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PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Domestic honeybees affect the performance of pre-dispersal seed predators in an alpine meadow

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Abstract

Flowering plants interact simultaneously with mutualistic pollinators and antagonistic herbivores such that plant-mediated interactions between pollinators and herbivores must exist. Although the effects of herbivores on pollinator behavior have been investigated extensively, the effect of pollinators on herbivore performance has seldom been explored. We hypothesized that insect pollinators could improve the survival and growth of pre-dispersal seed predators by increasing seed production. We tested this hypothesis along three transects radiating from well-established apiaries in an alpine meadow by supplementing pollination in sites close to and distant from apiaries and subsequently examining seed production of the dominant nectariferous plant species *Saussurea nigrescens* (Asteraceae) and the performance of three dominant pre-dispersal seed predators (tephritid fly species). Pollen supplementation (1) significantly increased seed set and mass of developed seed per capitulum (i.e., flowerhead) in the distant but not the close sites, (2) did not change the survival and growth rates of the smaller-bodied species (*Tephritis femoralis* and *Campiglossa nigricauda*) at either site, but (3) improved the performance of the larger-bodied seed predator (*Terellia megalopyge*) at distant sites but not close sites. In addition, the larger-bodied tephritid fly showed higher infestation rates and relative abundance in the close sites than in the distant sites, whereas the smaller-bodied species had lower relative abundances in the close sites and similar infestation rates in both site types. These observations demonstrate contrasting effects of plant mutualists on the performance of antagonists with potential consequences for population sizes of insect herbivores.

Keywords Pollen limitation · *Apis mellifera* · Tephritid fly · Pollinator-herbivore interaction · Insect community

Introduction

Angiosperms interact simultaneously with mutualistic pollinators and antagonistic herbivores (Sauve et al. 2016). Hence, it has been long recognized that plant-pollinator-herbivore interactions are critical to understanding the ecology and evolution of the species involved (Herrera et al. 2002; Fontaine et al. 2011). For example, studies have shown that herbivores may induce changes in concentrations of volatile plant organic compounds or nectar alkaloids, which likely reduce plant attractiveness to pollinators (Adler et al. 2006; Kessler et al. 2011). Herbivores have also been shown to change plant morphology (Strauss et al. 1996; Poveda et al. 2005) and phenology (Pilson 2000) in ways that influence pollinator visitation rates and, consequently, plant fitness (Lucas-Barbosa 2016).

Yet, whether and how plant pollinators affect herbivore performance remains largely unknown. Pollinators can interact with herbivores either directly or indirectly.

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For example, pollinators compete directly for similar floral resources with non-pollinator herbivores, such as pollen beetles (Cook et al. 2004) and nectar-robbing insects (Irwin et al. 2010). It is also possible that pollinators improve seed production and hence indirectly and positively affect the survival and growth of pre- or post-dispersal seed predators by providing food resources. In addition, pollinators may decrease nutrient allocation to vegetative organs or increase resistance to herbivory, thereby negatively affecting the performance of leaf and stem herbivores (Obeso 2002; Aizen et al. 2014).

There is good reason to expect positive effects of plant pollinators on the performance of plant seed predators. Many angiosperm species are partially or totally self-incompatible (e.g., 73% of the 571 Asteraceae species whose breeding systems have been tested, Ferrer and Good-Avila 2007), such that their seed production is positively correlated with pollinator visitation frequencies (Ashman et al. 2004). In a similar vein, the survival, growth, and development of pre-dispersal seed predators largely depend on the amount of seeds produced per flowerhead, as has been demonstrated for tephritid flies in Asteraceae plants (Headrick and Goeden 1998; Aluja and Norrbom 1999). Moreover, the larvae of many seed predators (e.g., tephritid flies), particularly larger-bodied species, are reported to experience food limitation, with negative effects on survival and growth (Fenner et al. 2002; Xi et al. 2017).

We determined whether pollinators could positively affect performance of pre-dispersal seed predators using a pollinator-seed predator-plant (domestic bees-tephritid flies-Asteraceae) system in an alpine meadow in the eastern Tibetan Plateau. Asteraceae species are diverse and abundant in the meadow and domestic honeybees (*Apis mellifera*) and tephritid flies (Diptera: Tephritidae) are the major agents of plant seed production (Mu et al. 2014) and seed damage (Xi et al. 2015, 2016), respectively. This system is ideal because fly oviposition occurs on capitula before pollination, and larval flies subsequently consume developing seeds as they grow and pupate within capitula, thereby excluding the confounding effect of tephritid flies preferring pollinated or unpollinated plant capitula. Moreover, intensive apicultural practices introduce huge numbers of apiaries (Mu et al. 2014), such that there are steep gradients in honeybee density from the apiaries to distant sites. We took advantage of these pollinator density gradients, and conducted pollen supplementation experiments, in which we examined seed set and seed production in a dominant nectariferous Asteraceae species (*Saussurea nigrescens*), as well as the survival rate and body growth of three pre-dispersal tephritid seed predators. The objective of this study was to test whether the high density of domestic honeybees improved plant seed production and the survival and growth of the tephritid flies.

Materials and methods

Study site

This study was conducted in Hongyuan County (32°48′N, 102°33′E; altitude ≈ 3500 m), Sichuan Province, China, on the eastern part of the Tibet Plateau. The climate is characterized by long and cold winters and short and cool summers with a mean annual temperature of 0.9 °C and a mean annual precipitation of 744 mm, which occurs mainly during May to August. The soil is often high in organic content (250 g kg^{-1}), but low in total N (8 g kg^{-1}) and P (5 mg kg^{-1}) (Liu et al. 2011).

The meadows are mostly dominated by sedges including *Kobresia setchwanensis* and Asteraceae species are particularly diverse (> 30 species) in this study site. Previous studies have shown that the pastures are taxonomically diverse in insect herbivores (Xi et al. 2013) and pollinators (Mu et al. 2014), as well as detritivores (Wu et al. 2011).

Yaks (*Bos grunniensare*) are the most important livestock reared by local people. The meadows can be used as summer (mid-June to late September) or winter pastures (October to early June). Our field survey and experiments were carried out in the winter pastures, where yaks are not allowed to graze during the study period.

The study system

We studied a system consisting of a dominant Asteraceae species (*Saussurea nigrescens*), its pre-dispersal seed predators (tephritid flies) and pollinators (primarily domesticated honeybees *Apis mellifera* and native honeybees). *S. nigrescens* is a common perennial herbaceous species, 15–40 cm in height, bearing 2–5 capitula per plant with 37.2 ± 3.4 (n = 30, mean ± SD) florets per capitulum. Its flowering period ranges from late June to late August. Experiments have shown that seed production of this species relies heavily on insect pollinators, and that it does not produce viable seeds when its capitula are bagged (Mu et al. 2014). Bumble bees (*Bombus filchnerae*, *B. humilis*, *B. supremus*), native bees (*Apis cerana*, *Halictus* sp.), and several Lepidoptera and Diptera species are native floral visitors of *S. nigrescens* in our study area. However, because of vegetational changes due to heavy grazing (Xie et al. 2008) and a dramatic increase in apiculture, domestic honeybees (*Apis mellifera*) are currently the predominant pollinators (Mu et al. 2014). In addition, honeybee density is much higher at sites close to apiaries than at sites distant from apiaries. Visitation rates of *A. mellifera* on *S. nigrescens* have been found to be 100 times higher in sites around apiaries (> 3 per capitulum per hour) than in sites

5 km from apiaries (< 0.03 per capitulum per hour) (Mu et al. 2014). The most important nectariferous species is *Saussurea nigrescens*, which accounts for about 80% of the nectar collected by these domesticated honeybees (Mu et al. 2014).

Saussurea nigrescens suffers a great deal of seed damage due to pre-dispersal seed predators, including several tephritid fly species. These flies lay eggs in capitula before flowering, where they hatch, grow, and eventually mature by consuming developing seeds. *Tephritis femoralis*, *Campiglossa nigricauda*, and *Terrellia megalopyge* are three most common tephritid fly seed predators of *S. nigrescens*. *Tephritis femoralis* and *Campiglossa nigricauda* are multivoltine insects, having two to three generations each year. Their first generation is hosted by *Cremanthodium brunneopilosum* (Asteraceae) from late May to early July. Their second generation is hosted by *S. nigrescens*. Some individuals may have a third generation using *Saussurea stella* and *Cremanthodium lineare* as hosts. *Terrellia megalopyge* is a univoltine species that employs *S. nigrescens* as one of its primary hosts. In addition, *Teph. femoralis* and *C. nigricauda* overwinter in soils as adults, whereas *Terr. megalopyge* overwinters in soils as pupae after larvae crawl out of capitula and burrow into soils.

The body size of the three tephritid fly species differs considerably. Field surveys indicate that the fresh mass of female and male adult *C. nigricauda* are 7.6 ± 0.74 mg [$n = 39$; mean ± SD (the same hereafter)] and 5.5 ± 0.66 mg ($n = 23$), respectively. Likewise, the female and male adults of *Terr. femoralis* weigh 6.9 ± 0.54 mg ($n = 42$) and 5.2 ± 0.38 mg ($n = 36$), respectively. In contrast, the adult fresh mass of the female and male adults of *Terr. megalopyge* weigh 10.7 ± 0.62 mg ($n = 33$) and 8.3 ± 0. 74 mg ($n = 20$), respectively. The differences in body mass indicated that these species require significantly different amounts of food to complete their life cycles (see "Results" for details about seed consumption).

All three tephritid fly species are generalist pre-dispersal seed predators of Asteraceae plants. In addition to *S. nigrescens*, host species include *Anaphalis flavescens* (for *Teph. femoralis*), *S. hieracioides* (for *C. nigricauda*), and *S. leontodontoides* (for *Terr. megalopyge*). Nevertheless, *S. nigrescens* is the main host species for each of the three fly species. In turn, the three fly species account for > 80% of the tephritid flies infesting *S. nigrescens* capitula.

Field pollen supplementation experiments

We established three transects along honeybee density gradients. Each transect included two sites, one distant (6 km) and the other close (0.5 km) to a well-established apiary. The soil conditions and vegetation cover were generally similar among all six sites, and community plant species composition was also similar among all six sites (Mu et al. 2014).

At each site, 400 medium-sized capitula (one per plant) were selected while in the bud stage. These capitula were spaced at least 2 m from each other. At each site, 200 capitula were selected for the pollen supplementation treatment. The other 200 capitula were used as controls. Fresh pollen from nearby capitula (within 2 m) was harvested using a small soft paintbrush and parchment paper, and carefully transferred to the stigma of the capitula designated to receive supplementary pollen. Because florets within the capitula mature at different times, pollen supplementation was replicated four times every 3 to 4 days for each of the selected capitula to maximize pollination.

We harvested mature capitula (as indicated by the withering of flower tubes and the emergence of the hair-like modified calyx (i.e., the pappus) and brought them into the laboratory in late August when the pupae of multivoltine flies (*Teph. femoralis* and *C. nigricauda*) were about to finish eclosion and the final instar larvae of univoltine flies (*Terr. megalopyge*) were about to crawl out of capitula. Each capitulum was examined to determine whether it was infested by tephritid flies. The larvae or pupae were collected and were individually placed in 5 mL plastic tubes, so that their development into adults or pupae could be monitored. The newly emerged flies or pupae were refrigerated at 0 °C for 10 min and subsequently weighed on an electronic balance (0.0001 g precision). All emergent tephritid flies were identified to species using morphologically defining traits, and *Terr. megalopyge* pupae were identified by their unique red flat posterior spiracles. Moreover, the sex was recorded for each *Teph. femoralis* and *C. nigricauda* specimen (but not *Terr. megalopyge* because sex of the pupae cannot be determined). We employed molecular techniques to identify dead larvae to species owing to the absence of distinguishing morphological features. The total number of live and dead flies was 819. We calculated the mortality rate for each fly species as the number of dead individuals divided by the total number of individuals of the species.

We extracted DNA from every dead pupa or maggot, and amplified and sequenced their CoI (cytochrome c oxidase I), a DNA barcoding fragment commonly used for invertebrates following the methods of Xi et al. (2017). We then compared the sequences with a reference bank constructed by pooling all the CoI sequences of the tephritid fly species in our study area. The fly species were collected by cultivating pupae obtained from capitula and sweeping over the alpine meadow. As noted, specimens were identified to species using morphological criteria. Subsequently, we extracted DNA from the middle-right legs of each species and then amplified and sequenced their CoI. The reference bank included 31 species. All the sequences of the dead larvae and pupae in this study were successfully aligned to the specific species found in the reference bank.

To quantify the effects of site type and pollen supplementation on plant food availability and potential food limitation to larval tephritid flies, we investigated the seed production of host plants and the seed consumption by tephritid flies. As there is no endosperm within undeveloped seeds, the nutrients available to a larval fly within a capitulum are determined by the number and size of the developed seeds. We counted the number of developed (viable) and undeveloped seeds within 20 medium-sized capitula that were free from the attack of tephritid fly from each of the six sites under both ambient and pollen supplementation treatment respectively, and we calculated seed set as the proportion of developed seeds per capitulum. The seeds from each capitulum were stored at 65 °C for 48 h and subsequently weighed. Individual seed mass was calculated as the total dry mass of developed seeds per capitulum divided by the number of developed seeds. In the case of fly infested capitula, we recorded the total number of seeds (as indicated by the number of floral tubes) per capitulum and the number of unconsumed seeds (including both the developed and undeveloped ones), and subsequently calculated the number of consumed seeds as the total number of seeds minus the number of unconsumed seeds. It should be noted that because developed and undeveloped seeds occurred together within the capitulum (Pers. Obs. XX), we assumed that the larval flies consumed both developed and undeveloped seeds. Finally, capitulum infestation rates were calculated for each fly species as the total number of capitula infested by the fly species divided by the sample size (400 capitula from both ambient and pollen supplementation treatments) from each site.

Field insect survey

For each of the six sites, we surveyed the tephritid fly community using a standard sweeping net (diameter 38 cm) protocol during sunny days between 10:00 and 16:00, when flies were observed to be the most active. We conducted twenty sweepings in each sampling and a total of 5 samplings at each site from early June to late August. All of the tephritid flies collected were placed in plastic tubes and brought to laboratory for identification. Because the sampling efficiency was thought to vary with the person who did the sampling, we used species relative abundance to characterize the fly community. Species relative abundance was calculated as the number of collected individuals (for each of the three species) divided by the total number of tephritid flies.

Data analysis

We used generalized linear effects models (GLMs) to determine the effects of site type (close vs. distant), pollen supplementation, transect and their interactions on seed set (GLM with binomial error) of *S. nigrescens* and total number of seeds per capitulum and number of consumed seeds per infested capitulum for each of the three tephritid fly species (GLM with Poisson error). We included transect as a fixed blocking factor (with three levels) in each model. We then used general linear models (LM) to test whether the total seed mass per capitulum and individual seed mass differed according to site type and pollen supplementation as well as transect.

We employed LMs to determine the effect of fly sex (male vs. female), site type, pollen supplementation and transect on individual adult fresh mass of *Teph. femoralis* and *C. nigricauda*. As the sex of *Terr. megalopyge* pupae could not be identified by morphology, only the effects of site type, pollen supplementation and transect on individual pupal mass of *Terr. megalopyge* were determined. GLMs with binomial error were used to determine the effects of site type, pollen supplementation, and transect on mortality rate for each fly species as well as whether the infestation rates of each fly species differed significantly across transects. In addition, a paired Mann–Whitney *U* test (Wilcoxon rank test) was used to test whether the relative abundances of the three fly species differed significantly between the close and distant sites.

All data analyses were performed in R (R Core Team 2014).

Results

Pollen supplementation experiment

Seed set of *Saussurea nigrescens* was significantly lower in distant than close sites under ambient conditions ($Z = 2.63$, $P = 0.0085$; Fig. 1), and pollen supplementation significantly increased *S. nigrescens* seed set in the distant sites but not the close sites, as indicated by the interactive effect between site types and pollen supplementation treatments ($Z = 4.25$, $P < 0.001$, Fig. 1).

Likewise, developed seed mass per capitulum was significantly lower in the distant sites than the close sites ($F_{1,\,228} = 25.9$, $P < 0.001$) under ambient conditions, and pollen supplementation increased seed mass per capitulum in the distant sites but not in the close sites ($F_{1,234} = 8.07$, $P < 0.001$ for the interaction effect of site type and pollen supplementation; Fig. 1). However, the total number of seeds per capitulum (including both developed and undeveloped seeds) and individual developed seed mass were indistinguishable between site types ($Z = 0.75$, $P = 0.45$ and $F_{1,228} = 0.93$, $P = 0.34$, respectively) and pollen supplementation treatments ($Z = 0.75$, $P = 0.25$ and $F_{1,236} = 1.66$, $P = 0.20$, respectively, Fig. 1).

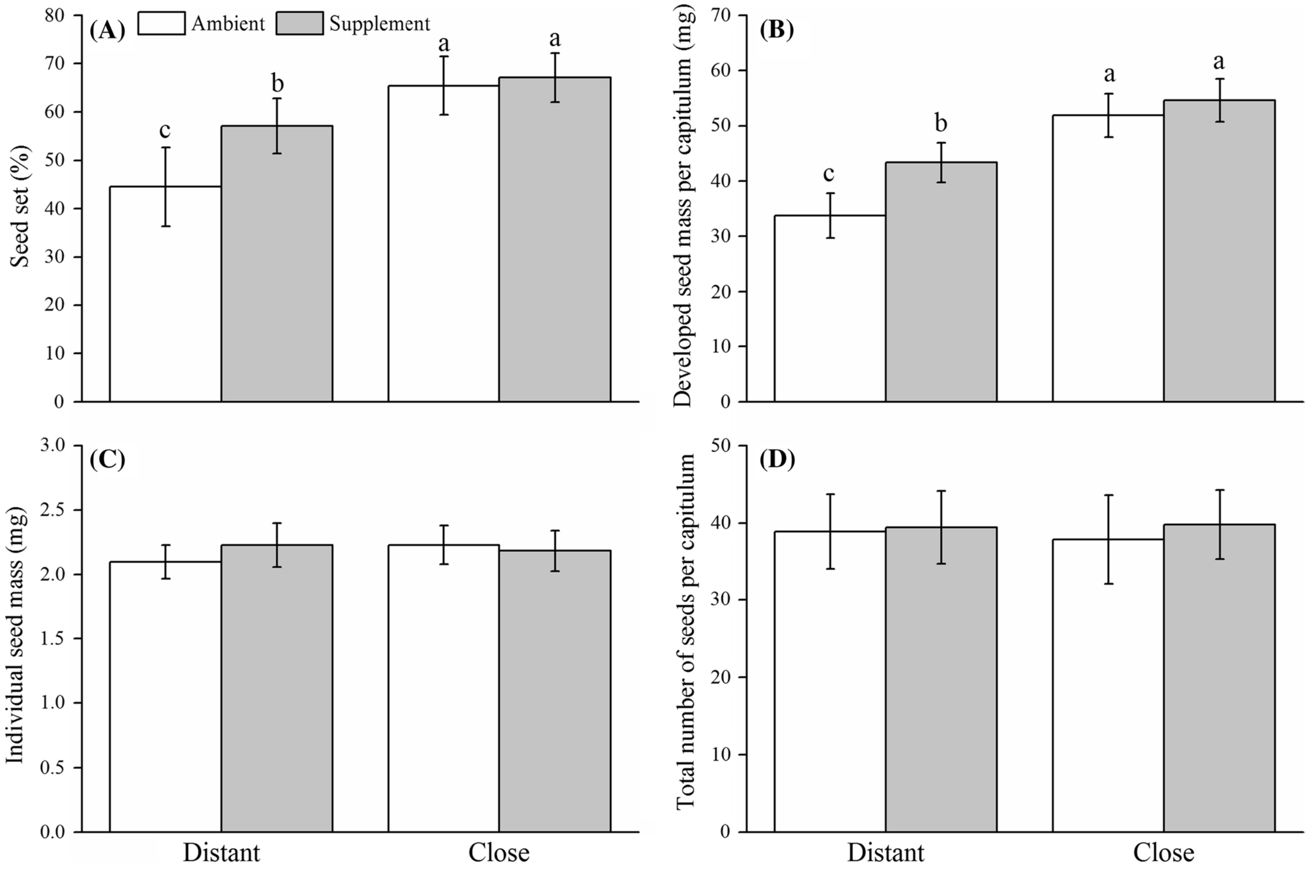

Fig. 1 Seed set (**a**), total seed mass per capitulum (**b**), individual seed mass (**c**), and number of ovules per capitulum (**d**) of *Saussurea nigrescens* under ambient and pollen supplementation treatments at distant and close sites along transects. The different letters above the columns indicate significant differences between treatments ($P < 0.05$; $n = 3$ sites for each treatment). The error bars denote standard errors

The number of consumed seeds (including both developed and undeveloped ones) per infested capitulum for each fly species was significantly larger in the distant sites than the close sites under ambient conditions ($Z > 2.58$, $P < 0.031$, Fig. 2). Pollen supplementation significantly decreased the number of consumed seeds (including both developed and undeveloped ones) per infested capitulum for each fly species in the distant sites but not in the close sites, as indicated by significant interaction effects between site type and pollen supplementation treatment ($Z > 5.77$, $P < 0.044$, Fig. 2).

We found no significant interactive effects between transect and site types or pollen supplementation treatment on seed set ($Z < 0.33$, $P > 0.67$), developed seed mass ($F_{2,228} = 0.70$, $P = 0.50$), or as seed consumption by three tephritid flies ($Z < 1.1$, $P > 0.29$), indicating similar patterns across the three transects.

The fresh adult mass of smaller-bodied fly species (*C. nigricauda* and *Teph. femoralis*) was indistinguishable between site types ($F_{1,204} = 0.63$, $P = 0.43$ and $F_{1,293} = 1.57$, $P = 0.21$, respectively; Fig. 3), whereas fresh pupal mass of the larger bodied fly species (*Terr. megalopyge*) was significantly smaller in the distant sites than the close sites ($F_{1,255} = 4.63$, $P = 0.032$, Fig. 3). Pollen supplementation had no significant effect on the fresh adult mass of the two smaller-bodied species in either the distant or close sites ($F_{1,204} = 0.47$, $P = 0.49$ and $F_{1,293} = 0.71$, $P = 0.40$, respectively; Fig. 3), but it increased fresh pupal mass of the larger-bodied species in the distant sites but not in the close sites, as suggested by the significant interaction effect between pollination and site types ($F_{1,255} = 5.3$, $P < 0.022$, Fig. 3).

The mortality rate was low (< 3%) and indistinguishable between distant and close sites for the two smaller-bodied fly species. However, the mortality rate was significantly higher in the distant than the close sites for the larger-bodied species ($Z = 2.12$, $P = 0.028$, Fig. 4). Pollen supplementation did not significantly affect the mortality rate of the two smaller-bodied species, but it significantly decreased the mortality rate of the larger-bodied species in the distant but not in the close sites, as indicated by the significant interaction effect between site types and pollen supplementation in the larger-bodied species ($Z = 3.46$, $P = 0.0087$, Fig. 4).

In addition, the effects of site type and pollen supplementation treatment on fly performance did not vary among

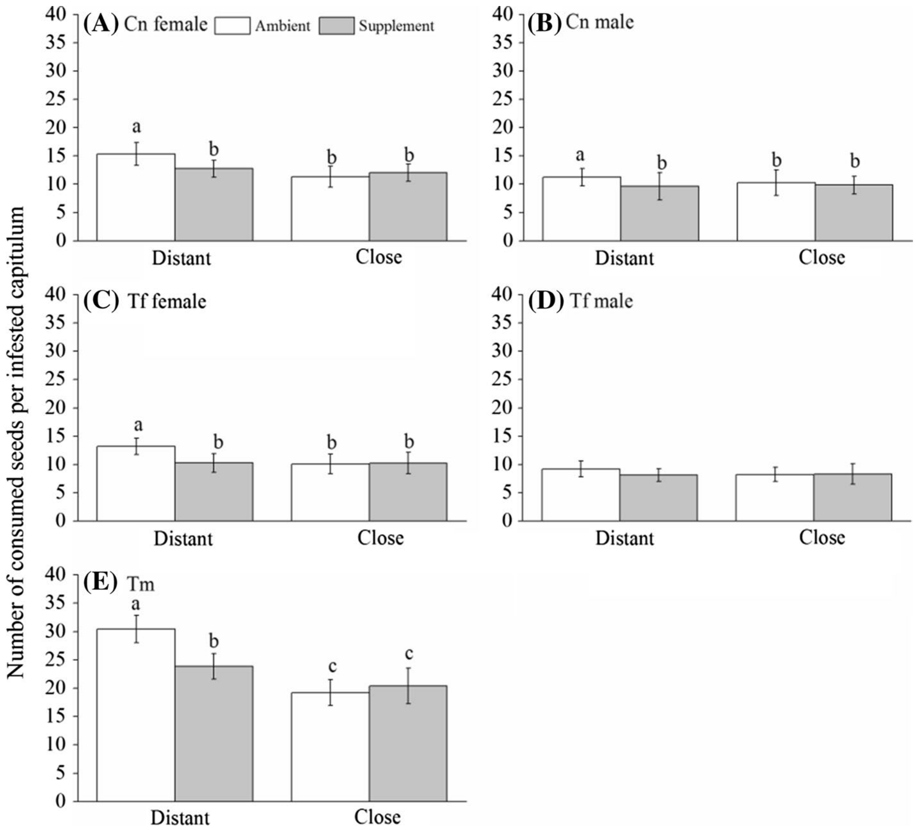

Fig. 2 Number of consumed seeds (including both developed and undeveloped) per infested *Saussurea nigrescens* capitulum by smaller-bodied tephritid flies including *Campiglossa nigricauda* (Cn, **a** and **b**), *Tephritis femoralis* (Tf, **c** and **d**), and larger-bodied *Terellia megalopyge* (Tm, **e**) under ambient and pollen supplementation treatments at distant and close sites along transects. The different letters above the columns indicate significant differences between treatments ($P < 0.05$). The error bars denote standard errors. Sample sizes are 39, 31, 37, and 34 in **a**, 22, 19, 24, and 29 in panel **b**, 48, 66, 47, and 41 in panel **c**, 26, 31, 36, and 22 in **d**, and 40, 45, 87, and 95 in **e** for each treatment, respectively

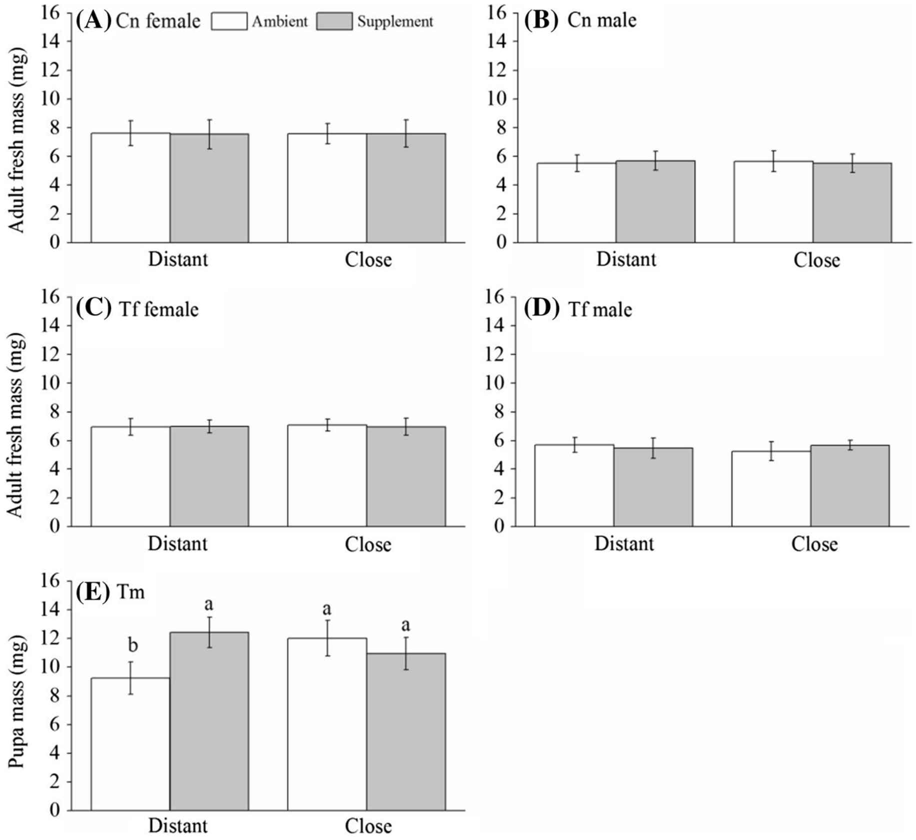

Fig. 3 Adult fresh mass or pupal mass of three common tephritid flies including smaller-bodied *Campiglossa nigricauda* (Cn, **a** and **b**), *Tephritis femoralis* (Tf, **c** and **d**), and larger-bodied *Terellia megalopyge* (Tm, **e**) under ambient and pollen supplementation treatments at the distant and close sites along transects. The different letters above the columns indicate significant differences between treatments ($P < 0.05$). The error bars denote standard errors. Sample sizes are the same as indicated for Fig. 2

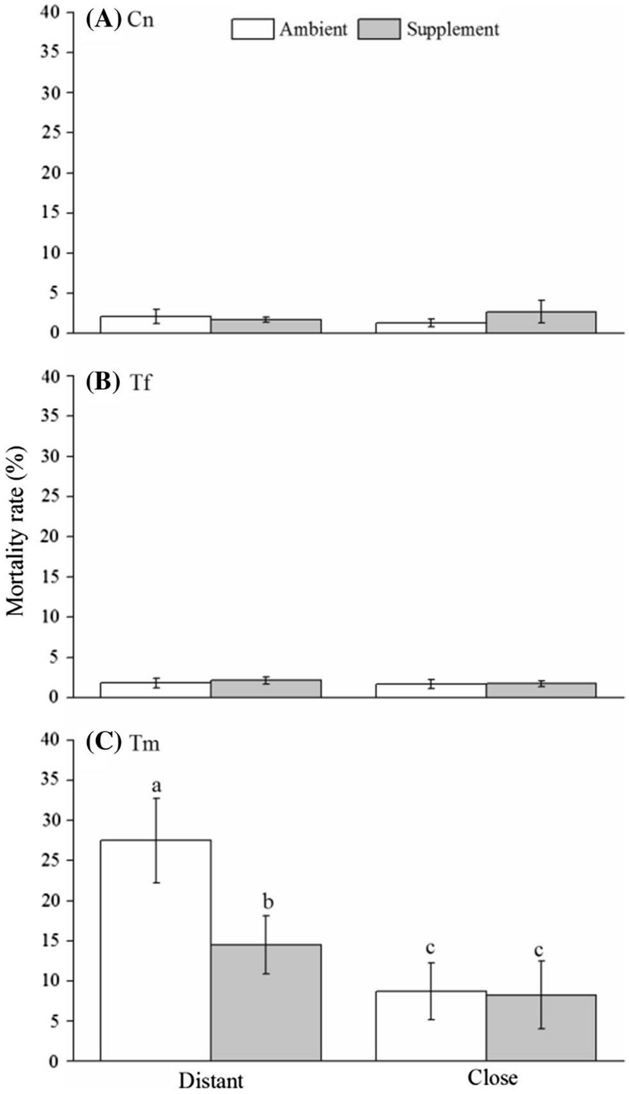

Fig. 4 Mortality rate of three common tephritid flies including smaller-bodied *Campiglossa nigricauda* (Cn, **a**), *Tephritis femoralis* (Tf, **b**), and larger-bodied *Terellia megalopyge* (Tm, **c**) under ambient and pollen supplementation treatments at the distant and close sites along the transects. The different letters above the boxes indicate significant differences between treatments ($P < 0.05$). No significant difference was found in (**a**) and (**b**). $n = 3$ sites for each treatment. The error bars denote standard errors

transects as no significant interactive effects between transect and site types or pollen supplementation treatment were found ($F < 0.90$, $P > 0.41$ for adult mass; $Z < 0.78$, $P > 0.44$ for survival rate).

Infestation rate and relative abundance of tephritid flies

The three fly species examined in our study accounted for > 80% of the total number of tephritid flies collected from *S. nigrescens* capitula. The infestation rate was indistinguishable between sites for the two smaller-bodied fly species ($Z = 0.51$, $P = 0.59$ and $Z = 1.47$, $P = 0.17$, respectively; Fig. 5), but it was 55% higher in the close sites than the distant sites in the case of the larger-bodied species ($Z = 10.34$, $P < 0.01$, Fig. 5).

Relative abundance was higher in the close sites than the distant sites for the larger-bodied fly species ($W = 0$, $P = 0.0079$, Fig. 5), but it was lower in the close than the distant sites for both of the smaller-bodied species ($W = 21$, $P = 0.09$ and $W = 23$, $P = 0.031$ for *Teph. femoralis* and *C. nigricauda*, respectively; Fig. 5).

Discussion

We have shown that high densities of domestic honeybees significantly improved the survival and growth of a relatively large-bodied tephritid fly species but not two smaller-bodied ones by increasing the seed production of the host plant species, *Saussurea nigrescens*. Consistent with the increased survival and growth of the larger-bodied species, the capitulum infestation rate and relative abundance of the larger-bodied species were greater in sites close to apiaries. These results demonstrate a significant positive and species-specific effect of pollinators on herbivores, indicating that pollinators serve as an important factor affecting the ecology of plant herbivores.

Pollen limitation is common among Asteraceae species (Ferrer and Good-Avila 2007), particularly in high altitude regions such as our study area where native pollinators are often scarce (Straka and Starzomski 2015). Several lines of evidence indicate that the increased seed production of *S. nigrescens* in the sites close to apiaries can be attributed to the high densities of domestic honeybees, which are the result of ongoing apiary practices that were not experimentally altered during our study. One line of evidence is that *S. nigrescens* has been demonstrated to be self-incompatible in the study site and its seed production largely depends on pollinator visits (Mu et al. 2014). Another is the observation that pollen supplementation increased seed set in the distant as opposed to close sites. This observation is easily explained because honeybee visits at sites near apiaries essentially “saturate” the seed-producing capacity of nearby plants. A third but ancillary line of evidence is that the vegetational composition and the soil water and nutrient availability were indistinguishable among all six sites, which essentially excludes water or nutrient limitations as factors confounding the effect of domestic honeybees on plant seed production. Indeed, individual seed mass and the total number of developed and undeveloped seeds per studied capitulum were indistinguishable among sites.

In light of our data, it is reasonable to argue that the increased seed set and seed production due to high densities

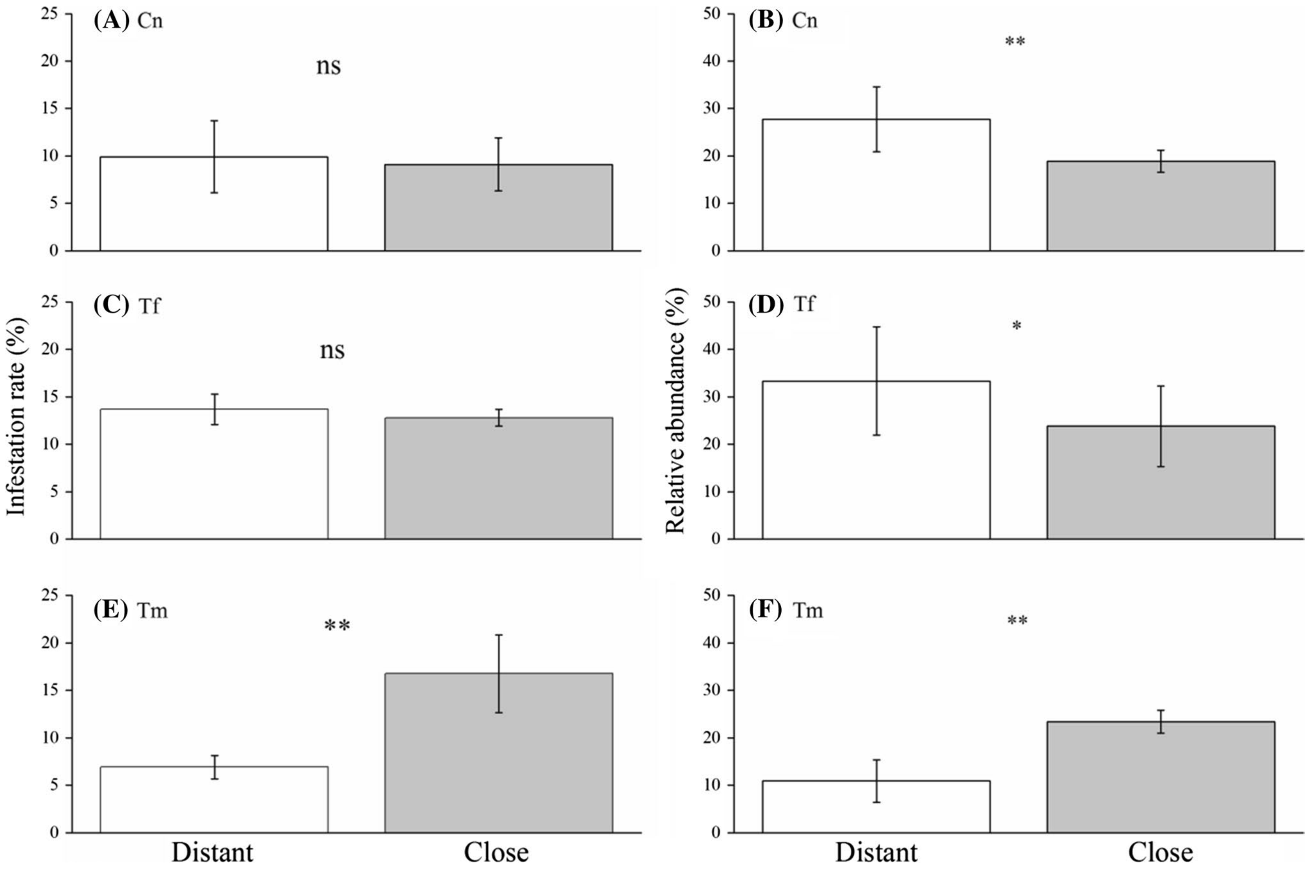

Fig. 5 Infestation rate of *Saussurea nigrescens* capitula and species relative abundance of three common tephritid flies including smaller-bodied *Campiglossa nigricauda* (Cn, **a** and **b**), *Tephritis femoralis* (Tf, **c** and **d**), and larger-bodied *Terellia megalopyge* (Tm, **e** and **f**) at the distant and close sites along the transects. **Indicate significant difference ($P < 0.01$); and *ns* not significant. $n = 3$ sites for each treatment. The error bars denote standard errors

of domestic honeybees would certainly improve the performance of the pre-dispersal seed predators because their larvae rely on the seeds within a single capitulum for their growth and survival (Xi et al. 2017). However, the effect of improved seed production on the three tephritid fly species was species-specific: it facilitated the growth in body mass of the larger-bodied species but not the two smaller-bodied species. We attribute this to the different food requirements among the three fly species. In the three sites distant from apiaries, the number of developed seeds per capitulum was on average 16.8. This is much smaller than the number of seeds consumed by the larvae of the larger-bodied *Terr. megalopyge* (30.4 seeds on average, including both developed and undeveloped ones) but it is larger than the number of seeds (including both developed and undeveloped) required by either of the two smaller-bodied species (< 15 seeds for both *C. nigricauda* and *Teph. femoralis*). Accordingly, an increase in seed production (in the close sites or under pollen supplementation) went "unseen" by the smaller-bodied seed predators because their food requirements were already saturated (e.g., in the distant sites without pollen supplementation), while the growth of the larger-bodied fly species was enhanced by improved seed production.

It is worth noting because that the "number of consumed seeds" here includes both developed and undeveloped seeds, the increase in seed set due to pollen supplementation in distant sites or domesticated honeybees around apiaries leads to a higher proportion of developed seeds consumed by larval flies and then less overall number of consumed seeds. This might partly explain why the larger-bodied species could sometimes survive in capitula containing a limited number of developed seeds. Nevertheless, we frequently observed vestiges of bracts being nibbled on in the capitula infested by the larger-bodied species, suggesting some degree of food limitation for the larger-bodied species.

The positive effects of pollinators on the performance of herbivores appear to have affected herbivore abundance. Although the soil conditions and vegetation structure are indistinguishable among sites (Mu et al. 2014), there was a relatively steep gradient in the abundance and the infestation rate of plant capitula by the larger-bodied fly species between the close and distant sites. In contrast, the abundance of the two smaller-bodied fly species was similar between sites. Note that although the higher species relative abundance is a correlate of the higher infestation rate, it can also be a consequence of increased survival rates that are in turn a correlate of an increase in seed production.

In summary, we have demonstrated that domestic honeybees have a significant effect on the survival and growth of pre-dispersal seed predators and thereby possibly have an indirect effect on herbivore abundances. Because pollen limitation is prevalent in flowering plants (Wilcock and Neiland 2002; Knight et al. 2005) and seed predators are diverse in natural communities (Lewis and Gripenberg 2008), our findings may have important implications. First, as one of the few studies that explicitly address the effect of pollinators on herbivore performance, our data indicates that pollinators must be regarded as a potent biological force affecting the adaptation of herbivores as well as the plant species they visit. It is thus reasonable to speculate that apiculture and the associated increased activity of domestic honeybees may have other important consequences on herbivores other than on pollination services, and thus deserves considerably more attention and further research (Moritz et al. 2005). Second, although it is generally appreciated that plant herbivores can negatively affect the behavior of pollinators, our data indicate that plant mutualists and antagonists can interact bi-directionally to affect plant fitness. This finding cautions against studying plant-mutualist and plant-antagonist networks separately rather than collectively. In addition, our data indicate that pollinators may indirectly affect the relative abundances of insect herbivore species, with potential consequences for insect communities. Such an indirect effect of plant mutualists on herbivore communities must be addressed further in future research.

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Compliance with ethical standards

Conflict of interest All of the authors declare no conflict of interest.

Data accessibility The data will be archived on the Dryad Digital Repository when the paper is accepted for publication.

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