




SHORT COMMUNICATION

The bumblebee *Bombus ardens ardens* (Hymenoptera: Apidae) visits white clover in orchards before Oriental persimmon blooms

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Abstract

Flowers on the ground of orchards can provide substantial resources for wild pollinators of orchard trees. Few studies, however, have examined the relative importance of groundcover flowers to orchard pollination by analyzing pollen on the body surface of pollinators. Oriental persimmon trees bloom within the longer blooming period of white clover, which is occasionally found as a flowering plant on the ground of persimmon orchards in Japan. The present study compared the insect species assemblage collected on persimmon flowers with that on clover. Before persimmon bloomed, *Bombus ardens ardens* and *Apis cerana japonica* were the major visitors of clover flowers. Once persimmon bloomed, the former was the most abundant bee that visited persimmon flowers over the flowering period. *Apis mellifera* was captured only on clover flowers. We found numerous clover pollen grains on the body surface of bumblebees captured on persimmon flowers, but far fewer persimmon pollen grains on bees that visited clover. These findings show that *B. ardens ardens* utilized the clover flowers under the orchards before persimmon bloomed.

Key words: *Diospyros kaki*, floor vegetation, groundcover plants, pollen, *Trifolium repens*.

Pollination service for fruit production is provided by a greater diversity of pollinators, including wild insects, than previously understood (Garibaldi *et al.* 2013, 2016). The European honeybee, *Apis mellifera* L. (Hymenoptera: Apidae), is the representative domesticated pollinator in worldwide fruit production; the species is highly effective due to numerous workers in each hive (Morse 1991). However, introducing honeybees and other managed bees into farms may damage local ecosystems and negatively affect the biodiversity of native wild pollinators (e.g., Huryñ 1997). Therefore, enhancing the pollination service performed by diverse wild pollinators seems to be the most sustainable method for several crops (Hoehn *et al.* 2008; Albrecht

et al. 2012); for example, the fruit set of coffee can be predicted by the number of wild pollinating species (Klein *et al.* 2003). Thus, it is becoming increasingly important to investigate wild pollinators' contribution to fruit production (Bretagnolle & Gaba 2015).

Flowers from native, cultivated, and alien species (Sargent & Ackerly 2008) around fruit trees can provide considerable floral resources to wild pollinators outside of the generally short flowering periods of fruits (Nicholls & Altieri 2013). Therefore, in addition to natural vegetation around orchards (Garibaldi *et al.* 2011; Norfolk *et al.* 2016), understory plants in orchards can also serve as important floral resources for wild pollinators (Kammerer *et al.* 2016). Karamaouna *et al.* (2019) reported that groundcover with suitable flowering species could be part of a sustainable olive crop management system, providing food for pollinating insects. However, if a trade-off exists between wild bees' flower-visiting frequency to fruit trees and that to groundcover plants, then increasing groundcover plants may cause a decline in pollination success of the fruits. A reliable

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way to investigate the effect of groundcover plants is to compare the pollen grains attached on the body surface of pollinators captured on the flowers of crops with those captured on surrounding vegetation.

A study by Nikkeshi *et al.* (2019) revealed that the wild bumblebee *Bombus ardens ardens* Smith (Hymenoptera: Apidae) is the most important pollinator of Oriental (or Japanese) persimmon, *Diospyros kaki* Thunb. (Ericales: Ebenaceae), in Hiroshima, Japan. In one of the orchards at the study site, white clover, *Trifolium repens* L. (Fabales: Fabaceae), was the dominant groundcover vegetation. This legume has been studied as a promising cover crop in orchards for regulating weeds, conserving water, providing nitrogen, and improving tree growth in apple orchards (Granatstein & Mullinix 2008), as well as suppressing aphids in apple orchards and psyllids in pear orchards by providing resources for the pests' natural enemies (Haley & Hogue 1990; Rieux *et al.* 1999). Leguminous cover crops such as *T. repens* are also bee-attractant plants (Lee-Mäder *et al.* 2020). Preliminary observations at our study site showed that a variety of insects including *B. ardens ardens* visited the flowers of *T. repens*, suggesting that this cover crop provides floral resources to pollinators of *D. kaki*.

The main objective of this study was to compare the flower visitors on *D. kaki* with those on *T. repens*. The insect species assemblages were identified by capturing flower visitors on both flower species from pre-anthesis until post-anthesis of *D. kaki*, and the pollen grains on the body surface of insects were then identified and counted to assess which insect species are involved in the pollination of *D. kaki*. We also discussed whether the insects that had visited the *T. repens* flowers switched to the floral resources of *D. kaki* when the trees started blooming.

Flower visitors were captured on *T. repens* and *D. kaki* in persimmon orchards at the Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organization (Hiroshima, Japan) in 2018 (for the procedures, see Appendix S1). No domesticated pollinators, such as *A. mellifera*, were placed in the orchards. Of the 399 insects captured on *T. repens* flowers, 396 were identified to the species level based on morphology and, if necessary, confirmed by using *CO1* DNA barcoding (Tables S1 and S2). The sequences were deposited in the DNA Data Bank of Japan (DDBJ accession numbers LC500071–LC500094). In total, 63 *Apis cerana japonica* Radoszkowski (Hymenoptera: Apidae) and 89 *A. mellifera* were captured on the flowers of *T. repens*, and 22 and 0, respectively, were captured on those of *D. kaki* on 17, 21, and 24 May (Table S1). The ratios of the numbers of these honeybee species captured on the two plant species differed significantly

(Fisher's exact test, $P < 0.0001$). Likewise, syrphid flies (Diptera: Syrphidae), which consisted of *Eupeodes corollae* (Fabricius), *Melanostoma mellinum* (L.), *Sphaerophoria indiana* Bigot, and *Sphaerophoria macrogaster* (Thomson), were collected only from the flowers of *T. repens* (Table S1). *Lasioglossum scitulum* (Smith) (Hymenoptera: Halictidae) was the only species in the genus that was captured on the flowers of *T. repens*, whereas *Lasioglossum japonicum* (Dalla Torre), *Lasioglossum mutillum* (Vachal), *Lasioglossum occidens* (Smith), and *Lasioglossum proximatium* (Smith) were also captured on the flowers of *D. kaki* (Table S1). The insect community captured on *T. repens* flowers on the five collection dates (10, 17, 21, and 24 May and 1 June 2018) was significantly different from that captured on *D. kaki* flowers on the three collection dates (one-way PERMANOVA nested by collection date; d.f. = 1, $F = 6.36$, $P = 0.019$). Non-metric multidimensional scaling illustrated the clear difference between the two insect communities that visited *T. repens* and *D. kaki* (Fig. 1). Due to its bell-shaped flowers and hard petals, the availability of *D. kaki* flowers for insects is restricted by body size and proboscis length (Miura 1982). Bumblebees are reported to prefer bell-shaped flowers, such as blueberry and blue heath (Macfarlane 1992; Kasagi & Kudo 2003). It is noteworthy that no *A. mellifera* was captured on

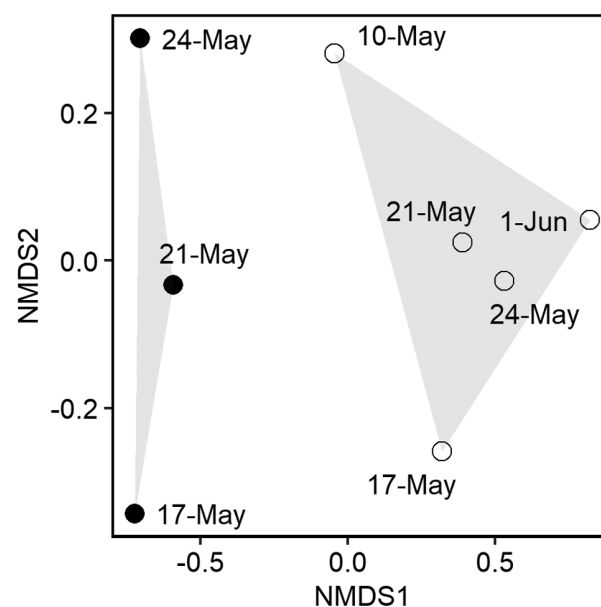


Figure 1 Non-metric multidimensional scaling (NMDS) plot showing the degree of similarity of insect communities that visited flowers of *Trifolium repens* (open circles) on five dates and those of *Diospyros kaki* (closed circles) on three dates in 2018.

D. kaki flowers over the flowering period, suggesting that this species was attracted by *T. repens* more than by *D. kaki*.

The proportion of the total number of bees in each functional group (at the species level for honeybees) captured on the flowers of *T. repens* and *D. kaki* are shown in chronological order in Fig. 2. For bumblebees, 192 individuals captured on both flowers were identified as *B. ardens ardens*, with one exception, *Bombus ignitus* Smith (Hymenoptera: Apidae). On 10 May, before *D. kaki* began to bloom, *A. cerana japonica* and *B. ardens ardens* were the major bee species captured on the flowers of *T. repens* (Fig. 2a). From 17 to 24 May, when *D. kaki* bloomed, *B. ardens ardens* was consistently dominant (>60%) among the bees captured on the flowers of *D. kaki* (Fig. 2b), while this species gradually decreased on the flowers of *T. repens* (Fig. 2a). On 1 June, when *D. kaki* had finished blooming, the bumblebee was not captured on the flowers of *T. repens*, and *A. mellifera* represented 89.4% of all captured bees. These data suggest that *B. ardens ardens* did not return to the flowers of *T. repens* when the flowering period of *D. kaki* terminated, even though *T. repens* was still in bloom. This bumblebee might have found other floral resources outside the orchard; *Deutzia crenata* Siebold et Zucc. (Cornales: Hydrangeaceae), which is potentially visited by bumblebees (Inoue *et al.* 2008), bloomed simultaneously with *D. kaki* around the orchards. Alternatively, colonies may have collapsed at the end of the breeding season.

The average number of pollen grains on the body surface of each functional group captured on the flowers of *T. repens* on 21 and 24 May is shown in Table 1. Pollen grains were counted after cutting away each sample's hind legs to exclude pollen loads. The

functional groups (at the species level for bumblebees and honeybees) used for the analysis (*A. cerana japonica*, *A. mellifera*, *B. ardens ardens*, and small bees) had large average numbers (>20,000) of *Trifolium* pollen grains. A smaller number of *Diospyros* pollen grains was found on the body surface of *B. ardens ardens*. In addition to *Trifolium* and *Diospyros* pollen, *Vicia* and *Vitis* pollen grains ($\leq 3,000$ and $\leq 5,000$, respectively) were detected on the body surface of some of the functional groups.

The average number of pollen grains on the body surface of each functional group captured on the flowers of *D. kaki* from 16 to 24 May was also calculated (Table 1). The numbers of *Diospyros* pollen grains on each functional group were described previously (Nikkeshi *et al.* 2019). Considerable numbers (average >8,000) of *Trifolium* pollen grains were found on the surface of *B. ardens ardens*, carpenter bees, and small bees. In addition to *Diospyros* pollen, *B. ardens ardens* possessed pollen of *Actinidia*, *Trifolium*, *Triodanis*, *Vicia*, and *Vitis*, which appeared to be pollen of *Actinidia deliciosa* (A.Chev.) C.F.Liang & A.R.Ferguson (Ericales: Actinidiaceae), *T. repens* (if not, *Trifolium dubium* Sibth. or *Trifolium pratense* L.), *Triodanis perfoliata* (L.) Nieuwl. (Asterales: Campanulaceae), *Vicia sativa* subsp. *nigra* (L.) Ehrh. (Fabales: Fabaceae), and *Vitis* spp. (Vitales: Vitaceae), respectively, based on the list of the flowering plants in and around the orchard (Table S3). This observation suggests that *B. ardens ardens* forages on a relatively wide variety of flower resources. In contrast, *A. cerana japonica* captured on *D. kaki* flowers possessed pollen of only two genera, *Diospyros* and *Trifolium*, implying that it visited the targeted plant species consecutively.

The numbers of pollen grains of *Trifolium* and *Diospyros* on the body surface of *B. ardens ardens* captured

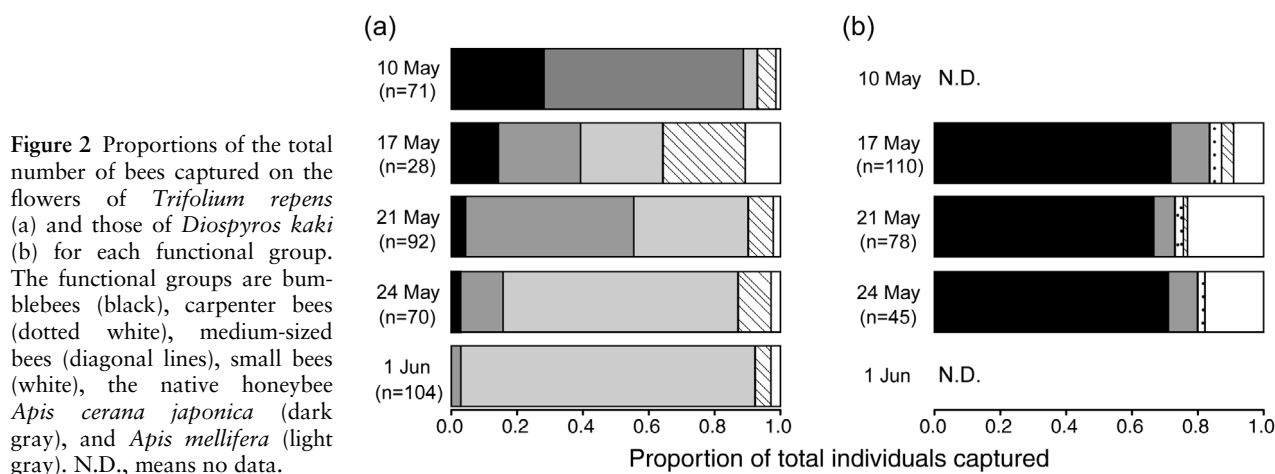


Table 1 Pollen grains on the body surface of flower visitors captured on the flowers of *Trifolium repens* and *Diospyros kaki*

Plant species	Functional group of flower visitors ^a	No. of analyzed individuals	No. of pollen grains of each plant genus ^b (average ± standard deviation)							
			<i>Actinidia</i>	<i>Diospyros</i>	<i>Lonicera</i>	<i>Trifolium</i>	<i>Triodanis</i>	<i>Vicia</i>	<i>Vitis</i>	Others
<i>T. repens</i>	<i>Apis cerana japonica</i>	5	0	0	0	45,232 ± 21,561	0	1,304 ± 2,021	0	8 ± 18
	<i>Apis mellifera</i>	4	0	0	0	20,630 ± 19,070	0	300 ± 353	280 ± 560	0
	<i>Bombus ardens ardens</i>	4	0	60 ± 95	0	29,230 ± 48,537	0	160 ± 219	40 ± 80	0
	Carpenter bees	0	—	—	—	—	—	—	—	—
	Medium-sized bees	0	—	—	—	—	—	—	—	—
<i>D. kaki</i>	Small bees	3	0	0	0	31,680 ± 20,930	0	253 ± 284	2,027 ± 3,204	0
	<i>Apis cerana japonica</i>	10	0	367 ± 468	0	53 ± 92	0	0	0	0
	<i>Apis mellifera</i>	0	—	—	—	—	—	—	—	—
	<i>Bombus ardens ardens</i>	20	852 ± 2,086	24,580 ± 27,071	0	8,615 ± 17,625	195 ± 872	2 ± 9	48 ± 86	40 ± 60
	Carpenter bees	4	0	24,305 ± 22,146	4,975 ± 9,883	8,095 ± 11,303	0	0	25 ± 50	90 ± 66
Medium-sized bees	3	0	12,547 ± 15,095	0	400 ± 500	0	0	0	0	
Small bees	4	0	8,733 ± 15,510	0	14,480 ± 27,891	0	0	788 ± 1,575	50 ± 100	

^aDescribed at the species level only for honeybees and bumblebees.

^bThe most probable species for each genus other than *Diospyros* and *Trifolium* is *Actinidia deliciosa* (kiwifruit; planted at the study site), *Lonicera japonica* (Japanese honeysuckle; weed), *Triodanis perfoliata* (common Venus' looking-glass; weed), *Vicia sativa* subsp. *nigra* (narrow-leaved vetch; weed), and *Vitis* spp. (Asian common wild grape; weed and grape planted at the study site), based on the list of plant species in bloom in and around the orchard (Table S3).

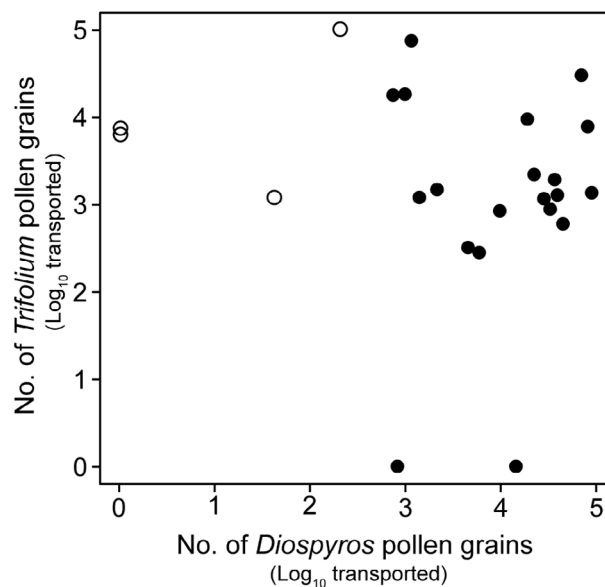


Figure 3 Numbers of pollen grains of *Trifolium* and *Diospyros* on the body surface of *Bombus ardens ardens* captured on the flowers of *Trifolium repens* (open circles) and on those of *Diospyros kaki* (closed circles).

on flowers of *T. repens* (4 individuals) and on flowers of *D. kaki* (20 individuals) were plotted (Fig. 3). The individuals from *T. repens* possessed no or small numbers of *Diospyros* pollen grains, whereas most of the ones from *D. kaki* possessed large numbers of *Trifolium* pollen grains. These findings demonstrate that *B. ardens ardens* had often visited the flowers of *T. repens* before visiting the flowers of *D. kaki* at our study site.

Both the number and the proportion of *B. ardens ardens* among all bees captured on the flowers of *T. repens* gradually decreased during the observation period, whereas this pollinator was dominant among those on the flowers of *D. kaki* throughout its flowering period. In addition, by identifying and counting pollen grains, the most prevalent pollen grains on the body surface of this bumblebee proved to be of two genera, *Diospyros* and *Trifolium*. Thus, it was conceivable that *B. ardens ardens* had visited the flowers of *T. repens* and switched its targeted floral resource to *D. kaki*. This was supported by the observation that most *B. ardens ardens* individuals captured on the flowers of *D. kaki* possessed large numbers of *Trifolium* pollen grains in addition to *Diospyros* ones.

In the present study, it was highly likely that *B. ardens ardens* foraged for *T. repens* flowers before the anthesis of *D. kaki* and for *D. kaki* flowers during its blooming period. The flowers of *D. kaki* might have been more attractive than those of *T. repens* for *B. ardens ardens*. Alternatively, competition with honeybees for *T. repens*

flowers might explain why *B. ardens ardens* gave more priority to *D. kaki* as a floral resource. In contrast, *A. mellifera* continued to forage for *T. repens* flowers after the anthesis of *D. kaki*. This difference in foraging strategy between the bee species is consistent with a report that bumblebees were quicker to abandon the degrading food source than were honeybees (Townsend-Mehler *et al.* 2011). To identify the switching mechanism of *B. ardens ardens* from *T. repens* to *D. kaki*, it would be necessary to compare the following before and after *D. kaki* anthesis: (i) the quantity and quality of the two floral resources; and (ii) visitation frequency of this bumblebee on both flowers.

Our results indicate that the groundcover *T. repens* attracts and supports the pollinator *B. ardens ardens* before the anthesis of *D. kaki*. Twenty individuals of *B. ardens ardens* were captured on *T. repens* flowers before *D. kaki* bloomed, suggesting that the orchard was attractive as a feeding site even before the anthesis of fruit trees; this bumblebee prefers to nest in forest (including secondary forest) and open lands at the foot of mountains (Katayama 2007; Ushimaru *et al.* 2008) and very likely lived in such locations around the orchard. However, *T. repens* flowers might compete with *D. kaki* flowers for pollinators and reduce pollination of the crop. In fact, negative effects of *T. repens* flowers on crop pollination in kiwifruit, apple, and cherry orchards have been reported (Clinch 1984; MacRae *et al.* 2005; Holzschuh *et al.* 2012). Therefore, further studies should examine whether growing *T. repens* on the ground of *D. kaki* orchards has a positive effect on sustaining the local population of *B. ardens ardens* and any significant effect on pollination of *D. kaki* by this species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Experimental procedures.

Table S1. Species compositions of visitors captured on the flowers of *Trifolium repens* and *Diospyros kaki* in 2018.

Table S2. Identification of 27 individuals (Andrenidae, Colletidae, Eumenidae, Halictidae, Ichneumonidae, Megachilidae, and Syrphidae) captured on the flowers of *Trifolium repens*.

Table S3. Plant species in bloom in and around the *Diospyros kaki* orchard.