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., Huryn 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 COI 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 mutilum (Vachal), Lasioglossum occidens (Smith), and Lasioglossum proximatum (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...
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 (≤3,000 and ≤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           | Functional group of flower visitors | No. of analyzed individuals | No. of pollen grains of each plant genus (average ± standard deviation) |
|-----------------|-------------------------------------|-----------------------------|--------------------------------------------------------------------------|
| *T. repens*     | Apis cerana japonica                | 5                          | 11.0 ± 20.93 ± 202.71                                                   |
|                 | Apis mellifera                      | 4                          | 0.8 ± 9.5                                                               |
|                 | Bombus ardens ardens                | 4                          | 0.6 ± 9.5                                                               |
|                 | Carpenter bees                      | 0                          | 0.2 ± 9.5                                                               |
|                 | Medium-sized bees                   | 10                         | 3.7 ± 4.66                                                             |
|                 | Small bees                          | 4                          | 0.2 ± 9.5                                                               |
| *D. kaki*       | Apis cerana japonica                | 4                          | 8.4 ± 2.96 ± 24.58 ± 27.07                                              |
|                 | Apis mellifera                      | 4                          | 0.2 ± 9.5                                                               |
|                 | Bombus ardens ardens                | 20                         | 4.8 ± 4.95 ± 4.97 ± 4.97                                               |
|                 | Carpenter bees                      | 4                          | 0.2 ± 9.5                                                               |
|                 | Medium-sized bees                   | 3                          | 0.2 ± 9.5                                                               |
|                 | Small bees                          | 4                          | 0.2 ± 9.5                                                               |

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).

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* before visiting the flowers of *D. kaki* at our study site.

Alternatively, competition with honeybees for attractive than those of *T. repens* for *B. ardens ardens*.
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. 2003; 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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REFERENCES

Albrecht M, Schmid B, Hautier Y, Müller CB (2012) Diverse pollinator communities enhance plant reproductive success. *Proceedings of the Royal Society B: Biological Sciences* 279, 4845–4852.

Bretagnolle V, Gaba S (2015) Weeds for bees? A review. *Agronomy for Sustainable Development* 35, 891–909.

Clinch PG (1984) Kiwifruit pollination by honey bees 1. *Tauranga* observations, 1978–81. *New Zealand Journal of Experimental Agriculture* 12, 29–38.

Garibaldi LA, Steffan-Dewenter I, Kremen C et al. (2011) Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecology Letters* 14, 1062–1072.

Garibaldi LA, Steffan-Dewenter I, Winfree R et al. (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339, 1608–1611.

Garibaldi LA, Carvalheiro LG, Vaissière BE et al. (2016) Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. *Science* 351, 388–391.

Granatstein D, Mullinix K (2008) Mulching options for northwestern organic and conventional orchards. * HortScience* 43, 45–50.

Haley S, Hogue E (1990) Ground cover influence on apple aphid, *Aphis pomi* DeGeer (Homoptera: Aphididae), and its predators in a young apple orchard. *Crop Protection* 9, 225–230.

Hoehn P, Tscharntke T, Tylianakis JM (2008) Functional group diversity of bee pollinators increases crop yield. *Proceedings of the Royal Society B: Biological Sciences* 275, 2283–2291.

Holzschuh A, Dudenhofer JH, Tscharntke T (2012) Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. *Biological Conservation* 153, 101–107.

Huryn VMB (1997) Ecological impacts of introduced honey bees. *The Quarterly Review of Biology* 72, 273–297.

Inoue MN, Yokoyama J, Washitani I (2008) Displacement of Japanese native bumblebees by the recently introduced *Bombus terrestris* (L.) (Hymenoptera: Apidae). *Journal of Insect Conservation* 12, 135–146.

Kammerer MA, Biddinger DJ, Rajotte EG, Mortensen DA (2016) Local plant diversity across multiple habitats supports a diverse wild bee community in Pennsylvania apple orchards. *Environmental Entomology* 45, 32–38.

Karamaouna F, Kati V, Volakakis N et al. (2019) Ground cover management with mixtures of flowering plants to enhance insect pollinators and natural enemies of pests in olive groves. *Agriculture, Ecosystems and Environment* 274, 76–89.

Kasagi T, Kudo G (2003) Variations in bumble bee preference and pollen limitation among neighboring populations: comparisons between *Phyllosticta caerulea* and *Phyllosticta aleutica* (Ericaceae) along snowmelt gradients. *American Journal of Botany* 90, 1321–1327.

Katayama E (2007) Bumblebees: Unknown Behavior and Life History. Hokkaido University Press, Sapporo. (In Japanese.).

Klein AM, Steffan-Dewenter I, Tscharntke T (2003) Fruit set of highland coffee increases with the diversity of
pollinating bees. *Proceedings of the Royal Society B: Biological Sciences* 270, 955–961.

Lee-Mäder E, Vaughan M, Goldenetz-Dollar J (2020) Agroforestry and cover cropping for pollinators. In: Gemmill-Herren B, Azzu N, Guidotti A (eds) *Towards Sustainable Crop Pollination Services: Measures at Field, Farm and Landscape Scales*, pp 105–126. FAO, Rome.

Macfarlane RP (1992) An initial assessment of blueberry pollinators in New Zealand. *New Zealand Journal of Crop and Horticultural Science* 20, 91–95.

MacRae AW, Mitchem WE, Monks DW, Parker ML (2005) White clover (*Trifolium repens*) control and flower head suppression in apple orchards. *Weed Technology* 19, 219–223.

Miura T (1982) On the hourly change of the pollinator association found in the Japanese persimmon, var. Saijo orchard in day-time. *Bulletin of the Faculty of Agriculture-Shimane University (Japan)* 16, 166–171. (In Japanese with English abstract.)

Morse RA (1991) Honeybees forever. *Trends in Ecology and Evolution* 6, 337–338.

Nicholls CI, Altieri MA (2013) Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. *Agronomy for Sustainable Development* 33, 257–274.

Nikkeshi A, Inoue H, Arai T, Kishi S, Kamo T (2019) The bumblebee *Bombus ardens ardens* (Hymenoptera: Apidae) is the most important pollinator of Oriental persimmon, *Diospyros kaki* (Ericales: Ebenaceae), in Hiroshima, Japan. *Applied Entomology and Zoology* 54, 409–419.

Norfolk O, Eichhorn MP, Gilbert F (2016) Flowering ground vegetation benefits wild pollinators and fruit set of almond within arid smallholder orchards. *Insect Conservation and Diversity* 9, 236–243.

Rieux R, Simon S, Defrance H (1999) Role of hedgerows and ground cover management on arthropod populations in pear orchards. *Agriculture, Ecosystems and Environment* 73, 119–127.

Sargent RD, Ackerly DD (2008) Plant–pollinator interactions and the assembly of plant communities. *Trends in Ecology and Evolution* 23, 123–130.

Townsend-Mehler JM, Dyer FC, Maida K (2011) Deciding when to explore and when to persist: a comparison of honeybees and bumblebees in their response to downshifts in reward. *Behavioral Ecology and Sociobiology* 65, 305–312.

Ushimaru A, Ishida C, Sakai S et al. (2008) The effects of human management on spatial distribution of two bumble bee species in a traditional agro-forestry Satoyama landscape. *Journal of Apicultural Research* 47, 296–303.

**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.