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ORIGINAL RESEARCH PAPER

A preliminary study on distributions and oviposition sites of *Drosophila suzukii* (Diptera: Drosophilidae) and its parasitoids on wild cherry tree in Tokyo, central Japan

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Abstract Distributions and oviposition sites of *Drosophila suzukii* (Matsumura) and its parasitoids on wild cherry tree were studied in early summer in a suburb of Tokyo, central Japan. Adults of *D. suzukii* occurred in the foliage layer as well as in the undergrowth layer. The number of *D. suzukii* that emerged did not significantly differ between wild cherry fruit collected from the foliage layer and those from the undergrowth layer. In addition, the number of *D. suzukii* that emerged per fruit decreased when fruit were left on the ground longer. It is therefore assumed that *D. suzukii* females rarely oviposit eggs in fallen wild cherry fruit. The *suzukii*-associated type of *Ganaspis brasiliensis* (Ihering) was the major parasitoid that emerged from *D. suzukii* in the study area. The rate of parasitism by this parasitoid did not significantly differ between larvae in fresh fruit from the foliage layer and those in fallen fruit from the undergrowth layer. This may also suggest that this wasp rarely attacks *D. suzukii* larvae in fallen fruit. Adults of the *suzukii*-associated type of *G. brasiliensis*, *Asobara* sp. TK1, and *Leptopilina japonica* that attack *D. suzukii* were mainly collected from the foliage layer. On the basis of the present results, some proposals for the control of *D. suzukii* were discussed.

Keywords *Drosophila suzukii* · Fallen fruit · Foliage layer · Fresh fruit · Parasitoids · Undergrowth layer

Introduction

Drosophila suzukii (Matsumura) is a pest of fresh fruit and causes serious economic loss in temperate regions of Asia, Europe, and America (Asplen et al. 2015; Kanzawa 1939). The current major control methods are insecticide application and net covering (Asplen et al. 2015; Beers et al. 2011; Bruck et al. 2011; Kawase et al. 2008; Yamakawa and Watanabe 1991), which incur some environmental loads and/or economic costs. As a method dealing with these shortcomings, biological control and integrated pest management have received much attention. In East Asia where *D. suzukii* has probably originated, the *suzukii*-associated type of *Ganaspis brasiliensis* (Ihering) (assigned as *G. xanthopoda* (Ashmead) in our previous papers) and *Asobara* sp. TK1 are potential candidates as biological control agents, because they are specialized *D. suzukii* parasitoids and their release would have a lower impact on local biological communities than the release of generalist parasitoids or predators (Daane et al. 2016; Kasuya et al. 2013; Mitsui et al. 2007; Nomano et al. 2015). These two parasitoid species are also potential candidates of classical biological control agents in Europe and North America where no effective predator or parasitoid against *D. suzukii* has been found (Asplen et al. 2015; Chabert et al. 2012; Gabarra et al. 2012; Kacsoh and Schlenke 2012; Rossi Stacconi et al. 2013).

To conduct integrated pest management, in addition, information on spatial distributions or habitats of pests and enemies is very important. It has been reported that *D. suzukii* oviposits in fresh fruit on trees and the *suzukii*-associated type of *G. brasiliensis* attacks *D. suzukii* larvae occurring in fruit on trees (Asplen et al. 2015; Kasuya et al. 2013; Mitsui et al. 2006). However, it is unknown whether *D. suzukii* and its parasitoids also inhabit the undergrowth layer and oviposit in fallen fruit or attack host larvae in

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fallen fruit. This is partly because spatial distributions of *D. suzukii* adults and the parasitoids were investigated by collections using traps baited with banana or by monitoring their emergence from fruit collected from fields (Asplen et al. 2015; Daane et al. 2016; Kasuya et al. 2013). Here we studied distributions and oviposition sites of *D. suzukii* and its parasitoids on wild cherry trees by net sweeping over the foliage and undergrowth layers of wild cherry trees, monitoring their emergence from fresh and fallen wild cherry fruit, and using oviposition trap baited with cherry fruit in Tokyo, central Japan.

Methods

Study sites

The study was conducted in Naganuma Park (about 0.4 km^2) located at the northern slope of a hillock in a suburb of Tokyo (35.7°N, 139.7°E). This park is mostly covered with a forest mainly composed of deciduous trees, but a narrow area at the boundary of residential area has been changed to lawn. Two species of wild cherry are found in this park, *Cerasus jamasakura* (Siebold ex Koidzumi) and *C. speciosa* (Koidzumi). The former mainly occurs in the forest area, while the latter mainly occurs in the lawn area. Fruit of these two cherry species are major natural resources of *D. suzukii* from late spring to early summer in central Japan (Mitsui et al. 2006, 2010). In the lawn area, *C. speciosa* trees extend branches downward to the height of 1 m above the ground, and therefore net sweeping in the foliage layer can be easily performed. Such cherry trees suitable for sweeping survey are not frequently found except in avenues or parks that are managed for viewing.

Wild cherry trees bear fruit in mid-April, and fruit changes color from green to yellow, red, and dark red with ripening. Green and yellow fruit is unripened and hard, and dark red ripened fruit sporadically falls on the ground. Females of *D. suzukii* rarely oviposit in green and yellow fruit (Mitsui et al. 2006).

Collection of adult individuals by net sweeping

To investigate distributions of adults of *D. suzukii* and its parasitoids on wild cherry trees, collections were carried out using insect net (360 mm in diameter). In the lawn area, net sweeping was performed over leaves and fruit in the foliage layer (1–3 m in height) and the undergrowth layer of *C. speciosa* trees for 2 h in the morning (from 5:15 to 7:15) and 2 h in the afternoon (from 13:40 to 15:40) on June 4, 2015 and for 4 h from 10:00 to 14:00 on June 2, 2016. The collection was not quantitative; net sweeping was carried out for more than 3 h in the foliage layer and less than 1 h in the undergrowth layer in both years. In the forest area, net sweeping was carried out only in the undergrowth layer approximately for 1 h in the evening on June 2, 2016. In the forest area, the foliage layer was out of the reach of insect nets (i.e., more than 3 m in height).

Emergence of drosophilids and parasitoids from field-collected wild cherry fruit

To investigate the oviposition site selection of *D. suzukii* and its parasitoids, their emergence from fruit occurring in foliage and undergrowth layers was examined. In the lawn area, fruit was collected from *C. speciosa* trees on June 4, 2015 and June 2, 2016 (10 trees in 2015 and 3 trees in 2016): from each tree, 100 fruits were collected from the foliage layer (1–3 m above the ground), and 100 fallen fruits were collected from the ground. In the forest area, three and four samples, each with 100 fallen fruits, were collected under *C. jamasakura* trees in 2015 and 2016, respectively. Collected fruit were placed in plastic boxes with paper towel (100 fruits per box), and kept at room temperature (22–25 °C) in the laboratory. Insects that emerged were identified to species level.

In addition, whether *D. suzukii* and some other drosophilid species oviposit in fallen wild cherry fruit was investigated in the forest area. All wild cherry fruit were removed from the undergrowth areas of wild cherry trees on May 27 and June 8, 2000, and newly fallen fruit in the undergrowth areas were marked with woody toothpicks on May 28 and June 9. Thereafter, 30 fruits were collected every day or on alternate days for 7 or 8 days. Emergence of drosophilids from these fruit was examined as described above. In this study, emergence of parasitoids was not examined.

Collection by oviposition trap

To examine occurrence and oviposition of *D. suzukii* at different heights, collections were conducted using wire-mesh traps baited with *C. speciosa* fruit (flesh + seed = 0.68 g on average) and "bing" cherry fruit [*Cerasus avium* (L.): 8.15 g on average] in 2015. Dark red ripened *C. speciosa* fruit was collected from trees in the lawn area and checked for oviposition by *D. suzukii*; if *D. suzukii* females oviposited, one or more small hole(s) with egg filaments were present on the fruit skin. Fruit without *D. suzukii* eggs were used as bait. "Bing" cherry fruit were bought from a shop. Six traps, each containing 20 *C. speciosa* fruit and two "bing" cherry fruit, were prepared; fruit in four traps were intact, whereas those in two traps were injured by knife. The reason why injured fruit were used in addition to intact fruit is to examine oviposition of non-*D. suzukii* drosophilids which prefer fermenting or decayed fruit (see "Results"); injury would enhance fermentation of decay.

Four trees were chosen for setting traps, two in the lawn area and two in the forest area. Traps were set at three heights, low (i.e., 50–100 mm above the ground), mid (2 m), and high (5.6–14.0 m). Traps with intact fruit were set on all trees, and traps with injured fruit were set on a tree in both the lawn and forest areas. Traps were set on May 31 or June 1, 2015. After a week, fruit in the traps were collected, placed in vials with tissue paper, and kept at room temperature in the laboratory. When insects emerged, they were collected and identified to species level.

Species identification

Drosophilids and parasitoids were usually identified to species level by morphology. However, *G. brasiliensis* in this area consists of three types, the *suzukii*-associated, *lutescens*-associated, and widely distributed types, which are hardly discriminated by morphology (Kasuya et al. 2013; Nomano et al. 2017). These types were discriminated by the nucleotide sequence of the *CO1* gene (Kasuya et al. 2013; Nomano et al. 2017).

DNA was extracted from each specimen using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Amplification was preformed with a pair of primers, 5′-GGT CAACAAATCATAAAGATATTGG-3′ (LCO) and 5′-TAA ACTTCAGGGTGACCAAAAAATCA-3′ (HCO) (about 600 bp) (Folmer et al. 1994). All amplifications were performed in 23-μL reaction volumes containing 1.3 mM $MgCl_2$, 0.042 mM dNTP, 2.6 μM primers, 0.042 U Ampli *Taq* DNA polymerase, and 2.4 μL 10× PCR buffer. PCR profile consisted of one cycle of denaturation (94 °C for 10 min), 35 cycles of denaturation (94 °C for 1 min), annealing (50 °C for 1 min), and extension (72 °C for 1.5 min), followed by one cycle of final extension at 72 °C for 12 min. Amplified products were diluted to 1 ng/μL and used as sequencing templates.

For all sequence reactions, Big Dye Terminator Cycle Sequencing Kit (ABI) was used. Sequencing was carried out with a 3100 Genetic Analyzer (ABI), utilizing the same primers used for PCR amplification.

In this study, the nucleotide sequence of the *CO1* gene of *Ganaspis* individuals collected by net sweeping in 2016 and a part of *Ganaspis* individuals that emerged from fruit in 2016 were determined. The *CO1* sequences of *Ganaspis* samples collected in 2015 were not determined. For the obtained sequences, genetic distances from the sequences of individuals of the *suzukii*-associated, *lutescens*-associated, and widely distributed types obtained in our previous study (Nomano et al. 2017) were calculated with Kimura's two-parameter model and pairwise deletion using Mega7 software (Kumar et al. 2016).

Statistical analysis

Statistical analyses were performed on the number of *D. suzukii* that emerged from fruit and the rate of parasitism by *G. brasiliensis* in the lawn area. Data from the forest area were not analyzed because sample size was small. The number of *D. suzukii* that emerged from fruit was compared between the undergrowth and foliage layers and between 2015 and 2016 by a generalized linear model (GLM) with a log link function and a Poisson error distribution (the number of *D. suzukii* was a response variable, and layer and year were explanatory variables). The rate of parasitism by *G. brasiliensis* was calculated on the basis of the assumption that the wasp emerged from only *D. suzukii* or from both *D. suzukii* and *D. lutescens* and compared between the undergrowth and foliage layers and between 2015 and 2016 by a GLM with a logit link function and a binomial error distribution (the rate of parasitism was a response variable, and layer and year were explanatory variables). The analysis was performed with R 3.3.2 (R Development Core Team 2016).

Results

Collection of adult individuals by net sweeping

In the lawn area, *D. suzukii* was collected from both foliage and undergrowth layers, whereas other drosophilids were almost exclusively collected from the undergrowth layer (Table 1). *Drosophila suzukii* was also collected from the undergrowth layer in the forest area as well as other drosophilids.

Five species of *Drosophila* parasitoids were collected, mostly in the lawn area (Table 1). In these parasitoid samples, 14 individuals assigned as *Ganaspis brasiliensis* by morphology were determined for the nucleotide sequence of the *CO1* gene. As a result, four were assigned as the *suzukii*-associated type, five were the *lutescens*-associated type, and five were the widely distributed type. All of these three types were collected from both layers. On the other hand, *Leptopilina japonica* and *Asobara* sp. TK1 were collected only from the foliage layer.

Emergence of drosophilids and parasitoids from field-collected fruit

Among drosophilids that emerged from wild cherry fruit, *D. suzukii* was the most abundant (Table 2). The GLM analysis on the number of *D. suzukii* that emerged per fruit showed that the effects of year and layer and the interaction of year and layer were significant (year: likelihood ratio $\chi^2 = 1540.59$, $df = 1$, $p < 0.001$; layer: likelihood ratio $\chi^2 = 81.74$, $df = 1$, $p < 0.001$; interaction: likelihood ratio

Table 1 Number of adult drosophilids and parasitoids collected by net sweeping in the foliage (F) and undergrowth (U) layers in lawn and forest

	Lawn				Forest
	F		U		U
	2015	2016	2015	2016	2016
Drosophilids					
D. suzukii	28	11	95	41	41
D. subpulchrella	0	1	0	0	1
D. lutescens	0	0	17	25	121
D. rufa	0	0	3	8	103
D. biauraria	0	0	3	6	6
D. triauraria	0	0	5	25	0
D. sternopleuralis	0	0	2	1	68
D. immigrans	0	0	1	7	16
Others	0	4	4	19	39
Parasitoids					
Ganaspis brasiliensis	3[a]		1[a]		
Suzukii-associated type		3		1	0
Lutescens-associated type		2		2	1
Widely distributed type		2		3	
Leptopilina japonica	1	2	0	0	0
Asobara sp. TK1	4	16	0	0	0
A. rufescens	0	1	0	1	0
Trichopria sp.	0	2	0	0	0

[a] The type was not determined

$\chi^2 = 180.9$, $df = 1$, $p < 0.001$); i.e., the number of emergence was much larger in fruit from the foliage layer than in those from the undergrowth layer in 2015, but the difference was quite small in 2016. Other drosophilids such as *D. lutescens* Okada, *D. rufa* Kikkawa & Peng, and *D. triauraria* Bock & Wheeler mostly emerged from fruit collected from the undergrowth layer.

Drosophila suzukii emerged most abundantly from fruit collected on the day of fall and its emergence decreased when fruit were left on the ground longer (Fig. 1). In contrast, *D. lutescens*, *D. rufa*, and *D. immigrans* Sturtevant did not emerge from newly fallen fruit but emerged from fruit that were left on the ground for a day or longer after fall (Fig. 1).

A total of 513 parasitoids emerged from samples of wild cherry fruit (50 individuals in 2015 and 463 individuals in 2016). Among them, *G. brasiliensis* was most abundant, followed by *Asobara japonica* Belokobylskij and *Leptopilina japonica* Novković & Kimura (Table 2). According to the nucleotide sequences of the *CO1* gene for 19 *G. brasiliensis* individuals that emerged in 2016, 18 were the *suzukii*-associated type and one from the undergrowth layer was the *lutescens*-associated type. The rate of parasitism by *G. brasiliensis* was 0.077 (0.076) in pooled samples for the foliage layer and 0.073 (0.054) in those for the undergrowth layer in 2015, and 0.197 (0.189) in those for the foliage layer and 0.274 (0.199) in those for the undergrowth layer in 2016 (the rates given out of parentheses were based on the assumption that the wasp emerged only from *D. suzukii*, and those in parentheses were based on the assumption that the wasp emerged from both *D. suzukii* and *D. lutescens*). According to the GLM analysis, the effect of year was significant (likelihood ratio $\chi^2 = 30.0$, $df = 1$, $p < 0.001$), but the effects of layer and the interaction of layer and site were not significant (layer: likelihood ratio $\chi^2 = 0.012$, $df = 1$, $p = 0912$; interaction: likelihood ratio $\chi^2 = 0.818$, $df = 1$, $p = 0.366$), irrespective of either assumption.

Collection by oviposition trap

No drosophilid individual emerged from intact fruit bait placed at the low height in the lawn area (Table 3). However, *D. suzukii* emerged from both intact and injured fruit placed at all heights. In contrast, *D. lutescens* emerged mostly from fruit placed at low height. Only two individuals of *A. japonica* emerged from fruit placed at low height in the forest area (data not shown).

Discussion

In the study area, *D. suzukii* is the only drosophilid species that inhabits and oviposits in the foliage layer (Tables 1, 2). Keesey et al. (2015) observed that *D. suzukii* females are more attracted to leaf odors compared to *D. melanogaster* Meigen females that prefer fermenting fruit, and they considered that this is the key characteristic that leads *D. suzukii* to forage not only the undergrowth layer but also the foliage and canopy layers. In this study, however, the number of *D. suzukii* adults collected from the foliage layer was not large, although net sweeping was performed for more than 3 h (i.e., the net was swung some hundred times); i.e., only 28 in 2015 and 11 in 2016 (Table 1). It is not certain whether the rate of fruit attacked by *D. suzukii* observed in this study, i.e., 0.36 (2015)–1.79 (2016) eggs per fruit (Table 2), can be explained by this adult density. It is possible that net sweeping in the foliage layer is not effective to collect *D. suzukii* adults or other insects.

The number of *D. suzukii* that emerged from wild cherry fruit was significantly larger in those collected from the foliage layer than in those from the undergrowth layer at least in 2015 (Table 2). In addition, the number of *D. suzukii* that emerged from fallen fruit decreased when they were left on the ground longer (Fig. 1). If *D. suzukii* females oviposit eggs in fallen fruit in addition to fresh fruit on trees, the number of *D. suzukii* that emerged from fruit would be larger in those collected from the undergrowth layer than

Table 2 Number (mean ± SD) of drosophilids and parasitoids that emerged per 100 fruits

	Lawn				Forest	
	Foliage layer		Undergrowth layer		Undergrowth layer	
	2015	2016	2015	2016	2015	2016
Drosophilids						
D. suzukii	36.0 ± 37.8	179.3 ± 45.2	5.1 ± 3.4	168.0 ± 64.1	30.0 ± 33.8	40.5 ± 20.6
D. lutescens	0.3 ± 0.7	9.0 ± 12.3	1.9 ± 3.0	86.3 ± 104.0	17.7 ± 6.7	34.3 ± 20.9
D. rufa	0	0	0	1.7 ± 1.5	0.7 ± 1.2	10.8 ± 7.4
D. biauraria	0	0	0	1.7 ± 1.5	0	0.3 ± 0.5
D. triauraria	0	0	0.6 ± 0.7	10.3 ± 11.2	0	0.3 ± 0.5
Others	0.3 ± 0.7	0	0.3 ± 0.9	1.0 ± 1.0	0	0.5 ± 0.6
Parasitoids						
G. brasiliensis	3.0 ± 2.9	44.0 ± 53.6[a]	0.4 ± 0.7	63.3 ± 29.7[b]	1.0 ± 1.7	0.8 ± 1.0
L. japonica	0.1 ± 0.3	1.0 ± 1.0	0	9.0 ± 5.3	0	2.0 ± 2.7
Asobara sp. TK1	0.5 ± 0.8	0.3 ± 0.6	0	0.3 ± 0.6	0	0
Asobara sp. TK2	0	0	0	0.3 ± 0.6	0	0
Asobara japonica	0	0	0	28.0 ± 40.7	0.3 ± 0.6	2.8 ± 2.5
A. rufescens	0	0	0	0.3 ± 0.6	0	0
A. rossica	0	0	0	0.3 ± 0.6	0	0

In the lawn area, 100 *C. speciosus* fruits were collected from both foliage and undergrowth layers of 10 cherry trees in 2015, and three trees in 2016. In the forest area, three and four samples, each with 100 *C. jamasakura* fruits, were collected in 2015 and 2016, respectively

[a] The nucleotide sequence of the *CO1* gene was determined in ten individuals, and all were identified as the *suzukii*-associated type

[b] Among nine individuals whose nucleotide sequence of the *CO1* gene was determined, eight were identified as the *suzukii*-associated type and one was the *lutescens–rufa–biauraria*-associated type

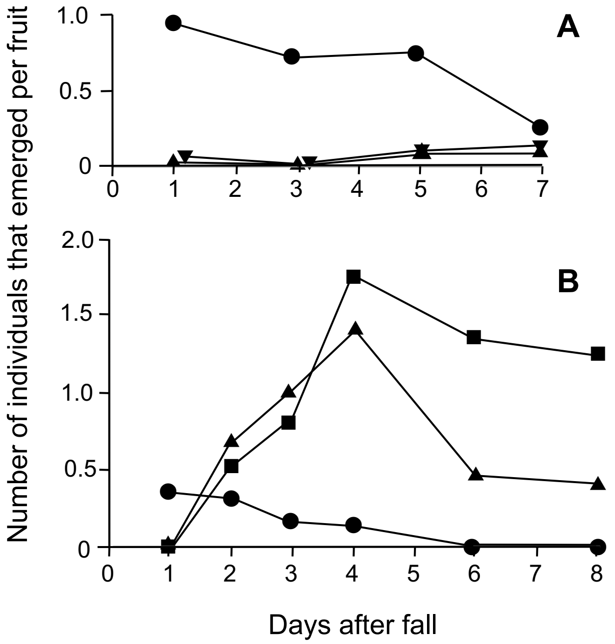

Fig. 1 Numbers of *D. suzukii* (filled circle), *D. lutescens* (filled triangle), *D. rufa* (filled downward triangle) and *D. immigrans* (filled square) that emerged per wild cherry fruit. Fruit that fell on the ground on May 28 (**a**) and June 9 (**b**) in 2000 were marked by toothpicks, and 30 fruits were collected every day or on alternate days for 7 or 8 days after fall. Collected fruit were placed in plastic boxes and the number of drosophilids that emerged from these fruit was examined in the laboratory

in those from the foliage layer and increase if fruit were left on the ground longer. In fact, *D. lutescens*, *D. rufa*, and *D. immigrans* that oviposit in fallen fruit increased in fruit that were left on the ground longer. Therefore, it is assumed that *D. suzukii* females rarely oviposit eggs in fallen fruit, although they frequently occur in the undergrowth layer. However, *D. suzukii* oviposited in fresh and injured cherry fruit that were placed close to the ground (Table 3). In addition, *D. suzukii* oviposits in fresh fruit placed in vials or bottles (Kasuya et al. 2013; Mitsui et al. 2006). These results suggest that *D. suzukii* females oviposit in fruit placed on or near the ground. They may avoid oviposition in fallen fruit that have started fermentation or decay. Further study is needed on this issue.

In the study area, the major parasitoid that attacked *D. suzukii* larvae in wild cherry fruit is the *suzukii*-associated type of *G. brasiliensis*, a specialist of *D. suzukii* (also see Kasuya et al. 2013). The rate of parasitism by this parasitoid did not significantly differ between larvae in fresh fruit collected from the foliage layer and those in fallen fruit collected from the undergrowth layer (Table 2). If *D. suzukii* does not oviposit in fallen fruit as mentioned above, this result suggests that this parasitoid also rarely attacks *D. suzukii* larvae in fallen fruit. Although the sample size was small, adults of this species were more frequently collected in the foliage layer (3 individuals in 2016) than in

Table 3 Number of drosophilids that emerged from fruit bait in oviposition traps at high, middle, and low heights of wild cherry trees in lawn and forest areas

	Lawn			Forest		
	High	Middle	Low	High	Middle	Low
Intact fruit (two sites each in lawn and forest areas)						
D. suzukii	20	14	0	3	5	6
D. lutescens	0	0	0	0	0	26
D. immigrans	0	4	0	0	0	0
Injured fruit (one site each in lawn and forest areas)						
D. suzukii	3	4	6	1	1	3
D. lutescens	0	0	92	1	1	10
D. rufa	0	0	3	0	0	1

Intact and injured *C. speciosa* fruit and "bing" cherry fruit were used as bait

the undergrowth layer. On the other hand, a somewhat larger number of *Asobara* sp. TK1 adults were collected from the foliage layer (16 individuals in 2016) (Table 1), but only few individuals of this wasp emerged in this study (Table 2). Further survey on the distribution of adult wasps is needed to understand their ecology.

Although sample size was small, adult wasps of the *suzukii*-associated type of *G. brasiliensis*, *Asobara* sp. TK1, and also *Leptopilina japonica* were collected almost entirely from the foliage layer. On the basis of the present results on the distributions of these parasitoids and *D. suzukii*, some implications for the control of *D. suzukii* are obtained. First, in the practice of integrated management, pesticide application to the foliage layer should be avoided, because specialist parasitoids mainly forage there. Second, fallen fruit should be removed from fruit farms, because *D. suzukii* larvae occur in them but they were rarely attacked by specialist parasitoids. However, this is a preliminary study based on field collection at one locality. Further study is needed to deepen our knowledge on ecology of *D. suzukii* and its parasitoids.

In the collection by oviposition trap in the lawn area, no drosophilid emerged from intact cherry fruit placed at the low height, although many drosophilids emerged from injured fruit placed at the low height. This difference may be attributable to the difference in the occurrence of ant [*Formica japonica* (Mochoulsky)]: many ants were observed in the traps with intact fruit but only few ants were observed in the traps with injured fruit. It has been reported that ants are one of the important mortality factors for drosophilid larvae (Lewis and Worthen 1992; Worthen et al. 1993). Ant predation may be an important factor to reduce *D. suzukii* populations.

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