

Community-wide impacts of early season herbivory on flower visitors on tall goldenrod

MITO IKEMOTO,¹ TAKASHI Y. IDA,^{1,*} SHUNSUKE UTSUMI² and TAKAYUKI OHGUSHI¹ ¹Center for Ecological Research, Kyoto University, Otsu, Japan and ²Field Science Center for Northern Biosphere, Hokkaido University Horokanai, Japan

Abstract. 1. The flower visitor community consists not only of pollinators but also of non-pollinators, such as florivores, thieves and predators that attack flower visitors. Although there is increasing evidence that early-season foliar herbivory influences pollinator visitation through changes in floral traits, few studies have explored indirect effects of foliar herbivory on community structure of the flower visitors. We examined how early-season foliar herbivory influences the flower visitor community established in late season.

2. We conducted an inoculation experiment using a lacebug (*Corythucha marmorata*), which is a predominantly herbivorous insect attacking leaves of tall goldenrod (*Solidago altissima*) in Japan.

3. Flower abundance significantly decreased when damaged by the lacebug. The numbers of pollinators, florivores and thieves were positively correlated with flower abundance, whereas predators were not. In response to flower abundance, florivores decreased on damaged plants. On the other hand, thieves increased on damaged plants, and pollinators and predators did not differ between damaged and undamaged plants.

4. When effects of flower abundance were excluded, foliar herbivory still influenced florivores negatively and thieves positively. This implies that factors besides flower abundance may have affected the numbers of florivores and thieves.

5. Community composition of flower visitors on damaged plants significantly differed from undamaged plants, although overall abundance, taxonomic richness and taxonomic evenness were unaffected by foliar herbivory in the early season. It is important to recognise that only evaluating species diversity and overall abundance may fail to detect the significant consequence of early-season herbivory on the flower visitor community.

Key words. Community-wide effect, flower visitor, functional group, herbivory, non-pollinator.

Introduction

Ecological communities are composed of many species with different functions. Flowers are among the important resource bases for shaping communities of arthropods with a wide range of functions, such as pollinators, florivores, thieves and predators that attack flower visitors. The functions of these flower

visitors on plant reproduction vary from positive via pollination to negative via florivory/predation (Strauss & Irwin, 2004). Pollinators increase plant reproductive success by transferring pollen, while florivores and floral larcenist, including thieves and robbers, reduce plant reproductive success by directly consuming viable gametes (e.g. stamen and pistil) and indirectly lowering attractiveness to pollinators (e.g. display and reward; Irwin *et al.*, 2001; McCall & Irwin, 2006). In addition, predators on flowers often decrease plant fitness through interfering with pollinator visitations (Muñoz & Arroyo, 2004; Goncalves-Souza *et al.*, 2008). Although most of the studies exploring flower visitor community focus on pollinators alone, non-pollinators

Correspondence: Mito Ikemoto, Center for Ecological Research, Kyoto University, 509-3-2, Hirano, Otsu, Shiga 520–2113, Japan. E-mail: m.ikemoto@ecology.kyoto-u.ac.jp

*Current address: Faculty of Science, Nara Women's University, Nara 630–8506, Japan.

are very common on flowers (Castro *et al.*, 2013; Zhang *et al.*, 2014).

Recent studies have shown that early-season herbivory on vegetative tissues affects temporally separated pollinator visitations later in the season (Bronstein *et al.*, 2007 for a review). Foliar herbivory in the early part of the season can decrease flower number (Karban & Strauss, 1993; Lehtilä & Strauss, 1997), flower size (Strauss *et al.*, 1996; Mothershead & Marquis, 2000) and pollen production (Quesada *et al.*, 1995; Strauss *et al.*, 1996). Furthermore, herbivory can change chemical compounds of flowers, such as volatile organic compounds (Kessler *et al.*, 2011), and alkaloids in nectar (Adler *et al.*, 2006). These studies demonstrated that herbivore-induced changes in floral traits decrease pollinator visits due to decreased attraction to pollinators. However, few studies have explored such indirect effects of foliar herbivory on a whole flower visitor community, including pollinators and non-pollinators (i.e. florivores, thieves and predators) (but see Smith *et al.*, 2015). It is well known that floral traits influence visitations of pollinators and non-pollinators similarly (e.g. Adler & Bronstein, 2004; Irwin *et al.*, 2004; Strauss & Whittall, 2006) or differently (Junker & Blüthgen, 2008). As leaf herbivory can change a wide variety of floral traits, as mentioned earlier, the early-season leaf herbivory may have a significant impact on abundance and/or species diversity of pollinators and non-pollinators, thereby altering community structure of flower visitors through changes in floral traits.

In this study, we examined whether early-season foliar herbivory by the lacebug, *Corythucha marmorata* Uhler (Hemiptera: Tingidae), can influence flower visitor communities on tall goldenrod, *Solidago altissima* Linnaeus (Asteraceae). In particular, we focused on the indirect effects via flower abundance, because changes in flower abundance are well known to change the number of flower visitors on *Solidago* plants (Genung *et al.*, 2010; Ikemoto *et al.*, 2011; Smith *et al.*, 2015). Specifically, we addressed the following two questions: (i) does foliar herbivory indirectly influence the arthropod visits via changes in flower abundance; (ii) do the effects of foliar herbivory vary among functional groups of flower visitors (i.e. pollinators, florivores, thieves and predators), and thus change the flower visitor community structure?

Materials and methods

Tall goldenrod, *Solidago altissima* L., is a perennial herb and was introduced to Japan from North America around 1900, and thereafter spread throughout Japan (Fukuda, 1982). The growing season in Japan is from March until October, with plants flowering from October until November, and setting seed from November to December. It is self-incompatible, with a highly diverse suite of insect pollinators, including species such as Hymenoptera, Diptera and Coleoptera (Werner *et al.*, 1980; Gross & Werner, 1983). A panicle inflorescence is formed by capitula consisting of pistillate florets for female and complete florets for bisexuals (Fig. 1). Only bisexuals provide nectar and pollen.

The lacebug, *Corythucha marmorata* (Hemiptera: Tingidae), is one of the most abundant herbivores on *S. altissima* in

Japan. It is an exotic insect from North America, and was first recorded in 2000 in Kobe, central Japan (Kato & Ohbayashi, 2009). *Corythucha marmorata* can utilise several plant species, including Asteraceae, Solanaceae and Convolvulaceae (Tsujino *et al.*, 2006). Both adults and nymphs pierce epidermis and feed on mesophyll of *S. altissima* foliage, leading to changes in leaf colour from green to yellow and withering leaves (Root, 1996). Thus, lacebug feeding causes chlorosis, thereby decreasing rates of gas exchange and photosynthesis due to parenchyma damage (Buntin *et al.*, 1996).

Experimental design

We conducted a field experiment in 2010 at a common garden in the Center for Ecological Research of Kyoto University (CER; 34°58'N, 135°57'E) in Otsu, central Japan. To examine the effects of early-season herbivory on floral traits and community structure of associated arthropods, we conducted an inoculation experiment using *S. altissima* and *C. marmorata*. We used plants from five genotypes, collected from a natural habitat within 15 km of CER during spring 2008, and which since then had been grown in the common garden until 2010. From 26 April to 2 May in 2010, we divided rhizomes into 7 cm segments and planted each segment in a pot (10 cm in diameter). In total, 307 ramets from five genets were grown in the greenhouse from 26 April to 4 July.

On 3–4 July, we selected 130 plants and transplanted them individually into bigger pots (20 cm in diameter). We randomly assigned half of the plants to the lacebug herbivory treatment, and the other half were herbivory-free as a control. All of the plants were transferred to four field cages (1.8 m × 1.8 m × 2.0 m), which were covered with a fine non-woven fibre cloth with high light transmissibility (Morishita Inc., Osaka, Japan). Each of two cages was assigned to herbivory treatment or control, and then each cage had 32 or 33 potted plants. On 5 July, four lacebugs (random mixture of adults and nymphs) were inoculated to each plant in the herbivory treatment. The lacebugs were collected from wild populations found on naturally growing tall goldenrods around CER. They could move freely between plants within the field cage. During the treatment, 18 inoculated and 28 uninoculated plants received accidental damage, and they were removed from cages. Subsequently, we obtained 47 damaged and 37 undamaged plants. The herbivory level (i.e. proportion of damaged leaves of each of the experimental plants) did not differ between the two cages (mean ± SD = 60.4 ± 25.5%; generalised linear model, $F_{1,45} = 0.01$, $P = 0.93$). Furthermore, such herbivory intensity is within the range of natural herbivory (24.3–86.0%) in nearby areas of the experimental garden (M. Ikemoto, unpublished). Hereafter, we refer to lacebug-inoculated and uninoculated plants as 'damaged plants' and 'undamaged plants', respectively.

Just before flowering (7–8 October), we placed the 84 experimental plants in the common garden to expose them to flower visitors. We set these potted plants in a 12 × 7 grid at 1 m apart. A single row of seven plants consisted of four damaged plants and three undamaged plants in a randomised order. One

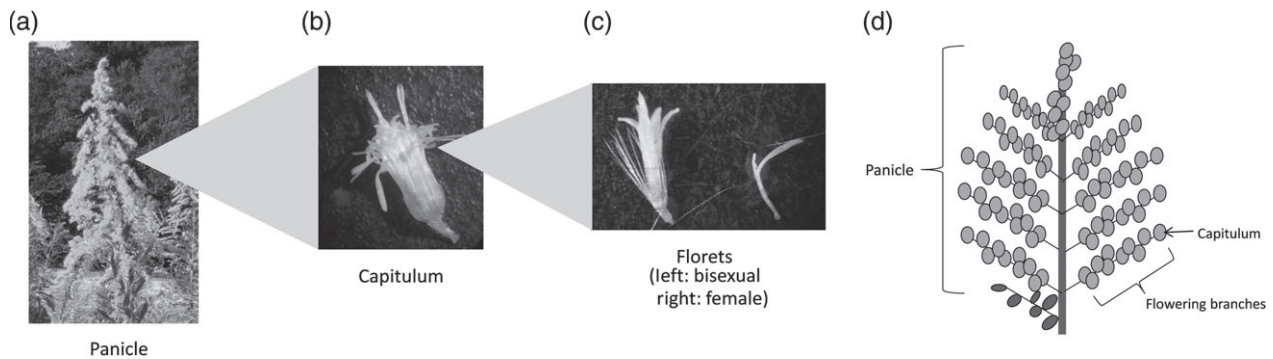


Fig. 1. Schematic presentation of the inflorescence structure of *Solidago altissima*. Panicle (a) consisted of small capitula (b), which contain bisexual florets and female florets (c). We measured the total length of flowering branches per plant (d).

undamaged plant was excluded from the analyses because it had fallen due to a storm.

Estimate of flower abundance

To estimate flower abundance, we measured the length of each peduncle (i.e. flowering branch) on 31 October and 1 November. As tall goldenrod has many small flowers (florets) and we could not count all the flowers, we estimated the total number of florets per plant as follows. First, we measured the length of all flowering branches (Fig. 1d) and calculated the total length of flowering branches per plant. Second, to assess the number of capitula cm^{-1} of flowering branches, we randomly collected four damaged and undamaged plants each, and counted the capitula number of their flowering branches ranging from 1.5 to 4.0 cm. Third, we estimated the number of florets per capitulum by counting the number of florets of all 64 capitula randomly collected from 12 plants (eight damaged and four undamaged plants). Neither the number of capitula cm^{-1} of flowering branches nor the number of florets per capitulum differed between the treatments (generalised linear models, capitula, $F_{1,12} = 1.34$, $P = 0.270$; florets, $F_{1,62} = 0.49$, $P = 0.488$). Then, the total number of florets per plant (i.e. flower abundance) was estimated as follows:

$$\begin{aligned} &\text{Total number of florets per plant} \\ &= (\text{total length of flowering branches, cm}) \\ &\times (\text{mean number of capitula } \text{cm}^{-1} \text{ of flowering branches}) \\ &\times (\text{mean number of florets per capitulum}). \end{aligned}$$

Survey of flower visitors

Censuses of arthropods visiting flowers were carried out from 14 October to 11 November. One person walked around checking all the plants in their entirety and recorded the number and morphospecies of all arthropods that visited inflorescences of the plants. A visit was determined as a single arthropod's contact with inflorescences. For each census, we checked all plants for 2 h day^{-1} with different time periods (10.00–12.00,

11.00–13.00, and 15.00–17.00 hours) on each observation date. We observed flower visitors during the daytime only because nocturnal visitors had not been found in our preliminary survey. In total there were 24 h of observations (three times from 10.00 to 12.00 hours, four times from 11.00 to 13.00 hours, and five times from 15.00 to 17.00 hours). We classified flower visitors into 14 taxonomic groups in terms of taxon and body size: large flies, small flies, syrphids, honeybees, solitary bees, large ants, small ants, butterflies, caterpillars, grasshoppers, stinkbugs, leaf beetles, ladybirds and spiders. Then, these taxonomic groups were classified into four functional groups, i.e. pollinators, florivores, thieves and predators (see Table S1), using behavioural traits of flower visitors with a focus on consequence of pollen transport. First, we referred to predators as arthropods sitting on inflorescences to attack flower visitors. Second, florivores feed exclusively on developing floral buds or flowers, including bracts, sepals, petals, stamens and pistils, as well as pollen and ovules (McCall & Irwin, 2006). Third, pollen-collecting arthropods were classified into pollinators as insects that transfer pollen to pistils without harming flowers (i.e. legitimate pollinators) and thieves that enter flowers non-destructively to consume nectar or pollen but transfer little pollen (Inouye, 1980). In our preliminary experiment, we collected flower visitors moving between flowers and we checked the presence/absence of pollen on their bodies using a stereomicroscope. Although ants carried some pollen, the pollen attached to ants will be inactive due to myrmecacin that is secreted from the metathoracic gland of ants (Beattie *et al.*, 1984). Thus, ants were classified into thieves because pollen transfer does not contribute to plant reproduction.

Statistical analysis

We analysed flower visitors at both taxonomic and functional group levels. We excluded visitors that were observed less than 10 times, such as Chrysopidae sp., Symphyta sp. and Parasitica sp. from all analyses. For each plant, we summed a cumulative number of flower visitors across observations.

We compared flower abundance per plant between damaged and undamaged plants using a generalised linear model (GLM)

with gamma distribution and ln-link function. The number of flower visitors was analysed using GLMMs with herbivory and flower visitor groups (i.e. functional group or taxonomic group) as fully crossed fixed effects, and involved individual plants as a random effect. As the number of flower visitors can be influenced by flower abundance, we analysed the number of flower visitors, considering the effects of flower abundance (as a covariate factor). We also modelled this without the covariate. From these two models, we examined hypotheses that lacebug herbivory influences flower visitor abundance, and that the number of flower visitors varies by herbivory-initiated changes in flower abundance. To facilitate interpretation, we present least-square means of herbivory effects, which adjusted for the effects of other components and their standard errors (Milliken & Johnson, 1984).

The community structure of flower visitors was compared using the following community properties: overall abundance, taxonomic richness, taxonomic evenness and community composition. Overall abundance was the cumulative number of all flower visitors per plant. Taxonomic richness was the sum of the observed number of taxonomic groups per plant. Taxonomic evenness was calculated by Shannon–Wiener's J' (Pielou, 1969). Then, we compared overall abundance, taxonomic richness and taxonomic evenness of flower visitors between damaged and undamaged plants. We used GLM with negative-binomial (ln-link function) for overall abundance and taxonomic richness, and normal distribution (identity-link function) for taxonomic evenness, respectively. Species richness is often dependent on the sample size (Gotelli & Colwell, 2001). Thus, to exclude the effect of abundance on taxonomic richness (i.e. sampling effects), rarefaction analysis was conducted by constructing rarefaction curves with 10 000 iterations (ECOSIM PROFESSIONAL version 1.2, Acquired Intelligence Inc., Kesey-Bear, Pinyon Publishing 2011, 2012, Jericho, VT, U.S.A.). The community compositions of flower visitors on damaged and undamaged plants were compared with permutational multivariate analysis of variance (PERMANOVA) with 10 000 permutations. This analysis was performed by two models with and without flower abundance as a covariate. To summarise the community compositions of arthropods visually, we used non-metric multidimensional scaling analysis (NMDS). For PERMANOVA and NMDS, we used Chao dissimilarity showing insensitivity to difference in sample size (Chao *et al.*, 2005).

PERMANOVA and NMDS were conducted using the package *Vegan* version 2.3-1 (Oksanen *et al.*, 2011) for R statistical software version 3.2.1 (R Core Team, 2015) and all other analyses were done with the GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc, 2013).

Results

Effects of early herbivory on flower abundance

Lacebug herbivory significantly decreased flower abundance ($F_{1,81} = 5.65$, $P = 0.020$). The damaged plants showed 21.1% decrease in flower abundance ($26\,691 \pm 14\,945$, mean \pm SD), compared with undamaged plants ($33\,836 \pm 11\,598$).

Effects of early-season herbivory on flower visitor groups

In total, we observed 1630 flower visitors (Table S1). The flower visitors consisted of pollinators (57.2%), thieves (23.7%), predators (11.7%) and florivores (7.3%). The most abundant taxonomic group was large flies (mainly *Stomorhina obsoleta*), comprising 32.3% of total visitors, followed by small ants (17.1%), syrphids (13.2%) and honeybees (10.3%).

Lacebug herbivory influenced the number of flower visitors differently among functional groups (herbivory \times group: $F_{3,243} = 3.74$, $P = 0.012$, Table 1a). The numbers of pollinators and predators did not differ between damaged and undamaged plants (Fig. 2a,d). The number of florivores decreased by 59.7% but that of thieves increased by 129.0% on damaged plants (Fig. 2b,c), compared with undamaged plants. When we added flower abundance as a covariate to the model, the number of flower visitors was influenced by flower abundance differently in each functional group [ln(flower abundance) \times group: $F_{3,223} = 4.80$, $P = 0.003$, Table 1b]. The numbers of pollinators, florivores and thieves increased with flower abundance, but predators did not (Fig. 3). When accounting for flower abundance, lacebug herbivory still decreased the number of florivores and increased the number of thieves (herbivory \times group in Table 1b; pollinators, $t_{250} = 0.06$, $P = 0.954$; florivores, $t_{298} = 2.69$, $P = 0.008$; thieves, $t_{318} = -4.64$, $P < 0.0001$; predators, $t_{313} = 0.46$, $P = 0.643$).

In the taxonomic group level analysis, herbivory effects on the number of flower visitors were variable among taxonomic groups (herbivory \times group, $F_{13,969} = 2.46$, $P = 0.003$; Table 1c). The number of pollinators, such as large flies, syrphids, honeybees, butterflies and solitary bees, did not differ between damaged and undamaged plants (top panels in Figure S1). The numbers of thieves of small ants and leaf beetles were increased by 148.8% and 244.7% on damaged plants and undamaged plants, respectively (top panels in Figure S2). This is also the case in small flies (thieves) (top panels in Figure S2), although no statistical significance was detected ($t_{1111} = -1.66$, $P = 0.097$). In contrast, the number of florivore caterpillars showed an 83.0% decrease on damaged plants (top panels in Figure S2). When we took flower abundance into account, each taxonomic group responded to the number of flowers in a taxon-specific manner [ln(flower abundance) \times group; herbivory \times group interaction; Table 1d]. The numbers of large flies, honeybees, caterpillars, grasshoppers, small ants, leaf beetles and ladybirds increased significantly with flower abundance (bottom panels in Figures S1 and S2). The numbers of small ants, leaf beetles and small flies were still significantly increased, and the number of caterpillars was decreased, by foliar herbivory when we took flower abundance into account (middle panels in Figure S2).

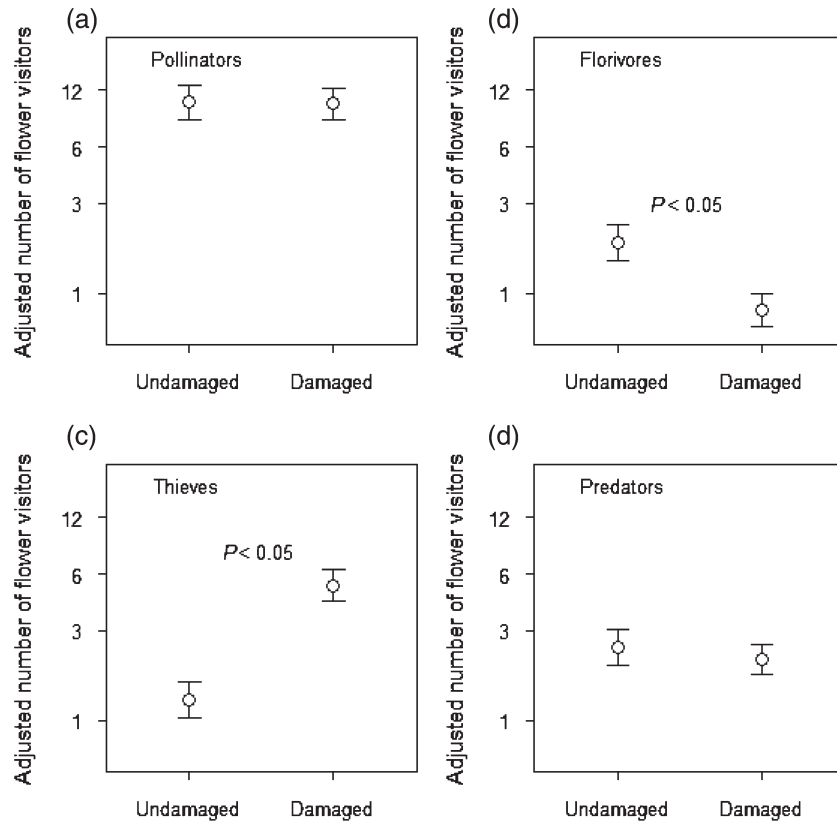
Effects of early-season herbivory on flower visitor community

Overall abundance, taxonomic richness and taxonomic evenness did not differ between damaged and undamaged plants (overall abundance, $F_{1,81} = 0.15$, $P = 0.701$; taxonomic richness, $F_{1,81} = 2.40$, $P = 0.125$; taxonomic evenness, $F_{1,81} = 1.60$, $P = 0.210$, Fig. 4). The obtained 95% confidential intervals of rarefaction curves were overlapped between damaged and

Table 1. Results of generalised linear mixed models for the effects of herbivory (damaged and undamaged plants), group (taxonomic and functional groups), their interactions on the number of arthropods, and plant identity as a random factor.

	Functional group		Taxonomic group	
	(a) Without covariate model	(b) With covariate model	(c) Without covariate model	(d) With covariate model
Herbivory	$F_{1,81} = 0.43$	$F_{1,80} = 0.45$	$F_{1,83} = 0.20$	$F_{1,89} = 1.53$
Group	$F_{3,243} = 23.52^{***}$	$F_{3,221} = 4.90^{**}$	$F_{13,969} = 23.62^{***}$	$F_{13,971} = 2.79^{**}$
Herbivory \times group	$F_{3,243} = 3.74^*$	$F_{3,233} = 9.84^{***}$	$F_{13,969} = 2.46^{**}$	$F_{13,950} = 3.70^{***}$
Ln(flower abundance)	–	$F_{1,79} = 46.09^{***}$	–	$F_{1,92} = 52.54^{***}$
Ln(flower abundance) \times group	–	$F_{3,223} = 4.80^{**}$	–	$F_{13,968} = 2.84^{**}$
Plant identity	$X^2_1 = 53.09^{***}$	$X^2_1 = 16.62^{***}$	$X^2_1 = 58.96^{***}$	$X^2_1 = 12.81^{**}$

The number of arthropods was analysed using two models with or without ln(flower abundance) as a covariate. *** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$.

**Fig. 2.** Effects of herbivory on the number of flower visitors (least-square means \pm SE) of: (a) pollinators, (b) florivores, (c) thieves, and (d) predators. Note the log scale on the Y-axis.

undamaged plants, indicating that the taxonomic richness was not different between damaged and undamaged plants, regardless of sample size. On the other hand, community composition of flower visitors significantly differed between damaged and undamaged plants at both taxonomic and functional group levels (PERMANOVA: taxonomic group, pseudo- $F_{1,81} = 3.13$, $R^2 = 0.037$, $P = 0.022$; functional group, pseudo- $F_{1,81} = 5.97$, $R^2 = 0.069$, $P = 0.017$, Fig. 5). When we performed PERMANOVA including flower abundance as a covariate to assess whether the differences in community composition were brought about by flower abundance or other traits, the herbivory treatment still had

significant effects on community composition between damaged and undamaged plants (taxonomic group, pseudo- $F_{1,80} = 3.36$, $R^2 = 0.040$, $P = 0.015$; functional group, pseudo- $F_{1,80} = 6.64$, $R^2 = 0.077$, $P = 0.013$). On the other hand, flower abundance did not have significant effects on the community composition at taxonomic and functional group levels (taxonomic group, pseudo- $F_{1,80} = 0.92$, $R^2 = 0.011$, $P = 0.484$; functional group, pseudo- $F_{1,80} = -0.31$, $R^2 = -0.004$, $P = 0.809$). This indicates that changes in floral traits other than flower abundance may have resulted in the difference in a community composition of flower visitors between damaged and undamaged plants.

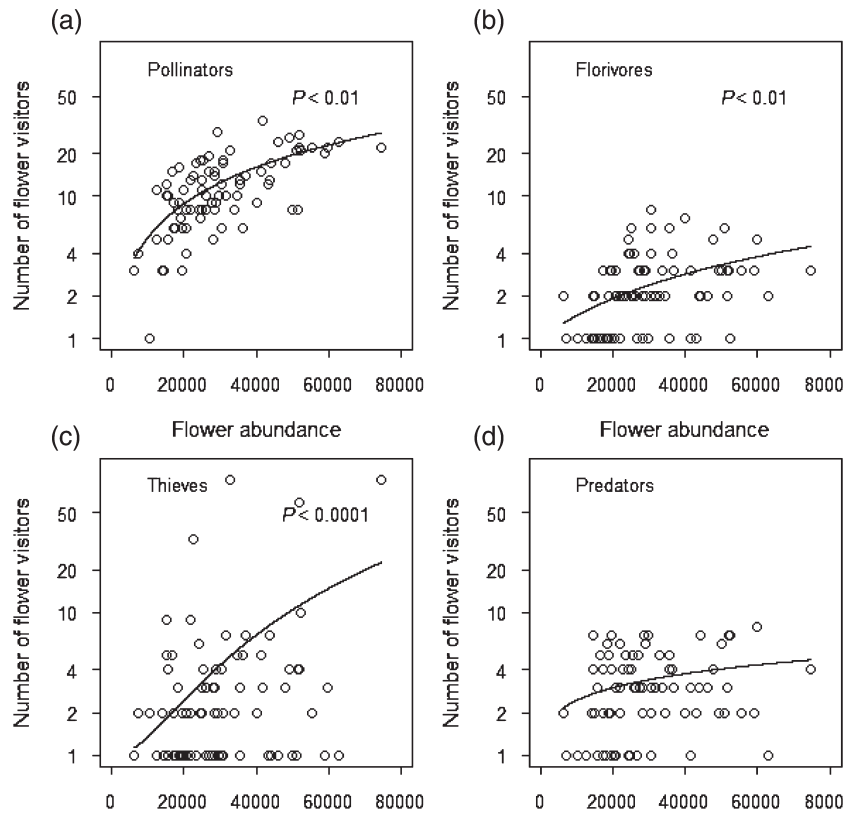


Fig. 3. Relationships between flower abundance and number of: (a) pollinators, (b) florivores, (c) thieves, and (d) predators. Solid lines indicate the regression of flower visitors on flower abundance based on the generalised linear mixed model (GLMM) using residual pseudo-likelihood estimation. The Y-axis indicates the number of visitors + 1. Significance levels (test for $b = 0$) of coefficients of $\ln(\text{flower abundance})$ in the GLMM are shown. Note the log scale on the Y-axis.

Discussion

This study clearly demonstrated that early-season foliar herbivory decreased flower abundance of *S. altissima*. Also, foliar herbivory indirectly influenced the abundance of florivores negatively and thieves positively, which, in turn, altered community composition of flower visitors.

Difference in indirect effects of foliar herbivory on flower visitors

Leaf damage by lacebugs decreased flower abundance of tall goldenrods, which is consistent with Root (1996) documenting that herbivore pressure early in the season decreased inflorescence mass of tall goldenrod. All of the flower visitor groups except predators positively responded to flower abundance (Fig. 3). Previous studies have reported that both pollinators and non-pollinators are largely dependent on flower abundance (Brody & Mitchell, 1997; Lay *et al.*, 2011).

As the lacebug herbivory reduced flower abundance, we can expect that the number of flower visitors would decrease on damaged plants. Contrary to our expectation, the number of pollinators did not decrease on damaged plants, indicating that the early-season herbivory may be less important in determining

the visitation of pollinators and predators. In contrast, the numbers of thieves and florivores were influenced by the lacebug herbivory in a different manner, even after we removed the effect of flower abundance in the model (Table 1). Thus, unknown herbivore-induced changes would reduce plant attractiveness towards florivores, but enhance it towards thieves (Fig. 2b,c). A number of studies reported that foliar herbivory can induce defensive compounds in floral tissues and nectar (Adler *et al.*, 2006; Euler & Baldwin, 1996) and alter sugar composition of nectar (Bruinsma *et al.*, 2014) and floral volatiles (Kessler *et al.*, 2011; Bruinsma *et al.*, 2014). A decrease in florivores on damaged plants may be explained by the induced defensive chemicals, although no studies have examined whether foliar herbivory can induce chemical compounds in goldenrod's tall flowers. On the other hand, there was an increase of thieves on damaged plants. These results suggest that florivores and thieves may respond to different plant cues or the same cues in a different manner. Alternatively, florivores that destroy capitula may decrease the visitation of thieves. However, thief abundance was not negatively related to florivore abundance ($b = 0.05$, $t_{81} = 0.23$, $P = 0.820$), indicating that thieves were not likely to avoid florivores. Further research is needed to understand why florivores and thieves responded to damaged plants differently.

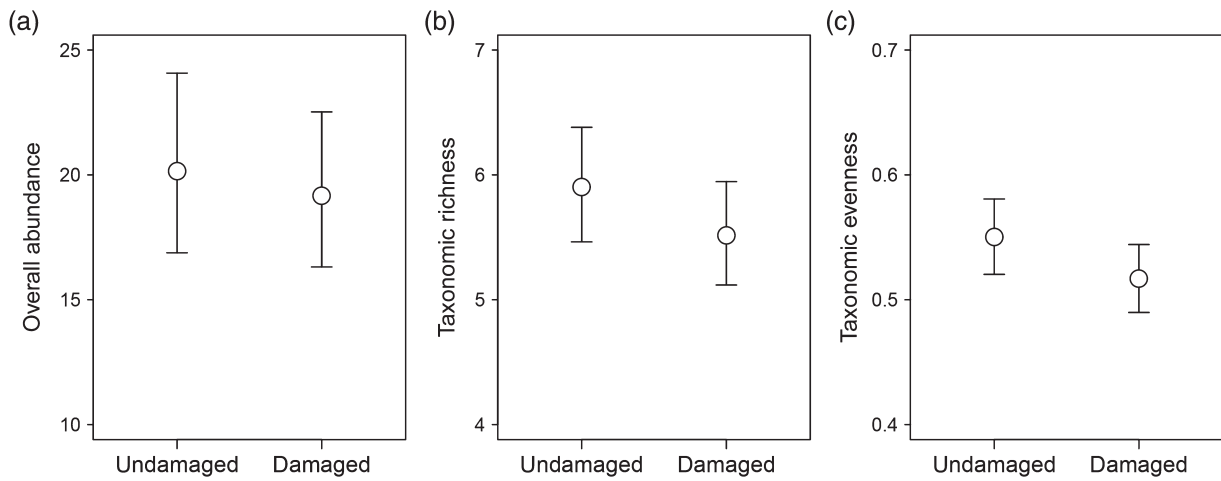


Fig. 4. Effects of herbivory on: (a) overall abundance, (b) taxonomic richness, and (c) taxonomic evenness per plant. Least-square means (\pm SE) on damaged and undamaged plants are presented.

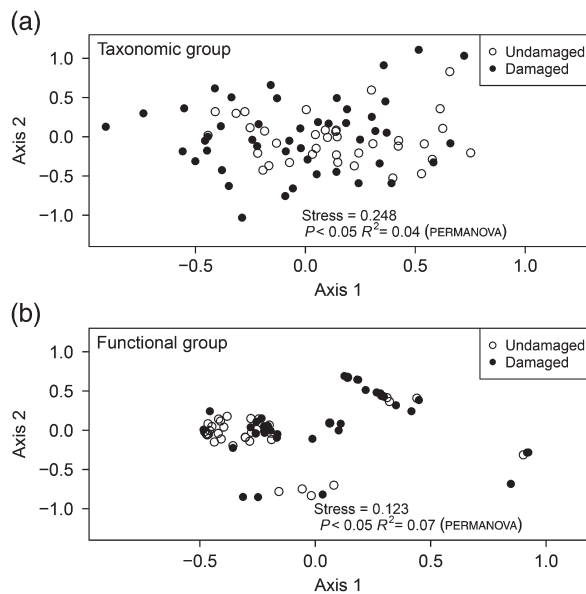


Fig. 5. Non-metric multidimensional scaling analysis ordination of community composition of flower visitors in the taxonomic group (a) and the functional group (b) on damaged and undamaged plants.

Community-wide impacts of foliar herbivory

Early-season herbivory did not influence the overall abundance, taxonomic richness and taxonomic evenness of flower visitors. On the other hand, the community composition was significantly altered by lacebug herbivory at the taxonomic and functional group levels. This pattern is most likely explained by different responses of major flower visitors. As mentioned earlier, some florivores decreased but thieves increased in response to lacebug damage, and thus effects of foliar herbivory on overall abundance, taxonomic richness and taxonomic evenness may be diminished. In contrast to these community metrics, community

composition was significantly altered by the lacebug herbivory, which was not due to changes in flower abundance, suggesting that unknown herbivore-induced plant phenotypes may have influenced the community composition.

There is a growing appreciation of how early-season herbivory influences later community structure through herbivore-induced phenotypic plasticity in plants (Ohgushi, 2005, 2012; Poelman *et al.*, 2008), although most of the past studies have focused on arthropod communities depending on vegetative tissues. Two studies explored the herbivory effects on arthropod communities on *Solidago* plants. Ando *et al.* (2011) found that aphid feeding on *S. altissima* in the early season decreased abundance and changed the community composition of herbivorous insects, but did not change species richness late in the season. In addition, elk browsing on *S. velutina* altered species richness, abundance and community composition of flower visitors (Smith *et al.*, 2015). Both of the studies, coupled with our study, clearly indicate that early-season herbivory undoubtedly influences communities of subsequent arthropods, including herbivores and flower visitors. In addition, our results revealed that the significant consequences of early-season herbivory on flower visitor communities were due to different responses of functional groups of flower visitors.

Our study also suggests that changes in functional groups of flower visitors may influence plant reproductive success. For example, early-season herbivory decreased the number of ovules (i.e. potential reproductive capacity) and florivore visits, which may indirectly decrease reproductive success. The former may be partially compensated by the latter. On the other hand, thieves may decrease seed production of damaged plants, because they indirectly interfered with pollinator visitation due to decreased floral attraction in *Corydalis ambigua* (Zhang *et al.*, 2014). However, such a negative impact of thieves on pollinators may be negligible because pollinator abundance did not differ between damaged and undamaged plants, irrespective of a significant change in thief abundance (Fig. 2c). Thus, future research should explore how herbivore-mediated indirect effects

on flower visitor communities affect plant reproductive success, paying more attention to the different roles of functional groups of flower visitors.

In conclusion, our results clearly indicate that different responses of functional groups to herbivore-induced plant phenotype played a critical role in determining community composition of flower visitors, including both pollinators and non-pollinators, and that traditional biodiversity metrics such as richness and evenness may fail to detect changes in the community structure when functional groups respond differently to foliar herbivory.

Acknowledgements

We would like to thank Y. Ando for providing clones of *S. altissima* and helpful advice on the experimental design and cultivation. We thank A. Kawakita and K. Tsuji for identification of flower visitors. Thanks are also due to R. F. Bode and A. Valtonen for valuable comments on this manuscript. We appreciate the two anonymous reviewers and an associate editor for insightful comments and suggestions on this manuscript. This study was supported by a Grant-in-Aid Scientific Research of the Ministry of Education, Culture, Sports, Science, and Technology (B-25291102, 16H04842) to TO, the Global COE program (A06) to Kyoto University, and Research Fellowship of Japan Society for the Promotion of Science to MI (13J00550).

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12372

Figure S1. Effects of herbivory on the number of pollinators, and relationships between the number of pollinators and flower abundance. UD and D refer to undamaged and damaged plants, respectively. Top and middle panels (a–j) show least-square means (\pm SE) of the number of visitors in the generalised linear mixed model (GLMM) without and with $\ln(\text{flower abundance per plant})$ as a covariate, respectively. Middle panels present the number of pollinators adjusted for the variations in flower abundance. The bottom panels (k–o) show relationships between the number of each pollinator and flower abundance per plant. Solid lines indicate the regression lines of each pollinator and flower abundance derived from the GLMM with negative binomial distribution. Probability indicates significance levels (test for $b = 0$) of coefficients of $\ln(\text{flower abundance})$ in the GLMM. Note that the log scale on the y-axis on the top and middle panels and the Y-axis indicates $\ln(\text{number of visitors} + 1)$ on bottom panels.

Figure S2. Effects of herbivory on the number of florivores, thieves and predators (a–r) and relationships between the number of visitors in each functional group and flower

abundance (panels from s to aa). Explanations are same as Figure S1.

Table S1. Arthropods observed on *Solidago altissima* flowers.

References

- Adler, L.S. & Bronstein, J.L. (2004) Attracting antagonists: does floral nectar increase leaf herbivory? *Ecology*, **85**, 1519–1526.
- Adler, L.S., Wink, M., Distl, M. & Lentz, A.J. (2006) Leaf herbivory and nutrients increase nectar alkaloids. *Ecology Letters*, **9**, 960–967.
- Ando, Y., Utsumi, S. & Ohgushi, T. (2011) Community-wide impact of an exotic aphid on introduced tall goldenrod. *Ecological Entomology*, **36**, 643–653.
- Beattie, A.J., Turnbull, C., Knox, R.B. & Williams, E.G. (1984) Ant inhibition of pollen function: a possible reason why ant pollination is rare. *American Journal of Botany*, **71**, 421–426.
- Brody, A.K. & Mitchell, R.J. (1997) Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. *Oecologia*, **110**, 86–93.
- Bronstein, J.L., Huxman, T.E. & Davidowitz, G. (2007) Plant-mediated effects linking herbivory and pollination. *Ecological Communities: Plant Mediation in Indirect Interaction Webs* (ed. by T. Ohgushi, T. P. Craig and P. W. Price), pp. 75–103. Cambridge University Press, Cambridge, U.K.
- Bruinsma, M., Lucas-Barbosa, D., Broeke, C.J.M., ten Dam, N.M., van Beek, T.A., van Dicke, M. *et al.* (2014) Folivory affects composition of nectar, floral odor and modifies pollinator behavior. *Journal of Chemical Ecology*, **40**, 39–49.
- Buntin, G., Braman, S., Gilbertz, D. & Phillips, D. (1996) Chlorosis, photosynthesis, and transpiration of azalea leaves after azalea lace bug (Heteroptera: Tingidae) feeding injury. *Journal of Economic Entomology*, **89**, 990–995.
- Castro, S., Loureiro, J., Ferrero, V., Silveira, P. & Navarro, L. (2013) So many visitors and so few pollinators: variation in insect frequency and effectiveness governs the reproductive success of an endemic milkwort. *Plant Ecology*, **214**, 1233–1245.
- Chao, A., Chazdon, R.L. & Shen, T.J. (2005) A new statistical approach for assessing similarity of species composition with incidence and abundance data. *Ecology Letters*, **8**, 148–159.
- Euler, M. & Baldwin, I.T. (1996) The chemistry of defense and apparency in the corollas of *Nicotiana attenuata*. *Oecologia*, **107**, 102–112.
- Fukuda, I. (1982) Distributions and population structures of North American plants, *Solidago altissima* L. and *S. gigantea* AIT. introduced into the Japanese Islands. *Science Reports of Tokyo Woman's Christian University*, **32**, 675–690.
- Genung, M.A., Lessard, J.-P., Brown, C.B., Bunn, W.A., Cregger, M.A., Reynolds, W.M.N. *et al.* (2010) Non-additive effects of genotypic diversity increase floral abundance and abundance of floral visitors. *PLoS ONE*, **5**, e8711.
- Goncalves-Souza, T., Omena, P.M., Souza, J.C.S. & Romero, G.Q. (2008) Trait-mediated effects on flowers: artificial spiders deceive pollinators and decrease plant fitness. *Ecology*, **89**, 2407–2413.
- Gotelli, N. & Colwell, R. (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379–391.
- Gross, R.S. & Werner, P.A. (1983) Relationships among flowering phenology, insect visitors, and seed-set of individuals: experimental studies on four co-occurring species of goldenrod (*Solidago*: Compositae). *Ecological Monographs*, **53**, 95–117.

- Ikemoto, M., Ohgushi, T. & Craig, T.P. (2011) Space-dependent effects of floral abundance on flower visitors. *Journal of Plant Interactions*, **6**, 177–178.
- Inouye, D. (1980) The terminology of floral larceny. *Ecology*, **61**, 1251–1253.
- Irwin, R.E., Brody, A.K. & Waser, N.M. (2001) The impact of floral larceny on individuals, populations, and communities. *Oecologia*, **129**, 161–168.
- Irwin, R.E., Adler, L.S. & Brody, A.K. (2004) The dual role of floral traits: pollinator attraction and plant defense. *Ecology*, **85**, 1503–1511.
- Junker, R.R. & Blüthgen, N. (2008) Floral scents repel potentially nectar-thieving ants. *Evolutionary Ecology Research*, **10**, 295–308.
- Karban, R. & Strauss, S.Y. (1993) Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. *Ecology*, **74**, 39–46.
- Kato, A. & Ohbayashi, N. (2009) Habitat expansion of an exotic lacebug, *Corythucha marmorata* (Uhler) (Hemiptera: Tingidae), on the Kii Peninsula and Shikoku Island in western Japan. *Entomological Science*, **12**, 130–134.
- Kessler, A., Halitschke, R. & Poveda, K. (2011) Herbivory-mediated pollinator limitation: negative impacts of induced volatiles on plant–pollinator interactions. *Ecology*, **92**, 1769–1780.
- Lay, C.R., Linhart, Y.B. & Diggle, P.K. (2011) The good, the bad and the flexible: plant interactions with pollinators and herbivores over space and time are moderated by plant compensatory responses. *Annals of Botany*, **108**, 749–763.
- Lehtilä, K. & Strauss, S.Y. (1997) Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. *Oecologia*, **111**, 396–403.
- McCall, A.C. & Irwin, R.E. (2006) Florivory: the intersection of pollination and herbivory. *Ecology Letters*, **9**, 1351–1365.
- Milliken, G.A. & Johnson, D.H. (1984) *Analysis of Messy Data*, Vol. 1. Van Nostrand Reinhold, New York, New York.
- Mothershead, K. & Marquis, R. (2000) Fitness impacts of herbivory through indirect effect on plant–pollinator interactions in *Oenothera macrocarpa*. *Ecology*, **81**, 30–40.
- Muñoz, A.A. & Arroyo, M.T.K. (2004) Negative impacts of a vertebrate predator on insect pollinator visitation and seed output in *Chuquiraga oppositifolia*, a high Andean shrub. *Oecologia*, **138**, 66–73.
- Ohgushi, T. (2005) Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 81–105.
- Ohgushi, T. (2012) Community consequences of phenotypic plasticity of terrestrial plants: herbivore-initiated bottom-up trophic cascades. *Trait-Mediated Indirect Interactions: Ecological and Evolutionary Perspectives* (ed. by T. Ohgushi, O. Schmitz and R. D. Holt), pp. 161–185. Cambridge University Press, Cambridge, U.K.
- Oksanen, A.J., Blanchet, F.G., Kindt, R., Minchin, P.R., Hara, R.B.O., Simpson, G.L. *et al.* (2011) *Package 'vegan'* [WWW document]. URL <http://CRAN.R-project.org/package=vegan> [accessed on 11 January 2016].
- Pielou, E.C. (1969) *An Introduction to Mathematical Ecology*. John Wiley & Sons Inc., Hoboken, New Jersey.
- Poelman, E.H., van Loon, J.J.A. & Dicke, M. (2008) Consequences of variation in plant defense for biodiversity at higher trophic levels. *Trends in Plant Science*, **13**, 534–541.
- Quesada, M., Bollman, K. & Stephenson, A.G. (1995) Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology*, **76**, 437–443.
- R Core Team (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. [WWW document]. URL <http://www.R-project.org/> [accessed on 30 December 2015].
- Root, R. (1996) Herbivore pressure on goldenrods (*Solidago altissima*): its variation and cumulative effects. *Ecology*, **77**, 1074–1087.
- SAS Institute Inc (2013) *SAS OnlineDoc 9.4*. SAS Institute, Cary, North Carolina. [WWW document]. URL: <http://support.sas.com/documentation/94/> [accessed on 30 December 2015].
- Smith, D.S., Lamit, L.J., Lau, M.K., Gehring, C.A., Shuster, S.M. & Whitham, T.G. (2015) Introduced elk alter traits of a native plant and its plant-associated arthropod community. *Acta Oecologica*, **67**, 8–16.
- Strauss, S.Y. & Irwin, R.E. (2004) Ecological and evolutionary consequences of multispecies plant–animal interactions. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 435–466.
- Strauss, S.Y. & Whittall, J.B. (2006) Non-pollinator agents of selection on floral traits. *Ecology and Evolution of Flowers* (ed. by L. D. Harder and S. C. H. Barrett), pp. 120–139. Oxford University Press, Oxford, U.K.
- Strauss, S.Y., Conner, J.K. & Rush, S.L. (1996) Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *The American Naturalist*, **147**, 1098–1107.
- Tsujino, M., Tanaka, H. & Manabu, S. (2006) Seasonal prevalence in occurrence of *Corythucha marmorata* (Uhler) on tall goldenrod and the number of catches of the lace bugs by sticky trap. *Annual Report of the Kansai Plant Protection Society*, **130**, 129–130.
- Werner, P.A., Bradbury, I.K. & Gross, R.S. (1980) The biology of Canatidan weeds. 45. *Solidago canadensis* L. *Canadian Journal of Plant Science*, **60**, 1393–1409.
- Zhang, Y., Zhao, J. & Inouye, D. (2014) Nectar thieves influence reproductive fitness by altering behavior of nectar robbers and legitimate pollinators in *Corydalis ambigua* (Fumariaceae). *Journal of Ecology*, **102**, 229–237.

Accepted 13 October 2016

First published online 10 December 2016

Associate Editor: Andre Kessler