of trap	Treatments (liters/10 ares)	n	Spiders										Total	<i>Scritiothrips dorsalis</i> hood				
				Salticidae	Clubionidae	Thomisidae	Pisauridae	Oxyopidae	Theridioidea	Pholcidae	Tetragnathidae	Philodromidae							
2016	Plucking surface	Conventional 200 liters	3	71.0 ± 7.00b	6.7 ± 2.33	1.3 ± 0.33	0	0.3 ± 0.33	40.0 ± 5.03	1.3 ± 0.33	0.3 ± 0.33	0.3 ± 0.33	0	0	0	0	121.3 ± 4.33b	767.0 ± 34.12	
		Lower volume 40 liters	3	108.7 ± 2.47a	13.0 ± 3.51	1.0 ± 0.58	0.3 ± 0.33	1.3 ± 1.33	48.0 ± 5.13	0.7 ± 0.33	0.3 ± 0.33	0.3 ± 0.33	0.3 ± 0.33	0	0	0	173.3 ± 19.54a	691.0 ± 88.97	
	Bottom of leaf layer (25 cm depth)	No pesticide	3	124.7 ± 7.54a	11.3 ± 2.96	0	0.7 ± 0.67	1.0 ± 0.58	56.0 ± 3.51	0.3 ± 0.33	0.3 ± 0.33	0.3 ± 0.33	0.3 ± 0.33	0	0	0	194.3 ± 6.74a	930.7 ± 74.99	
		Conventional 200 liters	3	27.0 ± 1.73	5.0 ± 0.58b	2.0 ± 2.00	0	0	52.7 ± 0.67	0.7 ± 0.67	0.3 ± 0.33	0.3 ± 0.33	0.3 ± 0.33	0	0	0	87.7 ± 1.20b	295.0 ± 8.39a	
	2017	Plucking surface	Lower volume 40 liters	3	34.3 ± 3.58	9.7 ± 1.33ab	0	1.0 ± 0.58	0	43.7 ± 7.54	1.3 ± 0.33	0	0	0	0	0	0	90.0 ± 6.03ab	181.7 ± 36.97ab
			No pesticide	3	35.3 ± 5.17	14.7 ± 2.03a	1.0 ± 0.58	0.3 ± 0.33	0.3 ± 0.33	52.0 ± 3.79	1.0 ± 0.58	1.0 ± 0.58	1.0 ± 0.58	0.3 ± 0.33	0	0	0	106.0 ± 4.51a	204.3 ± 6.96b
Bottom of leaf layer (25 cm depth)		Conventional 200 liters	3	70.3 ± 0.67	8.7 ± 2.67b	2.7 ± 1.45	1.0 ± 0.00	0.3 ± 0.33	22.0 ± 5.03	0.3 ± 0.33	0	0	0	0	0	0	105.3 ± 5.81b	780.0 ± 55.14	
		Lower volume 40 liters	3	78.3 ± 7.13	15.7 ± 2.19a	4.3 ± 1.20	1.0 ± 0.00	0	24.3 ± 7.13	0.3 ± 0.33	0	0	0	0	0	0	124.0 ± 10.21a	745.3 ± 65.28	
Bottom of leaf layer (25 cm depth)		No pesticide	3	79.3 ± 5.70	13.3 ± 4.37a	3.7 ± 1.20	1.7 ± 0.33	0.3 ± 0.33	30.3 ± 3.18	1.7 ± 0.67	0	0	0	0	0	0	130.3 ± 1.67a	750.7 ± 58.07	
		Conventional 200 liters	3	26.0 ± 4.51b	8.0 ± 3.79	1.7 ± 0.88	0	1.3 ± 0.67	36.0 ± 6.66	1.0 ± 0.58	0.3 ± 0.33	0.3 ± 0.33	0	0	0	0	74.3 ± 11.35b	245.3 ± 29.41	
Bottom of leaf layer (25 cm depth)	Lower volume 40 liters	3	32.7 ± 2.33a	11.0 ± 2.08	3.0 ± 0.58	1.3 ± 0.88	0.7 ± 0.67	31.7 ± 6.23	2.7 ± 0.88	0	0	0	0	0	0	83.0 ± 7.77a	221.3 ± 22.85		
	No pesticide	3	27.3 ± 2.60ab	11.7 ± 4.48	2.0 ± 1.53	0	1.7 ± 0.67	31.3 ± 1.20	2.0 ± 0.58	0	0	0	0	0	0	76.0 ± 6.08ab	235.0 ± 22.65		

Data represent mean ± SE. Statistical significance of the differences among treatments was tested using Tukey's honest significant difference test. Different letters indicate statistically significant differences ( $P < 0.05$ ).



at the plucking surface between treatments (2016:  $F = 1.097$ ,  $df = 2, 22$ ,  $P = 0.3429$ ; 2017:  $F = 0.724$ ,  $df = 2, 23$ ,  $P = 0.4901$ ). In 2016, *S. dorsalis* were more abundant at the bottom of leaf layer following the 200-liter treatment compared with the 40-liter and control treatments ( $F = 3.53$ ,  $df = 2, 22$ ,  $P = 0.0376$ ); however, these difference were not significant in 2017 ( $F = 0.029$ ,  $df = 2, 23$ ,  $P = 0.9712$ ).

### Correlation Between Spider and *S. dorsalis* Populations and Tea Yield

Table 5 summarizes the correlation between the population sizes of spiders and *S. dorsalis* caught using white sticky traps. In 2016, no correlation was detected between the abundances of Salticidae spider and *S. dorsalis*. However, strong positive correlation was observed between Theridioidea spiders and *S. dorsalis* at the plucking surface. The total number of spiders did not show a correlation with that of *S. dorsalis*. In 2017, stronger positive correlation between abundances of Salticidae spiders and *S. dorsalis* was observed at the plucking surface than that at the bottom of the leaf layer. The total number of spiders, especially those belonging to the Theridioidea family, was well positive correlated with that of *S. dorsalis*. Tea yield did not differ significantly among treatments during the study period ( $P > 0.05$ ) (Table 6).

### Discussion

Tanaka et al. (2000) previously described the emigration of predators from plots that were saturated with pesticides and showed that predator reproductive rates may also be reduced following long-term pesticide use. In the present study, we found that the spider

population in a tea field varied in a similar way following a lower-volume pesticide application (40-liter treatment) and no pesticide application (control treatment). By contrast, there was a larger reduction in the spider population following the conventional (200-liter) treatment due to the leaf layer being covered with a thicker layer of pesticide and the residual toxicity for spiders after this treatment appeared to be approximately 2–3 wk, though this will likely vary between pesticides. We confirmed that the pesticide coverage at the bottom of leaf layer was significantly higher with the 200-liter treatment than that with the 40-liter treatment using both fenprothrin and permethrin. This loss of spiders was considered to be serious, particularly a few days after application, and indicates that a large number of spiders in the leaf layer will be lost when conventional pesticide application methods are used. By contrast, use of the lower-volume pesticide spray method that specifically targets the plucking surface may help to retain a more stable spider population.

In this study, we used the neurotoxic pyrethroid insecticides; neurotoxic substances are known to be affected to spiders (Pekár 2012). We found that diurnal, active hunting spiders, such as those belonging to the family Salticidae, were severely damaged by conventional pesticide applications, supporting the previous findings of Terada (1987). Goto et al. (1995) further found that while the abundance of hunting spiders (Clubionidae, Salticidae, and Thomisidae) was higher in pesticide-free fields than in conventionally sprayed fields, the reverse was true for web-weaving spiders (Theridiidae and Linyphiidae). Similarly, we found that the representation of Clubionidae spiders was lower following the 200-liter treatment compared with the 40-liter or control treatments. The number of Theridioidea spiders (which comprise both hunting and

**Table 5.** Correlation between the number of spiders and that of *Scritothrips dorsalis* Hood caught using white sticky traps

Year	Placement of trap	Treatments (liters/10 ares)	Spiders <sup>a</sup>		
			Salticidae	Theridioidea	Total
2016	Plucking surface	Conventional 200 liters	-0.16	0.58	0.00
		Lower volume 40 liters	-0.03	0.55	0.04
		No pesticide	0.15	0.69	0.27
	Bottom of leaf layer (25 cm depth)	Conventional 200 liters	-0.04	0.23	0.09
		Lower volume 40 liters	0.10	0.42	0.30
		No pesticide	0.10	0.33	0.26
2017	Plucking surface	Conventional 200 liters	0.53	0.41	0.55
		Lower volume 40 liters	0.43	0.31	0.45
		No pesticide	0.46	0.56	0.53
	Bottom of leaf layer (25 cm depth)	Conventional 200 liters	0.32	0.66	0.60
		Lower volume 40 liters	0.01	0.58	0.43
		No pesticide	0.17	0.33	0.31

We excluded few numbers of families.

<sup>a</sup>Data represent Spearman rank correlation coefficient.

**Table 6.** Tea yield (kg/10 ares) during study period

Treatments (liters/10 ares)	2015		2016		2017	
	Second	Third	Second	Second	Third	
Conventional 200 liters	484 ± 35.71	302 ± 21.13	576 ± 11.84	634 ± 10.21	450 ± 24.97	
Lower volume 40 liters	502 ± 16.07	288 ± 18.02	551 ± 32.58	605 ± 30.33	382 ± 37.49	
No pesticide	498 ± 27.44	286 ± 4.33	585 ± 24.84	571 ± 49.89	389 ± 29.87	
Statistical test	ns	ns	ns	ns	ns	

Data represent mean ± SE. Data in the third season in 2016 was not included because of excessive rain during time that was optimum for controlling pests. Based on ANOVA, differences in tea yield among growing seasons were not significant (ns).

web-weaving spiders) did not significantly differ among treatments in 2016 but was significantly lower following the 200-liter treatment than the other two treatments in 2017—though we do not know whether it was the family Theridiidae or Linyphiidae that mainly contributed to this difference and so this should be the subject of future research. The web-weaving type spiders, except those belonging to the Theridioidea family, are fewer in number in tea fields of Japan than the hunting type spiders (Goto et al. 1995, Uchiyama et al. 2011). The tendency of this study that the web-weaving type spiders were few agreed with previous study, and we thought that hunting spiders could be major in tea field of Japan.

The foraging behavior of hunting spiders enables them to encounter various pests (Nyffeler and Sunderland 2003). Hunting spiders wander throughout the leaf layer to increase their encounter rate with pests. However, although such active searching for prey may be the most successful hunting strategy for spiders (Young and Edwards 1990), it also increases the rate at which they encounter plots that are saturated with pesticides. A diverse assemblage of spiders may be more effective for biological control because different species will have different hunting strategies, habitat preferences, and periods of activity (Maloney et al. 2003). Therefore, spray methods that do not completely cover the leaf layer with pesticide and thus kill fewer hunting spiders will help maintain some of the natural enemies of tea pests.

Tanaka et al. (2000) previously stated that ‘Predators are exposed to pesticide solution or dust by three routes of uptake: direct uptake after exposure to droplets or dust of pesticides, uptake of residues by contact with contaminated surfaces of vegetation and soil, and oral uptake by feeding on contaminated prey’. Although we did not examine the pesticide sensitivity or prey consumption of spiders following the 40-liter treatment, we did find that this treatment preserved the spider abundance and controlled pest species while using only a small amount of pesticide. We predict that applying a lower-volume surface spray that targets only the plucking surface of the tea plant would allow tea leaves to function as a shelter that protects the indigenous natural enemies of tea pests. Hunting spider families (Salticidae, Clubionidae, Thomisidae, Pisauridae, Oxyopidae, and Sparassidae) accounted for approximately 86% ( $n = 309$ ) of the spiders captured in the control treatment and increased in abundance from July to September in 2017, during which time pesticides are sprayed three times under a standard recommended spray schedule. The lower-volume (40-liter) pesticide application may help prevent spiders and other insects from being eradicated by pesticides and actually allow their populations to expand.

Although helping to maintain the spider assemblage in a tea field through the use of a lower-volume spray treatment will likely have the indirect effect of improving the biological control of tea pests, the magnitude of these indirect effects requires further research. Therefore, it is important that the full assemblage of spider species is maintained, rather than just one species, to biologically control agricultural pests (Maloney et al. 2003, Wang et al. 2016). We also observed some positive correlation between the number of spiders and that of *S. dorsalis*, particularly in the case of Theridioidea and Salticidae, although this correlation did not depend on pesticide treatments. We predict that these spiders are occasionally attacked *S. dorsalis*. Moreover, the tea yield did not vary significantly during the study period. We speculate that the lower-volume pesticide sprays that specifically target the plucking surface slightly affected to the spider assemblages and also help spiders to suppress *S. dorsalis* easily. However, the abundance of the spiders and the plague has been estimated relatively by white sticky traps and that there could

be other prey-species that have not been taken into account. We have to investigate effects of other prey in the future.

Spiders are considered the most important natural enemies (Nyffeler and Birkhofer 2017). In tea fields, spiders ingest many species of pests and the amount of food that they ingested are affected by opportunity of encounter on prey (Kosugi 1999, 2003). To determine the abundance of different spider species in the tea field, we had to use two different capturing techniques. For example, it was difficult to catch Thomisidae and Pisauridae spiders using white sticky traps, but sufficient to do with the beating tray. We showed that spiders and *S. dorsalis* wandered from the plucking surface to the bottom of leaf layer, where the correlation between Theridioidea spiders and *S. dorsalis* was relatively high. Thus, targeting the plucking surface using the spraying method may help curb spiders’ activity at the bottom of leaf layer. Furthermore, spiders can indicate the success of environmental conservation in tea fields (Uchiyama et al. 2011). It is known that temporary habitat refuges enhance populations of arthropod natural enemies (Halaj et al. 2000). Since the tea leaf layer can provide a good refuge for spiders, it is important to encourage a denser leaf layer. Here, we found that the tea leaf layer acts as a useful shelter for spiders at the time of pesticide application, and that the lower-volume spraying method that targets only the plucking surface can help improve this sheltering function of the leaf layer. Therefore, to maintain healthy spider populations in tea cultivation, we should reduce the volume of pesticides used and restrict the spray area to only the plucking surface of the tea plants.

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